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PREFACE

This volume carries the proceedings of the Vahouny Fiber Symposium, which took place in Washington DC, in March of 1996. George V. Vahouny pioneered this conference, which was conceived as a means of exchanging information on the latest advances in fiber research. The conference was renamed in George Vahouny's honor after his untimely death.

In this volume we have included the reminiscences of Prof. A.R.P. Walker, one of the pioneers in the field, and discussion of the epidemiological findings relating dietary fiber to heart disease, cancer, and gastrointestinal disease. As the overall definition of fiber has broadened we have included for discussion other aspects of carbohydrate chemistry and metabolism. Complex carbohydrates were the subject of a full session and resistant starch and the role(s) of oligosaccharides were discussed later in the meeting.

Another area of fiber metabolism that is beginning to burgeon is that of the short chain fatty acids, and this too was the subject of presentations and discussion. In addition, the more familiar topics in dietary fiber were updated. Workshops devoted to specific areas of fiber research have now become a mainstay of these meetings, and they were repeated successfully.

Due to restraints on spending, the attendance at this meeting was somewhat smaller than usual but the discussion was lively and pertinent.

We are deeply grateful to our sponsors and contributors for their continuing interest in dietary fiber research, and for their generous support of this symposium.

David Kritchevsky
Charles Bonfield

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DIETARY FIBER IN HEALTH AND DISEASE

1

NUTRITIONALLY RELATED DISORDERS/DISEASES IN AFRICANS

Highlights of Half a Century of Research with Special Reference to Unexpected Phenomena

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1. INTRODUCTION

It is really a tremendous honor to have been asked to present highlights of half a century of research in Southern Africa, carried out by myself and numerous collaborators.

My interest in nutrition-metabolism-health/disease in Africa arose in the following manner. After graduating in 1934, principally in chemistry at the University of Bristol, for 3 years I first worked as an analytical chemist in the Bristol City Analyst laboratories. There I learnt much about the composition of numerous foods and beverages, and hence began wondering about their differing importance to health. In 1938, with my newly married wife, Betty, we emigrated to South Africa, largely because of the country-wide economic depression, and the great difficulty of even University graduates obtaining employment in the fields desired. In Germiston, and later in Johannesburg, I worked for 3–4 years in the laboratories of the Municipal Department of Health. One aspect of my duties was the analysis of various effluents and sludges from sewage, the end product of human metabolism. In the flow of raw sewage at the sewage works, one saw feces of various sizes and consistencies, hence the question — has such variability any significance to health? Thereafter, at the South African Institute for Medical Research, until now, half a century later, I have been interested in what happens in between, from the amount and nature of food eaten, the purpose it serves, to the ultimate passing of excreta.

Briefly, transition to this latter phase of employment arose as follows. In 1943, the South African Government, as a war-time measure, introduced a brown loaf of bread, "standard war bread," of 90% extraction rate, as the only bread available for consumption. At that time there was a South African National Nutrition Council, and it came about that I was

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temporarily seconded from my municipal post, to serve with this Council, in order to conduct nutritional studies on the war bread. The question was: "Could brown bread in any way be harmful to the mineral metabolism of the African population, whose intakes of mineral salts, such as calcium and iron, were already low?" The enquiry was prompted by the pioneer studies made on this subject by McCance and Widdowson,[1] in Cambridge. From the results of our balance studies, which were copies of their studies, it transpired that initially brown bread did indeed cause negative calcium and iron balances in both white and African subjects (prisoners). But over the weeks of observation, in time, equilibrium was reached, followed by positive balances.[2] This led us, *inter alia,* to doubt the adverse inhibitory role attributed to phytic acid.[3,4] It became clear from these and numerous subsequent studies that the body can adapt successfully to a wide range of dietary conditions.

It was these early investigations, especially the observations made on the African subjects, that gave rise to many areas of research in which I became an active investigator.[5–8]

Before describing some highlights of research, it is necessary, for better appreciation, to give some general information as background, with particular reference to the transitions occurring in the nutritional context, and in the health/ill-health experience of Africans.

2. THE CONTEXT

South Africa, a fifth of the area of the US, has the following populations — Africans, 30–35 million, Whites 6 million, Coloureds (Eur-African-Malay) 3 million, and Indians 1 million. As to future numbers of populations, while they will certainly continue to rise, the *rates* of increase of all the constituent populations are falling, because birth rates are decreasing. Indeed, at the extreme, in some urban African populations, with an average family of 2 or so, the birth rate has now fallen to near replacement level. It must be emphasized that in South Africa, African urban dwellers now compose approaching half of the African population — this by far is the highest proportion urbanized in any country in the Continent. It must be added that there is an increasing, and difficult to restrict, inflow of Africans from less prosperous neighbouring countries; such could be as many as 15 million or more.

3. CHANGES IN PATTERNS OF DIETS

The diet of rural dwellers, more especially those living traditionally, was, and still is in measure, low in energy, low in fat and protein intakes, but high in plant foods. In consequence, the fiber intake is high, about 20–35g or more daily. Nowadays, however, due to changes such as much improved transport and other services, the rural diet is slowly becoming westernized in several respects.[9]

In urban compared with rural dwellers, not unexpectedly, the diet has higher energy, fat, protein and sugar contents. It is still high in plant foods, save that the cereal products, chiefly maize meal and bread which form a large part of the diet, are now refined. Thus, compared to formerly, the diet is now one of higher energy, higher fat, but lower fiber content, about 10–15g daily.

It will be appreciated that the changes described among Africans parallel those previously experienced by most western populations, before and soon after the turn of the

present century.[10] As an example in the UK, in 1900, fat and sugar contributed a third of the energy intake; at present the proportion approaches two thirds.

4. NON-DIETARY TRANSITIONAL CHANGES

The most important of such changes which affect health/ill-health include — higher smoking frequency in African males, although not females; a higher alcohol intake, again, principally in males; and diminished physical activity in both sexes.[7,8] Understandably, apart from the impact of nutritional changes, each of the non-dietary factors listed can have a powerful negative affect on the general health/ill-health pattern.

5. RURAL AFRICANS — EXPECTED HIGH PREVALENCES OF NUTRITIONAL DISORDERS/DISEASES

Numerous on-going studies made on rural Africans have indicated the continuing commonness of protein-energy-malnutrition (marasmus, kwashiorkor), and of gastro-enteritis in the very young; and at a later age, pellagra, anemia (regionally, especially in areas where malaria and hookworm are endemic), and goiter (regionally). Our enquiries, past and present, made on causes of admission of adults to rural hospitals, have revealed infections, principally tuberculosis, to be common.[7,8,11] The most frequent of cancers were, and still are, those of the cervix, esophagus, and liver. Of the cancers of prosperity, there are still very low prevalences particularly of colo-rectal, breast and prostate cancers.[12]

6. URBAN AFRICANS — EXPECTED HIGH PREVALENCES OF NUTRITIONAL DISORDERS/DISEASES

Observations, particularly those made recently on urban dwellers, have indicated the current commonness of dental caries, obesity (in women), hypertension, and diabetes. In contrast to their far lower frequencies in rural areas, the current respective prevalences of these disorders/diseases in urban Africans have now become actually *higher* than those in the white population.[13]

7. UNEXPECTEDLY LOW PREVALENCES OF DISEASES IN AFRICANS

Understandably, in epidemiological studies, it is the why and wherefore, of the *unusual* findings which are the most exciting, and, accordingly, constitute the highlights of research interest. In our case, these include the very low prevalences of (1) gastrointestinal diseases — appendicitis, colonic polyps, and colon cancer, a situation which still prevails despite the greatly decreased intake of fiber; (2) coronary heart disease (CHD), whose very low rate obtains in spite of the high prevalence of many of the risk factors; (3) the rarity of hip fracture, and this despite the high prevalence of promotive factors, particularly low calcium intake; and (4) the apparent innocuousness to health of the still highly prevalent mild to moderate malnutrition in schoolchildren.

In contrast to the foregoing, a fascinating scenario is the commonness of iron overload, particularly in rural dwellers, which, possibly apart from cases exhibiting extreme overload, appears to have little or no pathogenicity.

Each of these topics will now be enlarged upon, because, to re-iterate, they rank among the author's highlights of collaborative research.

8. GASTROINTESTINAL DISEASES

The young African prisoners who were studied long ago in the brown bread investigations, voided a large amount of feces, 200–400g wet feces, daily, and they defecated more frequently, 2–3 times daily.[1] Comparable data on white subjects of the same age range were noted to be about 100g feces, with defecation usually once daily. The Africans by far had shorter mean transit times, compared with those of series of young white adults studied, namely, 18 versus 28–32 hours, using carmine as indicator.

What do these contrasting aspects of bowel behavior imply with regard to everyday health, and to bowel and other diseases? In the original prison context studied, it was found that whereas series of white prisoners often asked for laxatives for constipation, African prisoners never did (both series questioned in this respect numbered several hundred). In the previous 10 year period at the prison, there was only one case of appendicitis in thousands of African prisoners, whereas there had been 6 cases in the previous 3 years with white prisoners. In the history of the prison, no elderly African had developed colon cancer, nor, interestingly, had had an heart attack, in contrast to the not infrequent cases of both of these diseases noted among elderly white prisoners. All of these contrasting phenomena greatly stimulated our interest in the patterns of bowel physiology and pathology associated with dietary and other differences in our interethnic populations.[14]

Since the late 70's, there has been active collaboration in this field with Professor Isidor Segal, Head of Gastroenterology, Baragwanath Hospital (3000 beds), and Johannesburg Hospital. Enquiries revealed, *inter alia,* that appendicitis in African schoolchildren has only one-tenth of the frequency found in white children,[15,16] and that in African adults colon cancer has one-tenth of the frequency found in white adults,[17] as also noted in the South African Cancer Registry.[18] In agreement, is the absence of polyps even in elderly Africans, whose colon in appearance and elasticity resembles that of young whites.[19] Subsequently, investigations were made on inter-ethnic transit times,[20] fecal pH values,[21] and, more recently, fecal short chain fatty acids,[22] also the degree of absorption of maize and other cereals in Africans *versus* whites.[23]

In this field, some unexpected findings were encountered. An early experience concerned the ability of the huge majority of rural African schoolchildren to pass a stool on request! This ability still remains. Were children of western ancestors, also accustomed to a high fiber low fat diet, able to perform in this manner?

Arising from the contrasting behaviors noted, some highly practical questions arose. Could consumption of a very high fiber intake by whites in time confer a bowel *milieu intérieur* similar to that of Africans, and hence possibly lead to the prevention or the lessening of chronic bowel diseases? Could a high fiber intake reduce or even eradicate the presence of fecal mutagens, precursors of cancer?[24] In other but related fields, could a high fiber intake have any bearing on the low incidence of breast cancer in rural African women, an incidence which is a tenth of that in white women?[17,25,26]

Regarding the foregoing questions, it is interesting that in wartime countries in Europe, the involuntary changes in diet, which included a higher fiber intake, were associ-

ated with a fall in the occurrence of constipation, of dental caries scores, decreases in mortality rates from diabetes, and coronary heart disease,[27] also a reduction in the frequency of severe atherosclerosis.

Subsequently, considerable interest arose in the occurrences of cancers additional to colon cancer, which are very uncommon in Africans. Breast cancer is a huge current problem in western women.[18,26] In African, compared with white women, the former's protective factors include (1) reproductive behavior, namely, slower growth before and after puberty, later age at menarche, high teenage pregnancy, high parity and long periods of lactation and amenorrhea; (2) a diet of relatively low energy intake, and of low-fat and high-fiber contents, and (3) a high level of physical activity.[26] Pragmatically, from observations made, it would seem that should prevention of breast cancer in western women depend on compliance with a *number* of the protective factors listed, then its primary prevention not only in western but in some urban developing populations, would seem almost impossible, even with the increased motivation of those at risk familially.[28]

From the questions raised and the studies undertaken, interest arose in the epidemiology, risk factors, metabolism, and survival times of African patients with diet-related cancers, not only of the colon, but of esophagus, stomach, liver, prostate, and breast.[29–31] Together with helpers, many papers were published on survival times, a field of research barely touched upon locally, despite the huge differentials in the occurrence of many cancers between African and white populations.[32,33]

Because of the rarity of prostate cancer in rural African men, a number of collaborative studies were carried out with Peter Hill, of American Health Foundation, Valhalla, New York. It was found, *inter alia,* that a western diet fed to a series of rural African men caused a decrease in the urinary excretion of estrogens and androgens, and the plasma level of testosterone.[34]

From very recently undertaken unpublished studies of hospital records in Johannesburg and Durban, a depressing finding is the absence of significant *betterment* in the stage situation of cancers, compared with such prevailing 10 years ago. This indicates that no cancer prevention message, nor the need for early detection of treatment message, is getting through to Africans. This has led to the carrying out surveys of cancer knowledge (using modifications of overseas questionnaires) in young and older people in the different ethnic groups.

Since mortality from colon cancer remains very high in western populations, endeavors must continue to be made to try to learn more of the reasons why all African populations, despite considerable variations in foodstuffs eaten, suffer so very little from the disease.

9. CORONARY HEART DISEASE

One feature of the African prisoners studied long ago was the finding of large amounts of fat in the feces, up to 15g daily. This was considered to be largely endogenous fat, associated with a very high fiber diet.[35] This observation led to interest in fat intake, serum cholesterol level,[36,37] serum fibrinolytic activity,[38] the chemical composition and pathological grading of atherosclerotic lesions in aorta,[39] the epidemiology of the CHD,[40,41] interethnic electrocardiograms,[42] and prevalences of risk factors of CHD (e.g. hypertension), such as prevail in our different ethnic populations.[43]

In an investigation undertaken with Professor John Higginson, Department of Pathology of this Institute, on the pathological grading and chemical composition of aortas, it was

found that in pathological grading, a large proportion of aortas of elderly Africans resembled those of young whites; for most aorta of Africans were smooth with little evidence of plaques, ulceration or calcification.[39] Recent post-mortem observations by Dr A Shor, of the National Centre for Occupational Health, Johannesburg, have indicated there to be only a slight change in this respect, i.e. there are still very large differences in the extent of lesions in aorta and in coronary vessels in Africans compared with whites. Clearly, mankind does not *have* to age in these respects. It is the manner of life which regulates degeneration.

A crucial question, bearing on the foregoing, is — how uncommon is CHD in Africans, compared with its present occurrence in western populations? It was certainly known that it was of low occurrence, but not as low as it was found to be. Of residents of Soweto (3–4 million), adjacent to Johannesburg, the huge bulk of the population when need arises, attend Baragwanath Hospital. In 1994, 66 CHD patients were diagnozed,[44] although only 36 were locally resident. If all 36 died from the disease, then CHD would be responsible for only about 0.2% of the roughly 20,000 deaths occurring annually in Soweto — an extremely low proportion, even allowing for all of the uncertainties involved. In the recently reported Seven Countries Study,[45] for those in the countries Mediterranean, and inland, the age standardized 25 year CHD mortality rate percentages were 4.7% and 7.7%, respectively. Proportions reported for countries in Northern Europe and for US were far higher, 16.0% and 20.3%, respectively. These comparisons thus underline the rarity of CHD in urban Africans. In a case-control study, made with Professor P. Sareli, Cardiology Department, Baragwanath Hospital, and mentioned in a letter,[44] the only significant risk factor in African CHD patients was their low mean level of serum HDL cholesterol. The patients were found to be younger, males outnumbered females, and the clinical and laboratory features and complications of the disease were considered to be similar to those in the white population.

Remarkably, the very low mortality from CHD in urban Africans prevails despite their diet now being of higher energy and fat contents, but lower fiber content, and despite there being a high prevalence of obesity (BMI $\geq$ 30) occurring in 40–45% women (the prevalence of high BMI in men is very low, 8%). Additionally, there is a high prevalence of hypertension, increases in smoking practice (50% in men 10% in women), and diminished physical activity compared with that experienced in the past. The serum cholesterol level of patients averaged 5.5 mmol/L compared with 5.2mmol/L in unaffected urban dwellers;[46] mean HDL levels were 0.95 mmol/L and 1.55 mmol/L, respectively.

Should there really be surprise that CHD is very uncommon in urban Africans? After all, it was very uncommon in Afro-Americans in 1960–1970 (although now, their mortality rate has reached that of the white population[47]), and, among whites it was almost rare in the UK in 1910–1920.[48]

Because of the near universal impoverishment of populations in Africa,[49] it is highly unlikely that there will be a significant rise in mortality rate from CHD in the foreseeable future.[46]

10. HIP FRACTURE IN AFRICAN WOMEN OF 40 YEARS AND OVER

From the observations made on the low calcium intake of the African prisoners, interest arose in the intake of the element by Africans in general, also in the concentration of the element in breast milk,[50] in serum,[51] its content in bones (ribs and vertebral bodies),[52] and bone density indices in metacarpal and humerus.[53,54] Briefly, it became clear

that the low intake in the huge majority of Africans is not significantly deleterious to health. Calcium concentration in the breast milk of local African mothers is higher than that noted for breast milk in mothers in The Gambia.[55] Despite a low intake of the element the young African prisoners had excellent teeth. The latter observation led to our interest in diet and dental caries in interethnic children.[56,57] With collaborators, numerous comparative studies were made of caries scores in different populations. Currently, 70–80% of *rural* African schoolchildren of 10–12 years are caries-free. Many of such children come from very poor homes, with daily calcium intakes of 200–300 mg. It transpired, *inter alia*, from subsequent research studies, that sugar plays only a minor etiological role in caries development, in contrast to the current importance attached to it by many.[58,59]

Turning now to hip fracture — in western countries, the salient reason for the high calcium intake now being advocated by authoritative bodies is to ensure maximal bone density, to lessen osteoporosis, and ultimately to lessen proneness to hip fracture — whose incidence is increasing, with its associated high morbidity and mortality. Yet, contrary to orthodox expectation, among Africans, particularly among African women with their not only habitually low calcium intake, but with large calcium losses from high parity and long lactations, hip fractures are rare. In Johannesburg, in 1979, Solomon showed its incidence in Soweto to be one tenth of that in white women.[60] In a recent unpublished study made by Dr E Schnaid, at Natalspruit Hospital (900 beds), near Johannesburg, and serving about a million Africans, in observations extending over a 3 year period, this very low incidence of hip fracture was confirmed. Does calcium requirement vary with the context? Is its importance as the preponderant risk factor in hip fractures, over-rated? What exactly are the factors in the context of elderly African women which so clearly protect them?

11. LOW ANTHROPOMETRY AND ITS HANDICAP TO HEALTH

The young African prisoners' weights and heights for age showed marked shortfalls compared with the results of measurements made on young white adults. This led to intensive interest in the implications of lesser growth, so common in African children.[61,62] Using the criterion of being under the 5th centile of NCHS Reference Standards of weight and height for age,[63] numerous studies, occasionally with others, have indicated that 30–50% of African children, young and old, lie in this category, and can therefore be regarded as malnourished.[64] To what extent does such malnutrition impact on health experience?

First, among very young Africans, how much are unsatisfactory weaning foods to blame for faltering growth?[65] How much of their lesser attainment of growth is due to a lower energy intake? Or to other factors, such as adverse home circumstances? Certainly in pre-school children, up to 5 years of age, in many areas, nutritional intervention in those stunted and wasted has proved to be very rewarding, leading to reductions in morbidity and mortality.[66]

Among schoolchildren, lesser growth is often present in one third or more. In those with mild to moderate malnutrition (severe malnutrition (stunting and wasting) affects only 2–4%), how deleterious is it to the enjoyment of good health?[67–69] Is school-feeding mandatory?[70] Recently, exhaustive studies were made of a series of rural pre-pubertal African schoolchildren in respect of absenteeism, class position, distance traversed to school, nutrition at home and at school, detailed home socio-economic circumstances, habitat, etc. No single parameter, or set of parameters, was found to be clearly responsible.[71] Obviously, the situation is complex, as authorities such as Dobbing[72] and numerous others, have abundantly stressed. It would seem reasonable to suggest that the orthodox classification of schoolchildren below the

5th percentile of reference standards of growth, as malnourished *per se,* and at a health disadvantage, requires re-appraisal. Furthermore, it could be conjectured, from the results of animal experimental studies,[73,74] that an energy intake slightly lower than average, in youth, could actually help to prevent or lessen atherosclerosis in later life, reduce proneness to diet-related cancers, and extend longevity.

An important question is — does mild to moderate malnutrition prejudice physical activity of children, or of adults? It would be reasonable to think so. Yet, in early studies on rural African schoolchildren, using the 11 minute walk/run test, no significant difference was found in mean oxygen consumption level (VO_2 ml/kg/minute) between school pupils with and without the infection, of *schistosoma hematobium.*[75] Moreover, recent calculations showed there to be no significant difference in the mean performances of pupils below and above the 5th centile of NCHS reference standards. Additionally, in recent unpublished interethnic determinations, on 10–12 year children, regarding grip strength, no significant differences were found between the mean scores of African and white children, nor between the performance of African children below and above the 5th centile of growth. Evidently, in the contexts described, mild to moderate malnutrition in schoolchildren does not significantly prejudice physical activity.

Since the foregoing problem concerns a large proportion, and, in consequence, huge numbers of schoolchildren in developing populations, it is imperative to arrive at a clear decision as to whether the lesser growth depicted is, or is not, of significant public health importance.

12. HIGH IRON INTAKE AND IRON "OVERLOAD"

The finding of seemingly impossibly large amounts of iron in the feces of the African prisoners indicated that the iron must come from their food preparation vessels.[1] This led to the development and publication of the "iron overload" hypothesis, to explain the commonness of abnormal iron deposition, or siderosis, which previously had been reported in post-mortem studies on the liver, spleen, and other organs and tissues of African adults.[76] Formerly, these deposits had been attributed to malnutrition, pellagra, or other causes. The early studies of McCance and of Widdowson,[77] in Cambridge, and those of others, clearly demonstrated that an excessively high iron intake leads inevitably, although variably, to abnormal retention of the element.

Numerous studies were made on the iron content of maize, beans, and other foodstuffs, when cooked in the common three-legged iron "kaffir" pot, in paraffin cans, and also in large iron cauldrons, such as are used for the mass feeding of African workers on the gold mines. The highest iron concentrations were found in drinks of low pH value — sour porridge (*mageu*) and "kaffir" or sorghum beer. With Marianne Andersson and Theo Gerritsen, determinations were made on levels of iron, also of iron binding capacity, in the serum of groups of African miners, and of African villagers in various rural regions, including such in Swaziland. A large proportion of African adults were found to have elevated values, men being affected far more so than women. At that time, in the mid-1950's, Professor John Higginson, mentioned previously, became very interested in Bantu "siderosis".[78] Accordingly, numerous organs and tissues of Africans, from postmortems made at Baragwanath Hospital, were assayed for iron concentration, while John carried out the various histopathological examinations. From the results obtained in that particular study, the pathogenicity of iron overload was doubted, principally because no constant correlation was found between degrees of fibrosis and cirrhosis, and iron concentration.[79]

About a decade later, at the same hospital, there were reports of iron deposition being associated with scurvy, osteoporosis, diabetes, esophageal cancer, and heart failure.[80–82] The findings mentioned, however, made long ago, have not been confirmed, nor have case-control studies been pursued. No attempt has ever been made at village level to learn whether iron overload really contributes significantly to detectable morbidity and mortality in the community. In brief, is siderosis unequivocally disadvantageous to 1 in 10, 1 in 100, or 1 in 1000? In a recent contribution from Zimbabwe, it was concluded that iron overload could well be a causative factor in hepatocarcinoma, HBV infection being regarded as the primary factor.[83] Yet, even were iron overload contributory, mortality rate from liver cancer is low; in countries in Southern Africa, it is about 6–8 per 100,000 "world" population.[17,84] At present, in our appreciation, the noxiousness of iron deposition, although suggestive, remains unproven, and attempts to persuade Africans not to use iron vessels for the preparation of their foods and drinks lack justification.[85]

It is truly remarkable that huge differences in serum ferritin concentration can be consistent with outward good health, as with a series of vegetarian women studied in New Zealand, with a mean level of 13μg/L,[86] and, at the extreme, a group of African men in Cape Town who, at 45–54 years were found to have a mean value of 509 μg/L, and a median value of 285μg/L (Personal communication, Dr PL Jooste). Clearly, the bearing of very high values on current and future health must be elucidated.

In the western context, recently, the view has been expressed that a high level of serum ferritin and iron reserves could be promotive of the development of coronary heart disease.[87] In the African context, from the information already given, it will be apparent that siderosis has had no influence on the development of severe atheroma or of coronary heart disease, both of which are very uncommon.[88]

13. NUTRITION AND OTHER TRANSITIONS, GAINS, AND LOSSES IN AFRICANS

From the numerous observations made by ourselves and others, at the end of the day, it could be asked — among Africans, what are the benefits, and the drawbacks, from transition, which in any case is largely involuntary, rather than voluntary?

13.1. Positive Features

1. Major falls have occurred in infant mortality rate (in big cities rates are 20–25 per 1000 live births), in family size (the average number of children has halved or less), and marked increases in longevity, namely, to approximately 60 years.
2. Access to potable water has improved.
3. Clinic/hospital services have greatly improved in numbers and accessibility; benefits include considerable rises in immunization coverage in children.

13.2. Negative Features

1. The African population is still increasing rapidly, despite the fall in birth rate. This is occurring in a context of very high rates of unemployment and of crime.
2. Mortality rates from all western diseases are rising.
3. Dietarily, the lower energy, low fat/high fiber regimen of the past, has gone forever. Of dietary components, with prosperity, there *could* be a rise in the intake

of vegetables and fruit — this is the only dietary change likely to increase in protective capacity.

4. Smoking practice and excessive alcohol consumptions are rising.
5. Physical activity is falling.
6. Of immense adverse moment, tuberculosis is a huge and possibly increasing problem.[89] Crucially, HIV infection is increasing rapidly — 15% in parts of Natal KwaZulu,[90] and in some antenatal clinics in Zambia has reached 30%.[91] As a comparison, in Europe in 1347–1351, the Black Death caused the deaths of 30–50% of total populations.

14. ARCADIA, RESPECTING NUTRITIONAL DISEASES

This topic, which concerns the best experience of health in segments of societies past or present, is one of individual appreciation.

In Africa, from what has been described it will be apparent that the best circumstances of living were to be found in traditionally-living rural Africans, provided that they had enough food to eat, and that there was inter-tribal peace (infrequent in the past).[92] At such times, indeed, up to relatively recently, the elderly ailing African died not from degenerative diseases but from the same diseases as young adults, principally infections.[93]

In a western context, it is still possible, by following a particular way of life, to have far better health outlooks, specifically, lower standard mortality ratios (SMRs), compared with such experienced by the average. These ratios are relatively low for example, among vegetarians[94] and Seventh Day Adventists,[95,96] over half of whom are vegetarians. However, the most spectacular example, from personal knowledge, concerns the low SMR's of active Mormon priests and their wives — namely, cardiovascular disease 14 and 55, cancer 34 and 34, all causes 22 and 47, respectively, all compared with average Americans ranking as 100.[97] On this subject, far more intensive research is needed to identify which component or combination of components of life-style, make for the longest period of disease-free years, or, more appropriately, of years of "wellness".

Unfortunately, early suggested guidelines (mainly relating to fiber intake),[98,99] and later ones,[100,101] have achieved only limited adoption, at least among children.[102,103] Nowadays, scarcely anyone would wish to eat less in a world, at least in a western world, where food has never been more delectable, and is likely to become even more so. So, in view of almost universal indifference and of disinterest in the masses in both First and Third Worlds, it could be asked, pragmatically — does it really matter terribly much if people don't or won't take on a "prudent" lifestyle? In this connection, in the US, the concept of the "tyranny of health" ("do this, don't do that") has evoked considerable interest and some controversy.[104] Interestingly, one patient is reported to have countered his physician's health recommendations with "You know, Doctor, there are more things in life than good health".[105] In the UK, McCormick,[106] outspokenly, has taken the view that when advice is sought regarding health improvements, certainly every help possible must be given. Otherwise, he considers that apart from extremes (e.g. the heavy smoker, the very obese) "people should be encouraged to live lives of modified hedonism, so that they may enjoy to the full, the only life that they are likely to have."

To revert to Africans, and to underline what has already been indicated, this population, particularly urban dwellers, wish to pursue to the full, the experiences and practices of the white population. Only in respect of a possible rise in vegetables and fruit, with

prosperity, is nutritional betterment a possibility. The likelihood of any restriction respecting adverse non-dietary influencing factors is very remote.

There is one last point, in studies made on elderly villagers, urban dwellers too, some, despite adverse dietary and non-dietary factors, seemed to keep well consistently. Unfortunately, the subject of why the well keep well, whether regarding the individual or communities, has been poorly researched. How much is governed by genetics, by various life-style factors, by culture, etc? In 1962, John Ryle, of Cambridge,[107] one of the founders of social medicine, maintained that the study of those who keep in good health should be greatly increased. This subject has received minimal attention.

Finally, as will be appreciated, it has been a wonderful and exhilarating privilege to have been able to observe at first hand the contrasts and transitions between traditionally living rural Africans, and urban Africans whose highest aim, to re-iterate, at least in South Africa, is to fully emulate the way of life of the white population. Although our present health services can cope reasonably well with the current various disorders/disease scenarios depicted, this would certainly not be the case were there marked rises in the cancers of prosperity, and in CHD, or should the AIDS situation get out of hand, which alas could well happen. Furthermore, in the Continent of Africa, once population growth exceeds food production capacity, and this already prevails with a number of populations, who, accordingly, are facing "entrapment", then the conception of "Africa on the precipice"[49,108] could be a reality.

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THE EVOLVING EPIDEMIOLOGY OF FIBER AND HEART DISEASE

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1. INTRODUCTION

The diet:coronary heart disease (CHD) hypothesis has been a fixture in heart disease research for the last half of this century (Ascherio *et al* '95a). The dominant assumption behind this hypothesis has been that dietary fats and cholesterol influence CHD risk through their effects on various lipoproteins (Keys '65, Hegsted '65). However, a parallel line of scientific findings over the same period suggests that dietary fibers may also affect the risk for heart disease. The challenge for researchers and the public at large is to understand the role of fiber in context with the influences of other lifestyle factors (Ascherio *et al* '95a). Does the crude association of increased fiber intake with a lowered risk for heart disease merely reflect an inverse relationship with accepted risk factors such as fats or cigarettes (Sacks '93, Wynder '96)? Or do fibers have an independent effect on CHD risk, and, if so, do they exert that influence through recognized lipid pathways (LRC '84) or through other biologic mechanisms?

The fiber:CHD hypothesis was first propounded by physicians working in Uganda and South Africa (Walker '56, Walker '74, Burkitt '73, Trowell '78). They reported extreme interracial differences in CHD and other "western diseases" (Trowell & Burkitt '81). The prevalence of these diseases in Blacks varied directly with the degree of acculturation to western lifestyles, especially western diet (Burkitt '73, Trowell '78). Change to a western diet was characterized by sharp increases in the intake of fats and refined sugar and a concomitant rise in serum cholesterol levels. However, as long as fiber intake remained at traditionally high levels, heart disease rates stayed low (Trowell '72, '77).

2. EPIDEMIOLOGIC STUDIES BASED ON INDIVIDUAL LEVEL DATA

As provocative as these observations are, the strongest evidence for the fiber:CHD association have come from Western European and American cohorts (Morris *et al* '77,

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Kromhout *et al* '82, Kushi *et al* '85, Khaw *et al* '87, Fehily *et al* '93, Humble *et al* '93, Rimm *et al* '96, Burr *et al* '89). Most of these studies include white males sampled without regard to their cholesterol levels (Table 1) although some data are available for other groups (Table 2). This chapter presents a review of these studies. It also discusses two papers erroneously identified as having findings of no association (Garcia-Palmieri '80; Burr '82; Sacks '93) and two modes through which fibers are assumed to act.

2.1. Prospective Studies in Men Selected from the General Population

The first report of a protective association for dietary fiber came from a methodologic study that was not designed to specifically address the fiber:CHD hypothesis (Morris *et al* '77). The authors had recruited 337 "bankers and busmen" to help them develop "a practicable way of assessing individual diet...in a large scale prospective study of diet and CHD." Although they eventually abandoned the methodologic project, routine surveillance of morbidity and mortality among the cohort was maintained up to 20 years. When the authors reviewed the dietary data (from seven day diet records) and linked them to the CHD outcomes, they noted a 3 fold risk for men in the lowest fiber tercile relative to those in the highest tercile. The association was entirely explained by cereal fiber. Having also noted an inverse association between energy intake and CHD, they adjusted fiber intake for total energy, age, occupation, and length of follow-up and found that men who developed CHD had a mean cereal fiber intake of 2.42 grams (g) versus 2.84g expected on the null hypothesis ($p<0.005$).

The Zutphen cohort of the Seven Countries Study included 871 men who underwent a Burke-type cross-check dietary history in 1960 (Kromhout *et al* '82). During ten years of follow-up, 37 subjects died of CHD. Mortality from CHD was four times higher among men in the lowest quintile of fiber intake relative to men in the highest quintile in crude analysis; but the association disappeared after stepwise adjustment for dietary starch, vegetable protein and energy per kilogram of body weight. The authors do not discuss collinearity of dietary intakes as a possible explanation for the change in estimated effect. No analyses by soluble or insoluble fiber subtype are provided.

The Ireland-Boston Diet-Heart Study also measured baseline diets of 1001 middle-aged men by the Burke method (Kushi *et al* '85). Subjects were then followed for 19 years. In the original article on this cohort, the authors expressed crude fiber exposure in a nutrient density form and reported a significantly lower CHD mortality risk among subjects in the high fiber tercile compared with those in the low tercile ($RR=0.57$, $p<0.05$). Adjustment for systolic blood pressure (SBP), total serum cholesterol, ECG abnormalities, cigarette smoking, and alcohol intake did not appreciably change this finding. However, when fiber intake was expressed as a calorie-adjusted residual in a subsequent reanalysis (Kushi *et al* '87), the coefficient for fiber was reduced and no longer statistically significant ($p>0.17$).

The only test of the diet:CHD hypothesis that included both men and women (see below) comes from the Rancho Bernardo, California cohort of the Lipid Research Clinic (LRC) Prevalence Study (Khaw *et al* 1987). Unfortunately, it also relied on the weakest method for characterizing diet over a long period of follow-up (a single 24 hour recall at baseline.) Crude comparison of men in the extreme terciles found high fiber intake associated with a statistically significant 3 fold reduction in CHD risk. The age-adjusted relative risk for a six gram increase in total fiber was 0.76 (95% CI 0.58, 0.98). Single addition of other dietary variables to a model adjusted for age and sex did not change the coefficient for fiber. Adjustment for SBP, total cholesterol, fasting plasma glucose, body mass index (BMI), and cigarette smoking reduced this estimate to 0.85 (0.64, 1.11).

Table 1. Characteristics and findings from six prospective studies of the fiber:heart disease hypothesis in men from the general population

Authors/site(s)	# Subjects & age at baseline	Mean intake of total fiber (grams/day)	Yrs follow-up & endpoints (n)	Findings		Findings adjusted for
				Unadjusted/age-adjusted	Adjusted	
Morris *et al* (1977) England	337 30–67 yrs	17	"10–20 yrs" mort/morb (45)	low 3ile vs high 3ile total fiber RR = 3 cereal RR = 5	"...confirmed the importance and independence of...dietary fibre..."	age, cigarettes, weight, skinfold thickness, animal protein
Kromhout *et al* (1982) Zutphen	871 40–59 yrs	30	10 yrs mortality (27)	low 5ile vs high 5ile RR = 4	"...disappeared after multiple logistic regression analysis..."	starch, vegetable protein, calories/kg
Kushi *et al* (1985) Ireland-Boston	1001 30–69 yrs	12*	19 yrs mortality (110)	low 3ile vs high 3ile RR ≅ 2	nutrient density RR ≅ 2 ($p < .08$) calorie-adj residuals RR ≅ 1.25 ($p < .17$)	age, cohort, systolic BP, total cholesterol, ECG abnormality, cigarettes, EtOH
Khaw *et al* (1987) Rancho Bernardo	356 50–79 yrs	14	12 yrs mortality (42)	low 3ile vs high 3ile RR = 3 (1.3, 7.1)†	15% ↓ / 6 g ↑ ($p = .15$)	age, systolic BP, cholesterol, fasting, plasma glucose, BMI, cigarettes
Fehily *et al* (1993) Wales	2423 45–59 yrs	16	5 yrs mort/morb (148)	1.1 g higher in nonHD group ($p < .05$)	"...when standardized for total energy...disappeared"	age, BMI, smoking habit
Rimm *et al* (1996) USA health professionals	43,757 40–75 yrs	20‡	6 yrs mort/morb (734)	low 5ile vs high 5ile RR = 1.7 (1.3, 2.2)† (cal-adj residuals)	low 5ile vs high 5ile RR = 1.6 (1.1, 2.1)† 19% ↓ / 10 g ↑ 27% ↓ / 10 g ↑ cereal (cal-adj residuals)	age, BMI, smoking habit, sat fat, vit E, phys activity, EtOH, hypertension, hypercholesterolemia, profession, fam hx of MI

*Total fiber estimated as product of grams of crude fiber times 4.4
†Relative Risk (95% confidence interval)
‡Estimated from median of middle quintile

Table 2. Studies of the fiber:heart disease hypothesis in nonmale or nongeneral population cohorts

Subjects Authors/site(s)	# Subjects & age at baseline	Mean intake of total fiber (grams/day)	Yrs follow-up & endpoints (n)	Findings Unadjusted	Findings Adjusted	Findings adjusted for
Females Khaw *et al* (1987) Rancho Bernardo	503 50–79 yrs	12	12 yrs mortality (23)	low 3ile vs high 3ile total fiber RR = 3	33% ↓ / 6 g ↑ (p = .05)	age, systolic BP, cholesterol, fasting, plasma glucose, BMI, cigarettes
White male hypercholesterolemics (type 2a) Humble *et al* (1993) USA LRC placebo group	1801 45–59 yrs	16*	9 yrs mort/morb (249)	low 5ile vs high 5ile RR = 1.6 (p for trend = .003)	16% ↓ / 9 g ↑ (p < .07)	age, BMI, smoking habit, education, total calories, saturated fats, other fats, systolic BP, HDL, LDL, glucose tolerance (1 hr)
Secondary prevention of MI Burr *et al* (1989) Wales (DART)	1879 (avg 56.6 yrs)	Advice gp = 30 nonAdvice gp = 20	2 yrs mort/morb (276)	crude % mort/morb Advice gp = 14.7 nonAdvice gp = 12.4 RR = 1.23 (0.95, 1.6) (Adv / nonAdv)	RR = 1.25 (0.99, 1.65) (Adv / nonAdv)	hx of MI, angina, or hypertension; Evidence of heart disease; tx with antihypertensives, digoxin/ antiarrythmics, or anticoagulants

*Total fiber estimated as product of grams of crude fiber times 4.4

The Caerphilly Prospective Ischaemic Heart Disease Study included 2423 men who completed a food frequency questionnaire (Fehily *et al* 1993). Validation analyses based on seven day diet records found only modest correlation with intakes based on frequency methods (Pearson r's ranged from 0.3 to 0.4). Subjects were followed for five years. Although the absolute intakes of fiber were less in subjects who had a CHD event, the difference disappeared after adjustment for total energy by the nutrient density method. Comparisons of risks by quantile of fiber intake were not presented.

By far the strongest test to date comes from 43,757 men in the Health Professionals Follow-up Study (Rimm *et al* '96). Intakes were again based on food frequencies but validations based on two 1-week records found good to strong Pearson correlations for total fiber intake (r=0.68) and for consumption of major fiber sources (r's ranged from 0.51 to 0.95). Follow-up continued over six years. The authors "decoupled" fiber intake from total calories by basing their fiber quintiles on calorie-adjusted residuals (Willett *et al* '86). After adjustment for eleven covariates (see Table 1), a significant inverse trend in risk for CHD was noted ($p<0.004$) although only the highest fiber quintile subjects had a risk for CHD that differed significantly (RR 0.64; CI 0.47, 0.87) from those in the lowest quintile. When analyzed as a continuous exposure, each 10 gram increase in total fiber was associated with an RR for total CHD of 0.81 (CI 0.70, 0.93). Simultaneous control for soluble and insoluble fiber types showed a strong inverse effect limited to insoluble fibers. Of the various fiber sources, only the RR for cereal fibers was statistically significant (RR10 g=0.71; CI 0.54, 0.92). Restriction of the analysis to non-smokers did not change the results (Eric Rimm, personal communication).

2.2. Other Prospective Studies

2.2.1. Prospective Study of Women. To date only the Rancho Bernardo Study has included women (n=503). The estimated effect of fiber was similar both clinically and statistically for women and men (Khaw *et al* '87). Fortunately for the participants but unfortunately for researchers, there were only 23 deaths from heart disease for these women (even in this somewhat older cohort followed for 12 years). This compares with 42 in the smaller male cohort. Thus, the estimated effect of fiber in women is unstable given the low ratio of events to the number of terms (8) in the regression model. And as noted above, the authors relied on a single 24-hour diet recall, a sub-optimal method for measuring long term nutritional intakes.

2.2.2. Prospective Studies of Hypercholesterolemic Subjects. Humble and coworkers ('93) reported an inverse association between crude fiber intake and CHD in 1801 White males in the placebo arm of the LRC Trial (LRC '84). Two 24-hour recalls were combined to improve the accuracy of their nutritional data (Kushi '88, Willett '91). These were not independent measures of the same general diet because dietary counseling was given to all subjects between the first and third clinic visits when the two recalls were taken. This probably reduced the correlation of study nutrients (e.g., Spearman r for fiber from two periods = 0.28). However, group level comparisons demonstrated that while there was a general benefit of the dietary counseling in the cohort as a whole, only subjects who did not develop CHD had a increase in their fiber intakes. Age-adjusted rates of CHD showed a significant inverse trend over the quintiles of fiber intake ($p<0.003$). Age-adjusted Cox regression models estimated a 9.1% decrease in CHD risk per one gram increase in crude dietary fiber. Addition of terms for smoking, total calories, SBP, and HDL and LDL cholesterol reduced the estimated effect somewhat (% change in risk = 8.1; CI=−15.5, +0.1). Further adjustment for physical activity,

BMI, education, dietary cholesterol, post-prandial glucose level and the percentages of calories from carbohydrates, total fat and the various subcategories of fats had little effect on the estimated association of fiber and CHD.

Khaw and Barrett-Conner stratified their analysis for the hyperlipidemic and "random" samples in the Rancho Bernardo cohort (Khaw *et al* '87). A description of hyperlipidemic types in their study was not presented. The relative risk for each 6 g increment in total fiber (presumably adjusted for multiple risk factors) was 0.71 (p=0.03) in the 416 hyperlipidemic subjects compared to 0.78 (p=0.08) for the 443 randomly selected subjects. Assuming that each g of crude fiber equals 4.4 g total fiber (Bright-See *et al* '84), the RR for each gram of crude fiber in these hyperlipidemic subjects would be approximately 0.79 compared to the RR of 0.92 estimated for the type 2a hypercholesterolemics in the LRC placebo group (Humble *et al* '93). Again, the strong effect of fiber in the Rancho Bernardo cohort of hyperlipidemics may be spurious and could be explained by the small number of cases (n=38) relative to the number of terms in the regression model.

2.2.3. Randomized Clinical Trial of Men Who Survived Myocardial Infarction. The Diet and Reinfarction Trial (DART) in Wales is the only randomized controlled trial of the fiber:CHD hypothesis to date (Burr *et al* '89). The investigators employed a factorial design to examine the effects of a dietary intervention in the secondary prevention of myocardial infarction (MI). Two thousand and thirty-three men who had recovered from MI were randomized to receive or not receive advice on each of three dietary factors: 1) a reduction in fat aimed at improving the Polyunsaturated:Saturated (P:S) ratio, 2) an increase in fatty fish intake, 3) an increase in cereal fiber intake. The effect of the fiber advice was a doubling of fiber intake from cereal sources (from 9 to 19 g) while fiber from other sources remained unchanged at about 11 g per day in both groups. The authors do not present the distributions of covariates in the Advice and No Advice groups, but such factors should have been balanced by the randomization process.

After adjustment for 10 clinical risk indicators, the Cox model estimated an increase in total CHD events of 23% (CI –3%, +57%) in the Fiber Advice group. Although statistically nonsignificant, the direction of the estimated effect is counter to expectation, possibly due to the shortness of the follow-up period (two years). Early follow-up of men in the intervention arm of the LRC Coronary Primary Prevention Trial of cholestyramine (an artificial fiber) also suggested poorer outcomes which were reversed at the end of nine years (LRC '84). The requirement that subjects in the DART cohort had to have survived an MI also makes comparability of the DART study's findings problematic (Rimm '93). And the ~20 g average intake of total fibers in No Advice subjects is high relative to that of other groups studied (Table 1) suggesting the possibility of a threshold effect for fiber exposure. Still, the results for fiber from the DART study were largely disappointing because MI survivors are in special need of effective interventions. Also, negative results from a randomized trial such as this bolster the argument that positive findings from observational studies are explained by lifestyle factors (Wynder *et al* '96).

2.3. Studies That DO NOT Test the Fiber:CHD Hypothesis

Two other studies are sometimes cited as tests of the fiber hypothesis (Sacks '93). The first examined the association of diet and heart disease among men in the urban and the rural areas around San Juan, Puerto Rico (Garcia-Palmieri *et al* '80). The authors *inferred* a lower intake of fiber from the significantly lower intake of carbohydrates from legumes among urban dwellers relative to rural residents in Puerto Rico. They also sug-

gest lower fiber intake as one possible cause of the higher incidence of CHD among the urban subjects. However, they state explicitly, "No reliable estimate of dietary fiber intake could be made."

Burr and Sweetnam ('82) intended to test the hypotheses that risk of death from various diseases is reduced by a high intake of dietary fiber or by vegetarianism. However as they note, the fiber measure that they used to distinguish between High and Low intake groups (specifically, daily consumption of whole wheat bread) resulted in substantial overlap in fiber consumption between their "High" and "Low" fiber groups. Further, total fiber consumption averaged 21 grams in their Low Fiber group, substantially above the overall average intake of most cohorts that have been studied to date (Table 1). All groupings of the vegetarians and health food users had very low rates of CHD mortality, but the use of an SMR analysis prevents the direct comparison of outcomes in the study groups. Including further mention of these two studies only distracts from discussion of direct tests of the fiber:CHD hypothesis.

3. EPIDEMIOLOGIC EVIDENCE REGARDING POSSIBLE MODES OF ACTION

Heretofore, the assumed strongest mechanism for fiber has been through direct or indirect effects on serum lipids (Kritchevsky '87). This assumption is supported by evidence that fibers (especially soluble fibers) can lower serum cholesterol (Kritchevsky '87, Jenkins '93) and other evidence that lowering serum cholesterol levels decreases the risk for heart disease (LRC '84). The remainder of this paper examines whether results from the epidemiologic studies described above do or do not support this mechanism. Other chapters in this book address numerous mechanisms through which fibers might affect CHD risk.

3.1. Evidence for Confounding by Fat Displacement

Some have suggested that any effect of fibers on serum cholesterol can be explained by simple displacement of fats in the diet by fiber and not by any special cholesterol-lowering effect of the fibers themselves (Swain *et al* '90, Sacks '93, Wynder *et al* '96). If this is the case, then inverse associations between fiber and fat intake should be at least moderately strong in the cohorts described above. More importantly, even in cohorts where fat displacement may be observed, fat intake can only confound the estimated effect of fiber and CHD if *in the cohort under study* fat intake is associated with CHD. If *both* of these conditions are met, inclusion of one or more fat measures in a regression model should reduce or eliminate the estimated effect of fiber. If including the fat term does not alter the effect of fiber, fat is not a confounder. On the other hand, if one first models the effect of fat, then adds a fiber term and sees the fat effect reduced or eliminated, the original impression of an effect for fat is assumed to have been due to confounding by fiber. For fiber to be a confounder in this latter case, it must be a cause of the outcome under study.

Four studies described above provide data relevant to this question (Table 3). In the first report of a fiber:CHD link, Morris and coworkers include a bar chart with the percentages of total energy intake from animal protein, saturated fats, polyunsaturated fats, and sugar by tercile of cereal fiber intake (see Figure 3 in Morris *et al* '77). The percentage of calories from saturated fats is approximately 22% in all terciles. Approximately 4%

Table 3. Evidence regarding confounding role for fat displacement by dietary fibers in prospective observational studies

Authors/site(s)	Evidence for assessing role of fat displacement
Morris *et al* (1977) England	Percent calories from saturated & polyunsaturated fats did not vary by tercile of cereal fiber.
Khaw *et al* (1987) Rancho Bernardo	"(After) adjusting separately for...fats...dietary fiber remained a significant predictor of IHD and the relative risk was unchanged." [Total fat (p = 0.26) was not a significant predictor of IHD mortality in a model adjusted for age, sex, and fiber.]
Humble *et al* (1993) LRC placebo group	Inverse association between fiber quintile and % of calories from fat very slight [β for change in % Calories from Total Fats for each gram of crude fiber = –0.39% (95% CI = –0.09, –0.69)]. No difference in fat consumption at baseline between patients who did and did not develop CHD.
Rimm *et al* (1996) USA health professionals	Inverse association between grams of fat/day and quintile of dietary fiber. However, addition of fiber term to model eliminated the estimated effect of dietary fats whereas fiber effect was statistically significant.

of calories come from unsaturated fats in all three terciles. Morris and coauthors describe these associations with fiber tercile as "quite uninteresting." Their data for fat fail the first test for a putative confounder; i.e., association between the exposure (fiber) and the confounder (fat).

Khaw and Barrett-Connor ('87) test the confounding effect of fats by adding a term for total fat to a Cox regression model already containing terms for fiber, age, and sex. "(Fiber) remained a significant predictor of ischemic heart disease mortality and the relative risk was not changed: no other dietary variables were significant in the model with dietary fiber."

Humble *et al* ('93) report less than a two percent difference in the percentage of calories from total fat in the highest and lowest fiber quintiles in their study and discount the practical importance of such a difference (Figure 1). Subsequent regression analysis shows that although small, this difference is statistically significant (% change from quintile to quintile = –0.39, 95% CI = –0.09, –0.69). However, there is no difference in the proportion of calories from fat (37.2%) in hypercholesterolemics who developed CHD in their study and those who did not develop CHD (unpublished data). I.e., the data for percent of calories from fat in Humble *et al* may (barely) meet the first test for a confounder (association with the exposure), but they fail the second test (association with the outcome — CHD). Therefore, it is not surprising that inclusion of terms for calories from fat in their regression model did not alter the marginal effect of fiber found in their study. Results were similar for percent of calories from saturated fats (unpublished data).

Rimm and coworkers ('96) address the issue of fat substitution by citing from related work on the fat: CHD hypothesis in their Health Professional cohort (Ascherio *et al* '95b). In those analyses, each 5% increase in energy from saturated fat was associated with a 20% increased risk for total CHD (RR= 1.2; 95% CI = 1.0, 1.4) when fiber was not included in the model. However, addition of a fiber term to the model eliminated the estimated effect of saturated fats (RR= 1.0; 95% CI = 0.9, 1.2). The authors discounted the importance of any inverse association between fat and fiber intakes (Meir Stampfer, personal communication) and attributed the association between saturated fats and CHD to lower fiber consumption by men with high levels of saturated fats in their diets.

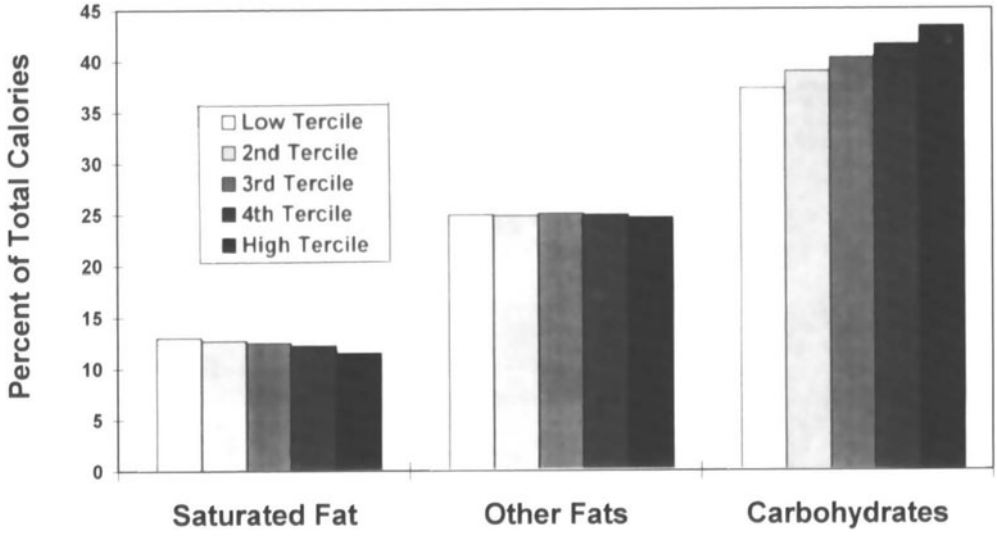

Figure 1. Percent of calories from saturated fats, other fats, and carbohydrates by fiber quintile among 1801 hypercholesterolemic men (based on unpublished data from Humble *et al*, 1993).

The editorial accompanying Rimm *et al* ('96) attributes the fiber:CHD association in the analysis to a "healthy lifestyle effect" (Wynder *et al* '96). They use the authors' own data showing trends in dietary cholesterol, saturated fat, smoking etc. over the fiber quintiles to support their argument, but these factors were controlled in the regression analyses. Wynder and coworkers also state that the use of energy-adjusted residuals in the analysis "tends to confound the effect of fiber with fat intake..." Inquiry with the first author reveals that the association between fat and fiber intakes was apparent for both energy-adjusted and unadjusted values and, therefore, was not the result of the adjustment procedure (Eric Rimm, personal communication). But regardless, inclusion of total fat or saturated fat in the regression model should have controlled for any such problem. The editorial also questions the statistical power of the authors' multivariate model even though most researchers envy those with 734 outcomes in a 43,757 member cohort.

Summing up the epidemiologic data for fat displacement (Table 3), although it may be reasonable to expect fibers to reduce heart disease through fat displacement, studies to date provide, at most, only weak evidence that such displacement occurs. More importantly, two studies provide evidence that fat does not meet the basic requirements for confounders, i.e., that fat be associated with both fiber intakes *and* risk for heart disease in the cohorts under analysis (Morris *et al* '77, Humble *et al* '93). And in two studies which modeled the independent effects of fat and fiber on CHD risk, inclusion fat did not alter the estimated effect of fiber (Khaw *et al* '87, Rimm *et al* '96).

3.2. Epidemiologic Evidence for Mediation through Lipid Pathways

The epidemiologic data can also elucidate whether part or all of the effect of fibers on CHD is mediated through their effect on serum lipids (Table 4). Again, the authors of the original report on fiber and CHD were the first to examine this question. They calculated correlation coefficients between lipid levels and all dietary variables and found

Table 4. Evidence of protection against heart disease for fiber subtypes or sources and possible mediation of effect throught serum lipid pathway(s) in prospective observational studies

Authors/site(s)	Effect noted for		Authors conclusion regarding mediation of protective effect through cholesterol pathway
	Cereals or insoluble fibers	Fruits/vegetable or soluble fibers	
Morris *et al* (1977) England	entirely for cereals	none	"...the association between...cereal fibre intake...and CHD is not mediated...in any simple way through plasma cholesterol concentrations."
Humble *et al* (1993) (LRC placebo group)	—	—	"...neither (HDL or LDL) associated with fiber..."
Rimm *et al* (1996) USA health professionals	"...strong inverse association..."	"...not for soluble fiber."	"reduction on CHD...larger than would be expected from the beneficial effects on serum cholesterol...suggests several ...biologic mechanisms"

"little or nothing" (Morris *et al* '77). Their conclusion was that the effect of cereal fiber intake "was not mediated or explained in any simple way through plasma cholesterol concentrations."

Humble and coworkers ('93) report no association between either HDL or LDL cholesterol and fiber intake (p values for trend over fiber quintiles were 0.111 and 0.475, respectively). Also, control for these factors in their multivariate analyses did not appreciably alter the estimated effect of fiber.

Rimm and coworkers ('96) were forced to address the question of possible mechanisms inferentially because they did not have serum cholesterol values at the time of their analysis (Eric Rimm, personal communication). In their paper, they first estimate the soluble portion in 10 g of cereal fiber (~2.5 g). Then, assuming that each soluble gram lowers cholesterol by 0.5 to 2%, they estimate an effect of 2.5 g of soluble fiber on serum cholesterol levels as a decrease between 1.25 and 5%. Finally, assuming that each 1% decrease in serum cholesterol predicts a 2% decrease in CHD, they calculate that each 10 g increase in cereal fibers should reduce CHD by between 2.5% and 10% (MRFIT Research Group '82). Their own data suggest a 29% reduction in CHD for each 10 g of cereal fiber; and they therefore conclude that fibers must act through several biologic mechanisms. While this conclusion may be reasonable, it seems strange that these authors first discount the protective effect of soluble fibers in general (see for example, the second sentence of the middle column on page 449 of Rimm *et al* '96) and then base their evaluation of action through the cholesterol pathway on the soluble fiber content of cereals.

Taken together, the (admittedly very limited) epidemiologic data to date challenge the view that dietary fibers act wholly or even mostly through their effects on serum lipids. Fortunately, there are numerous other possible mechanisms through which fibers may act to alter CHD risk. The reader is again directed to other parts of this book.

4. CONCLUSIONS

Data suggesting that dietary fiber can reduce the risk for heart disease have been accumulating steadily from observational studies. Even when not statistically significant, the

results tend to support a protective effect of fiber. These associations are usually reduced after adjustment for other factors, leading some researchers to suggest that other (lifestyle?) factors linked to fiber are the true causes. However, all evidence from epidemiologic studies to date fails to support displacement of fats by dietary fibers as an explanation of the fiber:heart disease link.

With the recent addition of results from the Health Professionals Follow-up Study (Rimm *et al* '96), the evidence for men, especially middle-aged white men, has become moderately strong. Results for other cohorts are generally consistent with findings for men from the general population, but more and larger studies are needed, especially for women and for high risk groups. These studies should also address the independent roles of fat and other life style factors in determining risk for heart disease. Replication of recent findings of a protective association with fiber coupled with no apparent effect of dietary fats (Khaw *et al* '96, Humble *et al* '93, Rimm *et al* '96) would challenge widely held assumptions about the relationship of diet and heart disease (Ascherio *et al* '95a).

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3

DIETARY FIBRE AND HUMAN CANCER

Epidemiological Data

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1. INTRODUCTION

Burkitt (1969, 1971, 1973) postulated that dietary fibre was a major protective factor against colorectal carcinogenesis, based on his studies in Africa. He hypothesised that the carbohydrate that reached the colon was readily fermented by the gut bacterial flora and that this resulted (a) in a modified gut bacterial flora that produced smaller amounts of tumour promoters, (b) in stool bulking that dilutes the carcinogens in the gut lumen, and that (c) speeded intestinal transit and thereby allowing less time for the luminal carcinogens to act. The hypothesis therefore revolves around the amount of fermentable carbohydrate reaching the colon. The earlier observations by Williams and Olmsted (1936) that stool bulking was related to the amount of complex carbohydrate (CC) fermented rather than the amount undegraded was consistent with this.

It is interesting to note that this hypothesis is based on the assumption that the major factor(s) responsible for colorectal carcinogenesis are delivered by the luminal rather than vascular route. This had been implicit in the hypothesis of Aries et al (1969) and Hill et al (1971) that large bowel cancer was caused by bile acid metabolites produced by bacterial action in the colon, and had been in its early years a cause of considerable criticism of that hypothesis. The evidence for a role for luminal factors includes:

a. The effect of diversion of the faecal stream (Cole and Holden 1959) which is to cause regression of adenomas.
b. The distribution of large adenomas and carcinomas around the large bowel (Morson et al, 1983), which is consistent with that expected from a luminal factor produced and concentrated during colonic transit. The even distribution of small adenomas is that expected from a causal agent delivered by the vascular route.

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c. The results of diversion of the bile from the duodenum to the caecum (Nigro 1981), which results in a shift of the tumours to points distal from the point of entry of the bile.
d. Germ-free animal studies (Reddy et al, 1974), which show that lack of a gut bacterial flora is highly protective against colorectal carcinogenesis in the dimethylhydrazine model.
e. The promoting effect of rectally instilled bile acids on the rate of development of colorectal cancers in rodent models (Reddy et al, 1974).

The hypothesis was reinforced by the observations of Roediger (1982) of the symbiotic relationship between the gut mucosa and the lumenal bacterial flora which provides its major nutrients.

Early attempts to test the Burkitt hypothesis were bedevilled by problems with the definition of dietary fibre, and with it's analysis. In early tables of food analyses "fibre" was crude fibre, the component resistant to digestion with weak acid and weak alkali, and consisted essentially of cellulose and lignin. In addition, the tables of McCance and Widdowson included a fraction termed "unavailable carbohydrate" which attempted to include all of the carbohydrate which was lost to the colon (and so unavailable for digestion in the small bowel).

Early epidemiological studies of the Burkitt hypothesis failed to support it; for example both Drasar and Irving (1973) and Armstrong and Doll (1975) found no relation between intake of "fibre" and the incidence or mortality from cancer of the colon or rectum. It was argued that this was due to the inadequacies of the analytical tools; in both cases the authors had used the food table data analyses for "fibre" rather than the "unavailable carbohydrate" data which, in retrospect would have been more appropriate. However, this led to a search for better definitions of and assay methods for dietary fibre.

The definition of "dietary fibre" which quickly emerged and was quickly accepted was that proposed by Trowell et al (1976); the new assay methods could then follow. These included the colorimetric assays of Southgate et al (1969), and the use of detergent extraction to separate cell wall polymers (Van Soest et al, 1967). Two types of method eventually emerged; one was based on the colorimetric or chromatographic analysis of the non-starch polysaccharides (NSP), eg Englyst et al (1982), whilst the other was based on the sequential removal of other components and weighing the cell wall residue (Asp et al, 1983).

However, at this time an element of confusion entered when it was finally demonstrated that not all starch was digested during small bowel transit but that some reached the colon (Stephen et al, 1983). Further, the amount of this "resistant starch", although very variable and dependent on cooking methods, age of plants etc, behaved in the colon similarly to hemicellulose and could greatly exceed in amount that of NSP. Consequently studies based on NSP greatly and variably underestimate the amount of fermentable carbohydrate reaching the colon and so fail to do justice to the Burkitt hypothesis.

The relation between intake of dietary fibre and cancer risk has been investigated epidemiologically in (a) studies comparing populations, (b) case-control studies, and (c) prospective or cohort studies.

2. POPULATION, OR ECOLOGICAL, STUDIES

In these, a group of populations is selected for which data exist on cancer risk and on dietary intakes. Cancer mortality data are usually available for countries; cancer inci-

Table 1. Population studies of diet and human cancer

Study	Observation
Drasar and Irving (1973)	No relation with fibre in 37 countries
Armstrong and Doll (1975)	No relation with fibre in 23 countries
Liu et al (1979)	Inverse relation with fibre that disappeared after controlling for cholesterol and fat intake
Eyssen and Bright-See (1984)	Results similar to Liu et al (1979)
Bingham et al (1979)	Very strong inverse correlation between colon cancer mortality in 15 UK regions and pentosan intake
Bingham et al (1984)	No relation between NSP intake and colon cancer mortality in UK regions
Jensen et al (1982)	Inverse relation between dietary fibre intake and colon cancer incidence in 4 Scandinavian populations

dence data are obtainable from cancer registries, but these only cover defined regions and complete national registration is only available for a limited number of countries.

Table 1 lists a selection of population studies and their findings. In many of these an estimate of total dietary fibre was attempted, for example Drasar and Irving (1973) made such an attempt and concluded that there was absolutely no relation between fibre intake and either colon or breast cancer risk. However, the estimate of fibre intake was crude, and more reliable estimates of relative risk came from the analysis of fibre-rich foods. Here a protective effect was seen for cereal intake. Liu et al (1979) found a protective effect of dietary fibre that disappeared when the analyses were corrected for total fat and cholesterol; however there was a strong protective effect of cereals. Eyssen and Bright-See (1984) found results similar to those of Liu et al, except that they did not find the strong causal relationship with cholesterol. On the basis of fibre assays based on the analysis of component sugars Bingham et al (1979) concluded that there was a very strong inverse relation between colon cancer incidence and cereal intake (the main source of pentosans), but in a later reanalysis (Bingham et al, 1985) which rigorously excluded all starch from the analyses the strong inverse correlation with pentosans disappeared and was replaced by a strong inverse correlation with uronic acids; the putative protection was therefore reassigned to the vegetable component of the diet. Armstrong and Doll (1975) showed a strong inverse correlation between some fibre-rich foods, in particular cereals, potatoes and pulses, and cancers of the colon, breast, endometrium and prostate (Table 2).

More recent studies of populations have given more mixed results. Farchi et al (1996) in a comparison of regions in Italy, found an inverse relation between fibre intake

Table 2. The relation between fibre-rich foods and cancer incidence (data from Armstrong and Doll, 1975). The relationships are given as correlation coefficients

		Fibre-rich foods		
Site	Sex	Cereals	Potatoes	Pulses
Colon	M	-0.52	-0.32	-0.31
Breast	F	-0.64	-0.39	-0.43
Endometrium	F	-0.58	-0.24	-0.62
Prostate*	M	-0.60	+0.40	-0.59

*Mortality

and cancer risk in general. However, Walker et al (1986) found no correlation between the fibre intakes of urban and rural South African white, black and Indian populations and their colon cancer risks.

3. CASE-CONTROL STUDIES

One of the first published case-control studies of fibre and colon cancer risk was by Modan et al (1975) who showed a protective effect of crude fibre; this is almost a unique result since most studies show no relation to crude fibre intake at al. They also showed an inverse relation with consumption of fibre-rich foods. Jensen (1989) reviewed 11 case control studies (Table 3), some of which involved colon cancer, some rectal cancer, and some colorectal cancer. For colon cancer two showed protection, four showed no effect and none showed a positive correlation; for rectal cancer one showed protection and two no effect while none showed a positive correlation; for colorectal cancer three showed protection and two a positive correlation with none showing no effect. Boutron et al (1991) reviewed 19 studies, 10 of which showed protection, 7 showed no effect and 2 showed a positive correlation (Table 3).

Dwyer (1993) carried out a meta-analysis of 13 case-control studies and found a significant protective effect against colon carcinogenesis.

4. PROSPECTIVE STUDIES

There has been a relatively small number of prospective studies of the relation between fibre intake and risk of colorectal or breast cancer. Willett et al (1990) showed that dietary fibre was protective against colorectal carcinogenesis in their prospective study of American nurses; in the same study they saw no relation between fibre intake and risk of breast cancer (Willett et al, 1987). Hirayama (1985) in his huge prospective study of more than 250,000 Japanese showed a strongly protective effect of green leafy vegetables against cancers of a range of sites as well as all sites. This was the main source of fibre in the Japanese diet other than rice (although the latter was in very much greater amounts).

5. THE IMPORTANCE OF RESISTANT STARCH

It is fair to state that the whole field of dietary fibre epidemiology should have been thrown into turmoil by the observation of Stephen et al (1983) that a proportion of the die-

Table 3. Summarized case-control studies of fibre intake and cancer risk

Study	Cancer site	Protection	Promotion	No effect
Jensen et al (1989)	Colon	2	0	4
	Rectum	1	0	2
	Colorectum	3	2	0
Boutron et al (1991)	Colorectum	10	2	7
Nutrition Soc (1993)	Colorectum	13	0	4

tary starch resists digestion during small bowel transit, and so reaches the colon where it behaves as hemicellulose. In terms of colon physiology, therefore, this fraction of the dietary starch behaves as dietary fibre and so, in any epidemiological study, should be counted as dietary fibre. This has, unfortunately, coincided with increasing efforts to exclude all starch from the analyses of dietary fibre (which is then defined as non-starch polysaccharide, NSP).

The seriousness of the problem was illustrated by Englyst and Kingman (1990). The digestibility of the starch from potato depends on the temperature at which it is served and the length of time since cooking; similar results were obtained with beans and other vegetables. In consequence, it is impossible to assess the amount of starch which will escape digestion without knowing the exact conditions under which the food is to be consumed. All we know is that, in view of the large amounts of starch in the diet, a variable and sometimes very large amount of starch will reach the colon, often dwarfing the amount of NSP. Since dietary fibre is often equated with NSP whilst the physiological effects depend as much on the starch polysaccharide entering the colon, a Working Party of the British Nutrition Foundation recommended in 1990 that the term dietary fibre be abandoned and replaced by CC.

There are indirect methods of assessing the amount of complex carbohydrate which reach the colon. In order to support the daily production of bacteria in the colon (estimated at 15 g) it has been estimated that at least 60–70 g/day of CC must be fermented (Cummings 1981; Stephen 1985). The normal daily intake of NSP in the western diet is approximately 15 g/day, which implies that on average three times as much starch as NSP reaches the colon. In the light of this it is remarkable that the data have so consistently shown a protective effect of NSP against colorectal carcinogenesis. This may be changing, however. In a recent study (Cassidy et al, 1994) there was a strong inverse correlation between colorectal cancer risk in 12 populations worldwide and starch intake, but none with NSP. This is a similar result to that of Irving and Drasar (1974) who found a protective effect of starchy foods but none with total fibre.

There is a further problem, Phillips et al (1995) showed that in the presence of large amounts of resistant starch in the diet there may be impaired colonic digestion of NSP. This may be important. Tomlin and Read (1990) showed that 9.5 g/day of resistant starch had no detectable effect on faecal physiology; this is reasonable if normally 60–70 g/day of CC reaches the colon. Phillips et al increased the resistant starch intake from 5.0 to 39.0 g/day (which should have increased the CC in the colon by approximately 50%), and observed a 50% increase in stool weight; however they also observed a 50% increase in stool NSP.

6. FIBRE-RICH FOODS

Although there is turmoil in the analysis of CC which call into question all previous observations on the relation between fibre and cancer risk, it is important to remember that the original food intake data on which the fibre intake estimates were based remain. When the relation between fibre-rich foods and cancer risk is studied the relationships look much more secure. Table 2 gives data from Armstrong and Doll (1975) relating intakes of some fibre-rich foods and cancers of the colon, breast, endometrium and prostate. Table 4 gives similar data from a number of studies and colon or rectal cancer mortality (Hill 1994); these show that cereals and pulses, in particular, are commonly observed to be protective

Table 4. The relation between colorectal cancer mortality and intakes of fibre-rich foods (Hill, 1995 for details and references)

Study	Fibre source	Correlation/coefficient
Drasar and Irving (1973)	Cereals	−0.31
	Potatoes	−0.11
Armstrong and Doll (1975)	Cereals	−0.70
	Pulses	−0.39
Howell (1975)	Cereals	−0.84
	Pulses	−0.76
Schrauzer (1976)	Cereals	−0.70
Yanai et al (1979)	Cereals	−0.50
Eyssen and Bright-See (1984)	Cereals	−0.73
	Pulses	−0.70
Liu et al (1979)	Cereals	−0.77
Bingham et al (1979)	Cereals	−0.96

against colorectal cancer. In contrast Table 5 lists some studies showing increased risks associated with high intakes of some starchy foods.

7. CONCLUSIONS

It is apparent that the relationship between fibre intake and cancer risk is far from clear. In some cases this may be because the populations compared were, in retrospect, not strictly comparable. An example here is the populations in South Africa studies by Walker et al (1986). In other cases it is clearly due to deficiencies in the methods of estimating fibre intake. I have suggested here that the attempts to clarify the analytical procedures by equating fibre to NSP have clouded rather than clarified the picture. We need new assays of the amount of CC entering the colon; until they give results compatible with the amounts needed to rationalize stool bacterial synthesis they should be treated with suspicion. The British Nutrition Foundation (1990) estimated the amount of CC in the UK diet; they concluded that there was 12.5 g/day NSP, 1.6 g/day of resistant starch (defined chemically) and 21.0 g/day of unavailable carbohydrate. But the estimated amount of colonic carbohydrate, based on the daily faecal bulk, is 60–70 g/day. It is unlikely that the bacteria in the gut can defy the first law of thermodynamics!

Table 5. The association between the intake of starchy foods and the risk of colorectal cancer (data from Nutrition Soc, 1993)

Study	Observation
Bidoli et al (1992)	Increased risk with higher consumption of bread
Benito et al (1990)	Increased risk with higher intake of potatoes and cereals
La Vecchia et al (1988)	Increased risk with more frequent consumption of starchy foods (pasta and rice)
Macquart-Moulin et al (1986)	Increased risk with higher consumption of pasta

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WHAT IS A HIGH FIBER DIET?

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1. ABSTRACT

There is no recognized definition of what constitutes a high fiber diet. Intakes of dietary fiber in different populations internationally vary widely from less than 20g to more than 80g per day. The types of foods contributing fiber also vary; in some countries cereals contribute the most fiber, in others leafy or root vegetables predominate. Vegetables have the highest fiber content per Kcal, and in most populations with fiber intakes over 50g, vegetables contribute over 50% of total fiber intake. In rural Uganda, where the fiber hypothesis was first developed by Burkitt and Trowell, vegetables contribute over 90% of fiber intake. An experimental diet, the "Simian" diet, has been developed to mimic as closely as possible using human foods, the diet consumed by our simian ancestors the great apes. It is also similar to the Ugandan diet in containing large amounts of vegetables and 50g fiber/1000Kcal. Though nutritionally adequate, this diet is very bulky and not a suitable model for general recommendations. Dietary guidelines are that fat intake should be <30% of energy, with a fiber intake of 20–35g/d. These recommendations are inconsistent with a high fiber diet because, for people consuming more than about 2400Kcal, low fiber choices for fruits and grains must be selected to keep dietary fiber intake within the range of 20–35g. In a 30% fat, 1800Kcal omnivorous diet, selection of wholemeal bread and whole fruit, results in a fiber intake over 35g/d, and for an 1800Kcal vegetarian diet, with substitution of modest amounts of peanut butter and beans for meats, dietary fiber intake goes up to 45g/d. Thus, if it is desirable to promote the use of unrefined foods, the recommended dietary fiber intake should be a minimum of 15–20g/1000Kcal.

2. INTRODUCTION

There is no definition of what constitutes a high fiber diet. Current dietary guidelines for Americans recommend a high carbohydrate diet which is rich in fruits, vegetable and whole grain cereals[1]. This advice is given in order to increase complex carbohydrate and die-

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tary fiber intakes. However, there is no definition of how much more fiber should be eaten, and no indication of a minimum or optimal range of fiber intake. A number of agencies, however, have given recommendations for fiber intake: the National Cancer Institute recommends 20–30g/d with an upper limit of 35g [2]. The American Dietetic Association[3] and the American Diabetes Association [4] both suggest that dietary fiber intake should be 20–35g/d.

The position taken in this paper is that a high fiber diet is beneficial for health, and that to achieve higher fiber intakes it is important to provide a specific recommendation for fiber intake. The desirable range of fiber intake suggested by some agencies, 20–35g/d, with an upper limit of 35g/d does not represent a high fiber diet, and, indeed, is inconsistent with the general aim of reducing fat and increasing carbohydrate intakes. To determine how much fiber should be consumed in a high fiber diet, and from what sources, fiber intakes in different populations throughout the world and in various experimental diets will be considered. Finally, dietary recommendations will be used to create sample diets to determine how much fiber such diets might contain.

3. FIBER INTAKES IN DIFFERENT POPULATIONS

Figure 1 shows some examples of fiber intakes in different populations around the world. The data for this figure represent actual intakes (rather than disappearance) and were drawn largely from a review by Bingham[5], supplemented with data from Australia[6], France[7] and Italy[8]. It can be seen that fiber intakes differ considerably not only in the

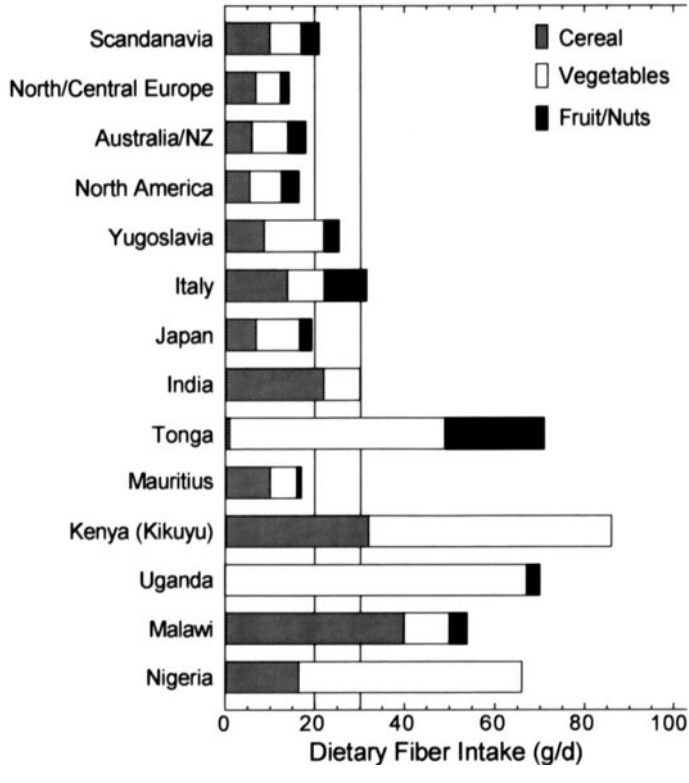

Figure 1. Dietary fiber intakes in different populations.[5–8]

amount, but also in the source of fiber. Bingham points out that previous data for Uganda and Kenya (the Kikuyu) showing fiber intakes of 130–150g/d were based on analyses in which the fiber was likely contaminated with starch, and, thus, the values were too high. She, therefore, revised the values based on more recent analyses. Nevertheless, the intakes in these populations of 70–90g/d is still 3–4 times more than consumed in North America and most other Western countries.

This highlights the fact that there is disagreement about the definition of dietary fiber, and the most appropriate method for measuring it. Until agreement can be reached, it will be difficult to know exactly how much fiber is being consumed and how much should be recommended. Different analytic techniques yield different values of fiber for the same food, because each analysis measures different things. Recently, Marlett and Cheung reviewed this issue and provided an interesting comparison of 5 different methods of analysis[9]. Since fiber values in the USDA database are based on the AOAC method[10], fiber values in this paper are based on the AOAC method.

Returning to Figure 1, it can be seen that most populations consume less than 35g of fiber per day, and that high fiber intakes range from 55–90g/d. The pattern of fiber intake also varies widely. In all populations, vegetables and cereals contribute 75% or more of total fiber, with fruit and nuts contributing a relatively small proportion. In most populations with a very high fiber intake, vegetables contribute most dietary fiber, with cereal fiber being very high only in Malawi. It is of interest that Uganda appears to stand out with over 95% of total fiber coming from vegetables. Rural Uganda, of course, was where Burkitt and Trowell made their observations leading to the development of the fiber hypothesis, and many of us may remember that Trowell often commented that the diet in Uganda consisted of very large platefuls of leafy vegetables. The amount of fiber in foods can be expressed in different ways; the usual way is to give the amount per serving. However, it is instructive to consider fiber intake per 100Kcal. Figure 2 shows the fiber content of the foods in the sample diets shown below expressed as grams per serving and grams per 100Kcal. Vegetables stand out as having a rather low fiber content when expressed as grams of fiber per serving, but the highest fiber content when expressed per 100Kcal. Thus, populations which rely on vegetables to provide energy, such as in rural Uganda, consume very large amounts of dietary fiber. What would such a diet look like? Is it possible to consume a diet extremely high in vegetables?

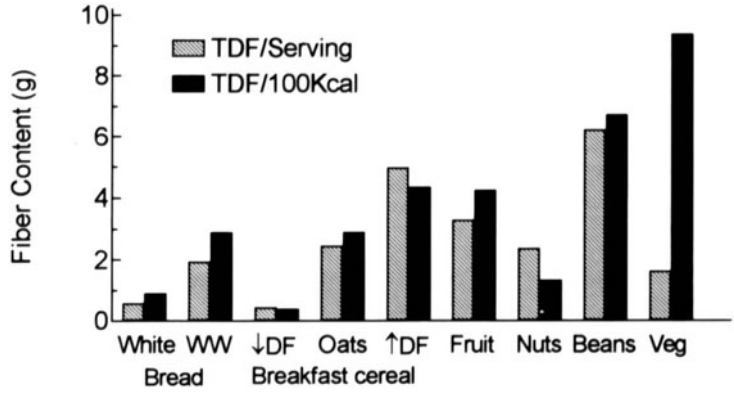

Figure 2. Dietary fiber (TDF) content of the foods used in the sample diets shown in Table 5 and Figure 4 expressed as grams per serving and grams per 100Kcal of food. TDF is according to the AOAC method.[10] WW = wholewheat bread; ↓DF and ↑DF = low and high fiber ready to eat breakfast cereals; Veg=vegetables.

Table 1. Sample 1800Kcal simian diet

Vegetables	2.3kg	Fruit	1.2kg
Cabbage	375g	Banana	225g
Broccoli	375g	Pears	225g
Eggplant	225g	Grapes	240g
Carrots	225g	Mango	248g
Peppers	225g	Blueberries	225g
Tomato	225g	Kiwifruit	75g
Pod peas	225g		
Green peas	188g	Nuts	
Onion	150g	Almonds	74g
Okra	75g		

4. THE SIMIAN DIET

In Toronto, we have been interested in a high vegetable diet because our nearest evolutionary ancestors, the great apes, in the wild, consume a diet which consists predominantly of leaves and shoots. If a simian diet consists of leaves, then, from an evolutionary argument, humans may be most suited to consuming a diet rich in leafy vegetables. Therefore, we have developed diets which are very high in vegetables with virtually no starch (Table 1), and compared their effects, in 2-week feeding trials, with those of consuming what we have called a neolithic diet, which is high in fiber and contains a variety of starchy foods (Table 2). Both of these diets contain 1800Kcal, are low in fat (23% energy) and adequate in protein (66g/d). The simian diet, with 2.3kg vegetables and 1.2kg fruit per day, contains over 90g fiber compared to 47g in the neolithic diet (Figure 3). In addition, the simian diet contains adequate amounts of nutrients (with the exception of vitamin B_{12}). Table 3 shows the calcium, iron, zinc and magnesium content of the simian diet compared to the sample omnivorous and vegetarian diets shown below. The simian diet is not being presented here as a model for general recommendations, but as a sample of an extreme experimental diet which shows that it is possible to consume 90g or more of fiber per day, similar to the diet in rural Uganda.

Table 2. Sample 1800Kcal neolithic diet

Cereals		Fruit	
Oats (raw)	50g	Raisins	38g
WW bread	72g	Dates	27g
Br rice (raw)	72g	Figs	16g
		Pears	119g
Vegetables		Apple	200g
Tomato	89g	Banana	165g
Olives	72g		
Broccoli	90g	Other	
		Yoghurt	129g
Legumes (raw)		Cottage cheese	124g
Chickpeas	22g	Olive oil	23g
Lentils	29g		

WW bread = wholewheat bread; Br rice = brown rice.

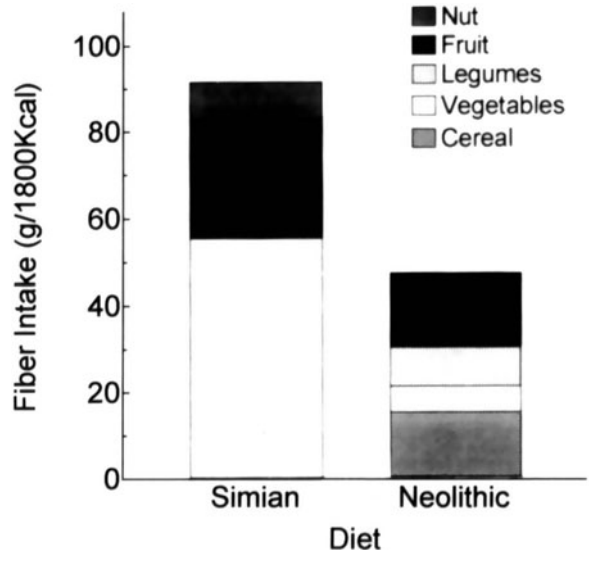

Figure 3. Dietary fiber content (grams/1800Kcal) of the experimental Simian and Neolithic diets shown in Tables 1 and 2.

One of the things Dennis Burkitt was famous for was showing pictures of very large stools passed by rural Ugandans. Preliminary experience with the simian diet shows that it is an extremely potent stool bulking diet. The mean daily stool output of 5 subjects on a simian diet containing an average of 90g fiber per day was over 900g/d. One subject produced over 1.7kg/d of stool. This, then, confirms Burkitt's observations that stools are very bulky on a diet containing very large amounts of vegetables!

5. DIETARY GUIDELINES FOR AMERICANS

Dietary guidelines for Americans are: eat a variety of foods; maintain healthy weight; choose a diet low in fat, saturated fat, and cholesterol; choose a diet with plenty of vegetables, fruits and grain products; use sugars only in moderation; use salt and sodium in moderation; and if alcoholic beverages are drunk, do so in moderation[11]. It is recommended that the diet contain ≤30% energy as fat, and ≤10% saturated fat. To achieve this, the recommendations suggest that most people should have 3–5 servings of vegetables, 2–4 servings of fruits, 6–11 servings of grain products, 2–3 servings of milk products and 2–3 servings of meats and alternates daily (Table 4). With respect to dietary fiber, it is recommended that fiber rich foods be consumed rather than fiber supplements, but no

Table 3. Mineral content of sample 1800Kcal high fiber diets

	Omnivorous	Vegetarian	Simian
Fiber	37 (5)	47	91
Calcium	1117	1280	953
Iron	32 (17)	17	22
Zinc	15 (4)	7	10
Magnesium	274	361	766

Table 4. Dietary guidelines for Americans (USDA, 1990)

Vegetables	3–5 servings*
Fruits	2–4 servings
Breads, cereals, rice, and pasta	6–11 servings
Milk, yogurt, and cheese	2–3 servings
Meats, poultry, fish, dry beans and peas, eggs, and nuts	2–3 servings

*1 serving:
Vegetables: 1c raw leafy or ½c of other kinds
Fruits: 1 med apple, orange or banana; ½c small/diced fruit, ¾c juice
Grains: 1 slice bread; ½ bun/bagel; 1oz RTE cereal; ½c cooked cereal/rice/pasta
Dairy: 1c milk/yogurt; 1½oz cheese
Meats: 2-3oz lean meat; 1egg, ½c cooked legumes, 2tbs peanut butter = 1oz meat

specific amount is provided. Agencies which recommend a range of intake for dietary fiber include the National Cancer Institute[2], the American Dietetic Association[3] and the American Diabetes Association[4]. They all agree that the optimum range of fiber intake should be 20–35g/d. The National Cancer Institute suggests that 35g/d is the maximum safe amount of dietary fiber which should be consumed.

To see a fiber intake of 20–35g/d is compatible with the Dietary Guidelines for Americans, we created sample diets containing the suggested maximum number of servings from the 5 food groups. We found that 5 servings of vegetables, 3 servings of fruit, 11 servings of grain products, 3 servings of dairy and 3 servings of meats yeilded about 1800Kcal and that a small amount of margarine could also be added, while keeping total fat intake within 30% of energy as recommended (Table 5). When low fiber foods were selected, ie. refined grains and fruit juice, the amount of dietary fiber was 15.8g (8.5g/1000Kcal). With selection of lower fiber vegetables, an even lower fiber intake could have been achieved. Nevertheless, if moderately high fiber foods are substituted into this diet, ie. wholewheat bread instead of white bread, a bran muffin for the blueberry muffin, moderately high fiber cereal such as oatmeal or raisin bran instead of cheerios, and 3 servings of whole fruit instead of fruit juice, the fiber content increased to 37g (20g/1000Kcal; Figure 4), which is more than the maximum safe

Table 5. Sample 1800Kcal omnivorous, low fiber diet

Breakfast	Snack
White toast — 2 slices	Blueberry muffin — 1 small
Margarine — 2 tsp	Apple juice — 5 oz
Cheerios — 1 cup	
2% milk — ½ cup	Dinner
Boiled egg — 1	Spaghetti (white) — 1 cup cooked
Orange juice — 7oz	Ground beef — 3 oz
	Carrots — ¼ cup
Lunch	Cauliflower — ¼ cup
White bread — 4 slices	Tomato — ¼ cup
Processed cheese — 4 slices	Broccoli — 1 cup
Ham — 2 slices	2% milk — 1 cup
Lettuce — 4 leaves	
Apple juice — 5 oz	

Vegetables: 5 servings; Fruits: 3 servings; Grains: 11 servings; Dairy: 3 servings; Meats: 3 servings; Kcal: 1850; Fat: 29.5% energy; Pro: 19.9% energy; Carb: 50.6% energy; Fiber: 15.8g

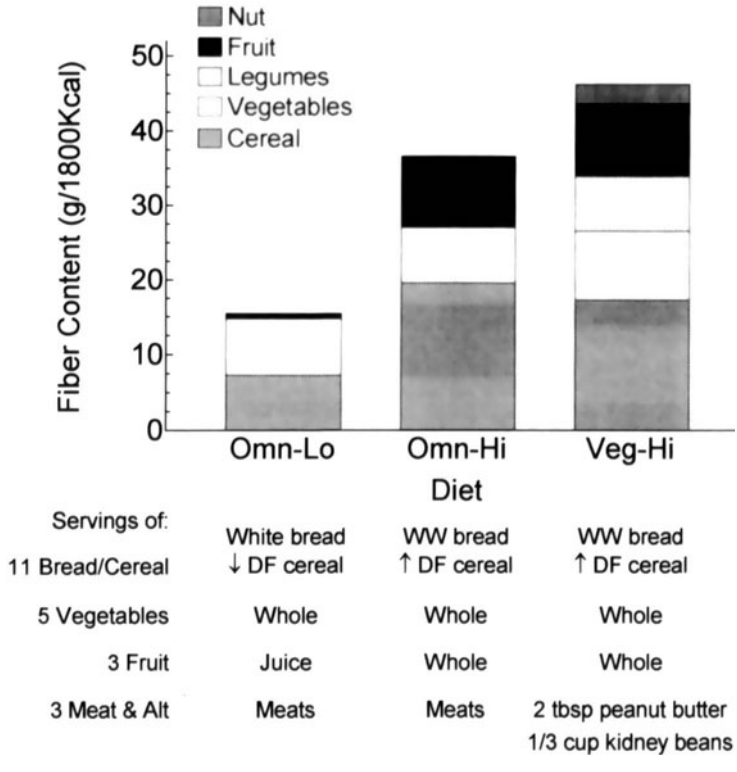

Figure 4. Dietary fiber content (grams/1800Kcal) and number of servings and types of foods in the 4 fiber-containing food groups of sample diets. Omn-Lo = omnivorous, low fiber as shown in Table 5; Omn-Hi = omnivorous, high fiber; Veg-Hi = vegetarian, high fiber. WW = wholewheat bread; ↓DF and ↑DF = low and high fiber. The vegetables are the same in all 3 diets (Table 5) except that in the vegetarian diet a carrot was substituted for the lettuce at lunch. In the 2 high fiber diets, 1 banana, 1 orange and 1 apple were substituted for the apple and orange juice (Table 5). In the high fiber diets, a bran muffin was substituted for the blueberry muffin. The same amount of white spaghetti was used in all 3 sample diets (Table 5).

amount recommended by the National Cancer Institute. If a vegetarian diet is selected, by replacing meats with cheese and a small amount of peanut butter and a small serving of beans, the fiber content of the diet increased to 47g (26g/1000Kcal), 33% higher than the safe maximum. It is easily possible to increase the fiber content of this diet even further by selecting a very high fiber breakfast cereal (such as a wheat bran cereal) and whole wheat pasta and using less cheese and more beans.

It should be emphasized that these sample diets contain only 1850Kcal. This is just under the average energy intake for 16–49 year old females (1890Kcal), but only 64% of the intake of males aged 16–49 years, 2875Kcal[12]. If adult men were to select a low fat diet, according to recommendations, containing 2900Kcal, low fiber foods would have to be selected to keep fiber intake within the range of 20–35g/d. Women, consuming only 1900Kcal, could also have an intake of fiber which exceeds 35g/d if they selected moderately high fiber foods. Thus, the recommendation to eat only 20–35g fiber per day is inconsistent with current dietary recommendations. If a diet is selected which contains 30% of less of energy as fat, it is impossible to eat plenty of vegetables, fruits, wholegrain cereals, breads, dry beans and peas, because low fiber foods must be selected to consume

Table 6. Recommended fiber intake

Minimum desirable:	15–20 g/1000 Kcal
Source:	Emphasis on a variety of foods, especially leafy vegetables, cereals, legumes and fruit.

only 20–35g of dietary fiber. Selection of wholegrain cereals, breads and dry beans, with plenty of vegetables and fruit, as recommended, results in a diet which contains at least 15–20g of fiber per 1000Kcal, and possibly considerably more.

6. CONCLUSION

There is little evidence that individuals consuming balanced diets rich in whole foods, such as vegetarians, have nutritional deficiencies[13–16]. Thus, to be consistent with general dietary recommendations, we suggest that the minimum desirable intake of dietary fiber should be 15–20g/1000Kcal, and the intake could be considerably higher with no deleterious effects. Dietary fiber should be obtained from a variety of foods, especially leafy vegetables, cereals, legumes and fruit.

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5

OVERVIEW ON COMPLEX CARBOHYDRATES

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Fifth Vahouney Conference on Dietary Fiber
Washington, DC

INTRODUCTION

The topic of Complex Carbohydrates can be a very complex subject. Every scientist working in the subject area has a pretty good idea of what complex carbohydrates are, but converting that knowledge to a streamlined word for word definition and adopting a commensurate public nutrition policy becomes significantly more difficult. Like most topics in the nutrition arena, sorting out the key health aspects of the complex of carbohydrates in the diet is no simple task. A great deal of work has been accomplished by a wide variety of researchers on the topic. This quantity of work by a multitude of researchers is necessary to arrive at scientifically valid conclusions regarding the health benefits that might be derived from consumption of any particular food component, not just complex carbohydrates.

Complex carbohydrates have a significant historical background that ties directly into a number of public health recommendations that have been made in the not too distant past. These recommendations have resulted in widespread public recognition and awareness of the need of individuals to increase their complex carbohydrate consumption. This has occurred at a time when there also has been increasing scientific awareness of the benefits of increased complex carbohydrate consumption in lieu of other food components such as excess fat.

The initial popular usage of the term "complex carbohydrates" seems to have occurred in the 1970's. At that point in time, the term "dietary fiber" was also coming into common usage amongst nutrition scientists worldwide. The two terms were very closely associated, i.e. recommendations were being made by health experts to consume diets "high in complex carbohydrates such as dietary fiber", or to consume diets of foods "high in dietary fiber and complex carbohydrates." Since that time, the term "complex carbohydrates" has proven to be more difficult to define with precision than the term dietary fiber, although we all have a good idea of what complex carbohydrates are. As a result, analytical methods for dietary fiber were developed, validated, and are now being routinely utilized for nutrition labeling on foods. Similar developments for complex carbohydrates and the labeling of complex carbohydrate content in products has not occurred. Although com-

Dietary Fiber in Health and Disease, edited by Kritchevsky and Bonfield
Plenum Press, New York, 1997

plex carbohydrates are deemed to be important to health, implementation of specific policies on food labeling and consequent promotion of their consumption has not been undertaken as yet.

In many ways, the current situation with complex carbohydrates is similar to that which occurred with dietary fiber. Both involved a number of scientific issues requiring resolution. As with any scientific issue that ultimately results in public discussion, debate, and resulting public policy, there will be scientific supporters of the issue, scientific detractors of the issue, and those scientists who change their minds (sometimes frequently) as the resolution to the issues are being sought. Current efforts at public education and awareness of the importance of complex carbohydrates through publicity and the use of proper food labeling are being offset, at least in part, by individuals and/or groups who would prefer that the use of the term were simply discontinued. This dichotomy of positions is not unexpected. A similar course of events took place as part of the general widespread acceptance of the need for increased dietary fiber consumption as part of a healthy diet. After initial widespread support of the term dietary fiber, the British Nutrition Foundation in 1990 indicated it would rather not see the continued use of the term "dietary fiber", i.e., make the term obsolete in the scientific literature, preferring instead to refer to complex carbohydrates of which dietary fiber is a fraction. Some would prefer to measure only a portion of dietary fiber, i.e., the "nonstarch polysaccharides" portion of the dietary fiber. This feeling was certainly not unanimous amongst the scientists working in the field. As Leeds stated in 1993 "A number of readers, particularly in the United Kingdom and other European countries will take issue with our discontinuing the use of the term "Dietary Fiber". There are two reasons for this, first the term "Dietary Fiber" is now reasonably well understood worldwide, whereas "nonstarch polysaccharide" is not. Secondly, nonstarch polysaccharide is not a term which easily trips off the tongue, nor is it easily incorporated into anything other than a rather clumsy book or newsletter title."

On the other hand, now, some 5 years later, we see the term dietary fiber is readily accepted and widely used with regularity by many of the scientists who were members of that task force. Dietary fiber continues to be the central subject of books and scientific articles. Many of these articles reflect the continuing research on dietary fiber; much of the research successful in reinforcing the reasons why diets high in dietary fiber are recommended. One need only look at the number of workshops and symposia on dietary fiber, to realize that the term dietary fiber is important and will continue to be so.

As was the case with dietary fiber, there currently is a small but vocal number of scientists recommending discontinuance of the use of the term complex carbohydrates. This suggestion appears to arise for many of the same reasons that discontinuation of the use of the term "dietary fiber" was suggested. Because of unresolved differences of opinion on small, almost trivial, details regarding the makeup and definition of complex carbohydrates, it seems easier to throw everything out and ignore the important strides that have been made in understanding this important component of our diet. The majority of scientist working in the field support continued research, promotion, and use of the term "complex carbohydrates".

As with any research area, informed scientists need to lead the advances in research and promote positive results for the general well being of the public. Unlike other areas of endeavor such as marketing or advertising where changes in opinion and changing fads are welcomed with regularity, most scientists and nutritionists are typically willing to bear with a subject until the key issues are resolved and the results used for the benefit of the human population. Alternatively those same researchers bear with the subject until it is proven that there is little of no potential public benefit to be gained and the subject can be

set aside. Numerous efforts by informed scientist are moving the complex carbohydrate subject forward. There are AOAC International activities relating to establishing a concise complex carbohydrate definition, and validating appropriate analyses to quantitate complex carbohydrates using that definition. Just as the term dietary fiber is now back in routine use, I predict that the term complex carbohydrates will be have full widespread usage and popularity within the next year or two.

CONSUMER AWARENESS

The public, i.e. the consumers of foodstuffs, are very aware of the need to increase the consumption of complex carbohydrates in their diets. This awareness has been raised by a variety of means. Perhaps the most effective factor in raising awareness has been regulatory organizations and other health promotion organizations who have issued recommendations that consumers should increase their intake of complex carbohydrates. These recommendations have in turn been picked up by the media, both print and electronic, and spread throughout the population via magazine articles, newspaper articles, and radio and television news flashes and information segments.

As indicated, the most important factor in raising awareness has been health recommendation made by government agencies, by health and nutrition organizations, and by health and nutrition professionals. These recommendations bear significant weight in the mind of the public and are especially effective when they impact individual health and education professionals such as doctors, nurses, and teachers. Some of the more significant of these recommendations are:

Dietary Goals for the United States — February 1977 — McGovern Commission. In February of 1977, the McGovern Commission of the United States Senate, headed by Senator George McGovern, as the number one priority goal, recommended that consumers increase their consumption of complex carbohydrates

In December of that same year revised guidelines were issued:

Dietary Goals for the United States — December 1977 — McGovern Commission. Increased carbohydrate consumption was moved to second place in priority, however, it was the number one priority in type of food consumed. Number one overall priority was a recommendation to decrease total caloric intake.

Surgeon General's Report on Health Promotion and Disease Prevention — 1979. The Surgeon General's office picked up on the advocacy of a good thing, issuing its report in 1979 with recommendations similar to that of the McGovern Commission Consumers should consume relatively more complex carbohydrates in foods such as whole grains, fruits, and vegetables. An updated surgeon general's report in 1988 carried a similar message.

Surgeon General's Report — Nutrition and Health — 1988. In this report, the recommendation was to increase consumption of complex carbohydrates and fiber via whole grains, fruits, vegetables.

Recommended Dietary Allowances — 10th Edition — National Research Council. In 1989, the National Research Council also recommended increased consumption of

Complex Carbohydrates, in this case Complex Carbohydrates referred to both digestible complex carbohydrates and the nondigestible component of food, dietary fiber.

Activity in recommending increased consumption of complex carbohydrates has not been limited to the United States in the years since 1977.

Nutrition Recommendations — 1990 — Health and Welfare Canada. In 1990, Health and Welfare Canada recommended an increase in complex carbohydrate consumption.

British Nutrition Foundation — 1990. Also in 1990, the British Nutrition Foundation made a similar recommendation, recommending an increase in the intake of a variety of foods which are rich in Complex Carbohydrates.

By 1991, there was sufficient consensus on complex carbohydrates as an important nutrient factor that the US Department of Agriculture and the US Food and Drug Administration proposed requiring the listing the quantity of complex carbohydrates on the nutrition label to educate consumers.

Nutrition Labeling and Education Act — Proposed Regulations — 1991. Complex carbohydrates were to have been defined as digestible dextrins and starches consisting of polymers of glucose of 10 or more saccharide units. However, when the final regulations were issued in 1993, the quantity of complex carbohydrates present in the food was not reflected on the label.

Nutrition Labeling and Education Act — Final Regulations — 1993. The USFDA and USDA felt that although increased consumption of complex carbohydrates by consumers was desirable from a health perspective, at the time the specific definition of, and adequate methodology for, complex carbohydrates had not been established.

Even as the proposed nutrition labeling regulations were being evaluated, and after the final regulations were issued, recommendations for increased complex carbohydrate consumption continued to be made.

Food Guide Pyramid — 1992. For instance the food pyramid of 1992, a document considered by many to be a key guidepost for consumers, emphasized eating plenty of Breads, Cereals, Rice, Pastas, Fruits, and Vegetables, all of which are valuable sources of complex carbohydrates. If one looks closely at the recommendations, it can be seen that three times as many servings of these foods are recommended as are the number of servings of other foods.

Dietary Guidelines for Americans — 1995. And most recently, the 1995 Dietary Guidelines for Americans, issued in early 1996, continue to recommend diets with plenty of grain products, fruits, and vegetable as important to good health. All these foods are high in complexcarbohydrates.

Informing consumers of the need to increase intake of complex carbohydrates has been very effective. In a survey reported in 1994 by the Kellogg Company, it was found that 50% of consumers knew something about Complex Carbohydrates and an additional 36% had heard about them. So nearly 7 out of 8 consumers were aware of this nutrition factor. Of those who claimed to know something about complex carbohydrates, 88% of the consumers knew it was important to their health. So the messages that have been put forth have been effective.

The tool that will ultimately provide consumers with the information they need regarding the level of complex carbohydrates they are consuming will be the nutrition label. An example to the information that the label might provide would be as follows:

Total Carbohydrates
  Complex Carbohydrates
    Dietary Fiber
      Soluble Dietary Fiber (Optional)
      Insoluble Dietary Fiber (Optional)
    Digestible Complex Carbohydrates (optional)
  Sugars
  Sugar Alcohols (optional)

SCIENTIFIC REPORTS

There are a number of scientific studies that emphasize the importance of Complex Carbohydrates in the diet. Some of these studies may not have been authored for the purpose of addressing the complex carbohydrate subject, nonetheless they contain information pertinent to the subject. Four of the more recent studies are: The James report on dietary fiber to the European Commission in 1993; The Stephen report on resistant starch to the International Life Sciences Institute Workshop on complex carbohydrates in 1994; The report by Schaefer et. al. in 1995 in the Journal of the American Medical Association regarding the effects of Low Fat *Ad Libitum* Diets; And the report by Rimm et. al. in 1996 in the Journal of the American Medical Association regarding the effects of fiber intake on coronary heart disease risk.

James Report

The James report was authored in 1993 to lend support to the idea that analysts and nutritionists ought to be measuring only a portion of dietary fiber, i.e. nonstarch polysaccharides rather than measuring dietary fiber in its entirety. However, the report concluded that there was no correlation between nonstarch polysaccharide consumption levels and colon cancer. On the other hand, the report also concluded that there was an inverse correlation with resistant starch consumption. However, it should be pointed out, that the resistant starch levels of the foods used in the correlation was never actually measured. The total starch content of the food was quantitated, then the total starch quantity was multiplied by 0.05 to obtain the resistant starch level, on the assumption that resistant starch was about 5% of total starch. We know of course, that the level of resistant starch varies with foods and the conditions used to process those foods making that a questionable assumption. Thus the true correlation was an inverse correlation between starch (whether digestible or resistant) i.e. a complex carbohydrate, and colon cancer. The impact of dietary fiber as a whole as opposed to the nonstarch polysaccharide portion of the fiber on colon cancer was not addressed.

Stephen Report

In a 1994 report to the International Life Sciences Institute Workshop on complex carbohydrates, Stephen pointed out that fermentable carbohydrates (e.g. resistant starch)

Table 1. Popualtion studies of diet and human cancer

Quintile	TDF (grams/day)	Relative risk
1	12.4	1.00
2	16.6	1.01
3	19.6	0.96
4	23.0	0.92
5	28.9	0.64

As can be seen, over the six year study period, the quintile of individuals of the study population that consumed the highest level of total dietary fiber had less than 2/3 the likelihood of significant ill heart health compared to the quintile that consumed the lowest amount of dietary fiber. If one looks at the results of the study relative to heart health response to different fiber sources, one can see that the three major sources of fiber, i.e. fruits, vegetables, and cereals each have a significant effect. Table 2 shows the data for fruit fiber.

reaching the colon where they become available for fermentation are more beneficial to the consuming body than are nonfermentable polysaccharides and other dietary fiber components. The increased fermentable substrate level available results in increased colonic fermentation which in turn increases butyric acid production, reduces colonic pH, and reduces production of secondary bile acids. All these changes in bowel environment have been shown to be beneficial. Fruits and vegetables alone do not supply a significant amount of starch or other fermentable substrate that reaches the colon, but rather supply relatively high levels dietary fiber on a dry weight basis. Therefore, to optimize fermentation, additional starches or complex carbohydrate sources such as legumes and grains are necessary in the diet to provide the colon with a fermentable feedstock. The digestible carbohydrates may need to be intimately bound into the dietary fiber for maximum effect.

Schaefer *et al.*

Schaefer and colleagues in 1995 reported that when 27 free living subjects were fed a low fat diet of equal caloric value to a normal fat diet, (i.e. equal calorie diets containing 15% total fat versus 35% total fat) the LDL/HDL cholesterol ratios and the total/HDL cholesterol ratios of the subjects increased significantly, not necessarily a desirable outcome. When the low fat diet regimen of the study was completed, the subjects were offered the same low fat diet, but were allowed *ad libitum* intake of the foods. During this

Table 2. The relation between fibre-rich foods and cancer incidence (data fromArmstrong and Doll, 1975). The relationships are given as correlation coefficients

Quintile	Fruit fiber (grams/day)	Relative risk
1	1.2	1.00
2	2.4	0.93
3	3.7	0.83
4	5.2	0.84
5	7.9	0.81

For fruit fiber, the quintile of individuals consuming the highest levels of fruit fiber experienced 1/5 fewer serious heart events during the course of the study. It is interesting to note that the most significant reduction in risk occurs with a modest increase in fruit fiber intake going from 1.2 to 3.7 grams.

Table 3. Summarized case-control studies of fibre intake and cancer risk

Quintile	Vegetable fiber (grams/day)	Relative risk
1	3.2	1.00
2	4.8	1.08
3	6.1	0.99
4	7.8	1.00
5	11.1	0.83

Looking at vegetable fiber, the relative risk of myocardial infarction does not decrease until high levels of fiber intake are reached, i.e. a daily intake of over 10 g to reduce the risk by 20% compared to fruit fiber where the reduction occurred at one third that level of intake. However, when we look closely at both the fruit and vegetable data it is apparent that it took a 3 fold increase in consumption of fruits to lower the relative risk by 15–20%. Likewise it appears that it takes a 3 fold increase in consumption of vegetables to achieve the same level of risk reduction.

phase of the study, 25 of the 27 subjects lost weight, and their total/HDL and LDL/HDL cholesterol ratios returned to either baseline ratios or lower. Thus, indications are that if a significant portion of the fat in the diet is displaced by carbohydrates, (in this case, adjusting the carbohydrate level from 49% of the diet to 68% of the diet), *ad libitum* consumption of the low fat diet actually results in weight reduction, cholesterol reduction, and positive improvement in the Total/HDL, and LDL/HDL cholesterol ratios.

Rimm *et al.*

Rimm et. al. (1996) reported a six year cohort study tracking the health related habits and health outcomes of almost 44,000 US male medical professionals. Foods intake of the individual subjects was monitored by a self reporting system. This system was audited by random detailing of diet records and by random checking of plasma carotenoid and tocopherol levels as an indicator of quantity of foods consumed compared to quantities indicated in self generated reports. The endpoint used to measure the health effects of the health habits was either nonfatal or fatal myocardial infarction. Table 1 shows a summary

Table 4. The relation between colorectal cancer mortality and intake of fibre-rich foods (Hill, 1995 for details and references)

Quintile	Cereal fiber (grams/day)	Relative risk
1	2.2	1.00
2	3.7	0.96
3	5.0	0.88
4	6.8	0.86
5	9.7	0.71

Cereal fiber consumption levels (see table 4) in contrast to fruit and vegetable fiber, show a relatively linear risk reduction relationship correlated with the amount of cereal fiber consumed. Cereal fiber consumption also shows the greatest effect per gram of fiber consumed. As in the case of fruit and vegetable fibers, it also appears that a 3 fold increase in cereal fiber intake reduces the relative risk of myocardial infarction by 15-20%. It does however, appear that on a gram for gram increase in fiber consumption basis, cereal fiber has a greater impact in lowering risk than do either fruit fiber or vegetable fiber (see table 5).

Table 5. Relative risk of myocardial infarction per 10 gram incremental increase in fiber consumption

Fiber source	Relative risk (95% conf interval)
Vegetables	0.88 (0.65–1.21)
Fruits	0.83 (0.62–1.10)
Cereals	0.73 (0.57–0.94)

When the consumption levels of fruit, vegetable, and cereal fibers are compared to one another on the basis of 10 gram incremental increases of daily dietary fiber intake, only the 10 gram daily increment of cereal fiber shows a statistically significant reduction in risk at the 95% confidence level. As was discussed in relation to the Stephen report above, cereals provide a significant level of digestible complex carbohydrates in addition to the dietary fiber they provide, while fruits and vegetables do not.

of the study results relating relative risk of myocardial infarction to quantity of total dietary fiber consumed per day with the subjects being separated into five equal groups respective to their fiber consumption levels. All relative risk figures have been adjusted for saturated fat level in the diet, vitamin E intake, age, body mass index of the subjects, physical activity level of the subjects, smoking, alcohol consumption, hypertension, hypercholesterolemia, family history, and profession.

CONCLUSION

Review of all the scientific studies relating increased complex carbohydrate consumption and improved good health is well beyond the scope of this brief synopsis. Such extensive reviews have been done by numerous authoritative health organizations and resulted in the many dietary recommendations to increase complex carbohydrate intake. Certainly, dietary fiber promotes good health, but dietary fiber alone is insufficient to explain the total positive health impact of high carbohydrate foods in numerous studies. The total complex of carbohydrates, including dietary fiber and digestible carbohydrates, is playing a significant role.

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6

DIETARY GUIDELINES AND COMPLEX CARBOHYDRATES

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In January, 1996 the Departments of Agriculture and Health and Human Services jointly released the 1995 Dietary Guidelines for Healthy Americans (1). Since 1980, review and publication of revised Dietary Guidelines has occurred every 5 years; however, the 1995 review is the first that was mandated by public law (Public Law 101–445, Section 3). The Guidelines, as released, are based on a report submitted to the Secretaries of these agencies that is developed by an 11 member Advisory Committee (2). However, the bulletin is reviewed and revised within the agencies before publication. The recommendations in the bulletin form the basis of Federal policy and education programs in food, nutrition and health and provide a working agreement between Agriculture and Health and Human Services for their programs. The written text of the guidelines is aimed at consumers; nonetheless, it is intended to reflect current scientific knowledge and has relied upon such reports as the Surgeon General's Report on Nutrition and Health, the National Academy of Sciences Report on Diet and Health and the tenth edition of the Recommended Dietary Allowances as well as current scientific literature (3–5).

A comparison of the 1985, 1990, and 1995 Dietary Guidelines is shown in Table 1. Since 1985, the committees have revised the bulletin to be more positive and action-oriented in its guidance. Between 1985 and 1990 USDA conducted a series of focus groups which illustrated that consumers were uncertain about the interpretation of the phrase "avoid too much" in the guidelines and often interpreted the guidelines as indicating that fat, sugar and sodium should be eliminated from the diet (6). The 1990 and 1995 Guidelines have emphasized moderation; however, nutrition educators caution that the meaning of the word moderation is not well understood by consumers. Several sections in the Guidelines contain reference to carbohydrates, including complex carbohydrates.

Both the Introduction of the bulletin and the section, Eat a Variety of Foods, refer to carbohydrates as an important source of energy and a factor to consider in energy balance. The Variety section recommends use of the Food Guide Pyramid as a dietary pattern. This section indicates that most of the energy consumed daily should be derived from foods at the base of the Pyramid (grain products, fruits and vegetables) as these foods are typically high in carbohydrates.

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Table 1. Dietary guidelines from 1985, 1990, 1995 as published by the Department of Agriculture and the Department of Health and Human Services

Nutrition and your health Dietary guidelines for Americans		
1985	1990	1995
Eat a variety of foods.	Same	Same
Maintain desirable body weight.	Maintain heathy body weight	Balance the food you eat with physical activity. Maintain or improve your weight.
Avoid too much fat, saturated fat, and cholesterol.	Choose a diet low in fat, saturated fat and cholesterol.	Choose a diet with plenty of grain products, vegetables, and fruits.
Eat foods with adequate starch and fiber.	Choose a diet with plenty of fruits,vegetables and grain products.	Same (see 3rd guideline, 1990)
Avoid too much sugar.	Use sugars only in moderation.	Choose a diet moderate in sugars.
Avoid too much sodium.	Use salt and sodium only in moderation.	Choose a diet moderate in salt and sodium.
If you drink alcoholic beverages, do so in moderation.	Same	Same

The section, Choose a Diet with Plenty of Grain Products, Fruits and Vegetables, was modified significantly between 1990 and 1995. In 1990 this section contained primarily information about foods that are sources of starch and dietary fiber and was the fourth guideline in the bulletin. In the 1995 bulletin, information about starch and fiber remain and has been updated based on new evidence regarding the role of dietary fiber in lowering risk of heart disease and cancer. In addition these foods are highlighted because of their content of other nutrients including carotenoids, folate, antioxidant nutrients, vitamins C and B-6, potassium, calcium, and magnesium and because these foods are typically low in fat, saturated fatty acids and cholesterol, depending on preparation methods. The bulletin also notes that plant foods contain other compounds that are currently being investigated for their potential role in lowering risk of chronic disease. This section emphasizes inclusion of grain products high in complex carbohydrates such as breads, cereals, pasta and rice as well as vegetables such as potatoes and corn and dry beans, which can be included as a meat alternative or as a vegetable serving. Based on this expanded description of the importance of plant foods and the recognition that most Americans consume less than the recommended number of servings of these foods, this recommendation was published as the third rather than fourth guideline.

The section, Choose a Diet Moderate in Sugars, refers to complex carbohydrates as starch and dietary fiber. The section notes that carbohydrates include both sugars and the complex carbohydrates, starch and dietary fiber, and that all carbohydrates except fiber break down to sugars during digestion. Other government agencies and scientific organizations have used the term "complex carbohydrates" to include oligosaccharides of a certain chain length, starch, starch plus dietary fiber, or as a concept which refers to foods which contain carbohydrates that were not added during formulation or processing (e.g. grains, fruits, dry beans, and vegetables) (7). The definition used in the Guidelines suggest that "complex carbohydrate" primarily refers to polysaccharides rather than simple sugars. If one views the guidelines as a whole, the recommendation to consume no more than 30% energy from fat indicates that most of the energy in the diet will be derived from car-

bohydrate. Likewise, the recommendation to consume sugars in moderation indicates that starch is the preferred carbohydrate energy source in the diet.

Dietary recommendations from other countries have also encouraged that carbohydrates provide the major dietary energy source (50% or more) and that emphasis be placed on energy from complex carbohydrates (8). In the United States the current food label does not directly provide Nutrient Facts about complex carbohydrate. This information is indirectly available through the label declaration of dietary fiber and other carbohydrates. The question remains whether this information is adequate for consumers to make informed choices about increasing their consumption of complex carbohydrates.

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CLASSIFICATION OF COMPLEX CARBOHYDRATES

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In 1991 the U.S. Food and Drug Administration, U.S. Department of Health and Human Services (FDA) and Food Safety and Inspection Service, U.S. Department of Agriculture (FSIS) proposed extensive food labeling changes, which included mandatory nutrition labeling for most foods, standardized serving sizes, and uniform use of health claims. The regulation stated that complex carbohydrates would be part of the information given on the nutrition label, to be used, as part of its mission in educating the consumer[1] . The regulations proposed that mandatory labeling of complex carbohydrates would be the sum of dextrins, saccharide units of 10 or more, and starches.

In 1992, FDA and FSIS called for a public hearing on the proposals. Ninety-two representatives from the food industry, the scientific community, and consumer groups presented their comments in person to a panel of FDA and FSIS officials. In addition to the oral comments, FDA received more than 40,000 others in writing, the largest number ever received in response to a proposed regulation. About 30,000 were form letters from organized campaigns. FSIS received 1,109 comments. An overwhelming majority came from consumer groups, industry, and medical groups asking for harmonization of FDA and FSIS labels.

The details called for by NLEA were often a problem for FDA rule-makers. For example, the law said the label shall have complex carbohydrates. In order to enforce the law, FDA would have had to come up with a way to measure complex carbohydrates. The problem was that there was no good definition for complex carbohydrates. Had the officials been working on nutrient list requirements *without* NLEA, they would not have included complex carbohydrates, but a more general term such as "carbohydrates other than sugar."

After the comment period closed and following considerable deliberations by FDA and the U.S.Department of Agriculture (USDA), the NLEA final regulations were issued in January, 1993[2]. The agencies concluded in their deliberations that complex carbohydrates could not be included on the label because of inadequate definition related to physi-

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ological effects and lack of sufficient analytical methodology. That is, no clear consensus on the definition of complex carbohydrates exists as it relates to physiological effect, health benefits, or dietary guidelines. The NLEA reaffirmed the legal basis for FDA's labeling initiative and established an explicit timetable for its implementation. Regulations pertaining to nutient labeling would become effective May of 1993 and nutrient content claims were to become effective in May 1994.

The dilemma that was presented was that the terminology, complex carbohydrates, had become very familiar to consumers through health related articles and discussions in the media. It was first brought to the attention of scientists by Cleave[3] and Cleave and Campbell[4], who found that few individuals in cultures with little or no industrial development suffer from a variety of illnesses compared to that of their counterparts in industrialized societies. They attributed this to the refining of foods, which removed carbohydrates, in industrialized societies. The Senate Select Committee on Nutrition and Human Needs (Senator McGovern, Chair), in 1977[5], first recommended that consumers increase their intake of complex carbohydrates and they felt that fruits vegetables and cereal grains were good sources of these carbohydrates. By the early 1980's, many of the affluent countries already had developed guidelines for nutrients. A World Health Organization study group recommended that persons should increase their intake of complex carbohydrates[6]. *Dietary Guidelines for Americans*[7] issued in 1985 also recommended that consumers increase their intake of complex carbohydrates paricularly starches. The *Surgeon General's Report on Nutrition and Health* in 1988[8] again recommended that consumers increase their consumption of complex carbohydrates and dietary fiber. The emphasis was again placed on increasing the individuals consumption of whole-grain cereal products, vegetables, and fruits. The 10th edition of *Recommended Dietary Allowances* of the National Research Council in 1989[9] coupled with *Diet and Health: Implications for Reducing Chronic Disease Risk* 1989[10], repeats the call for increased complex carbohydrate consumption (i.e. both digestible complex carbohydrate and dietary fiber). The importance of complex carbohydrates has also been the subject of consumer education and health recommendations in Canada where in 1990 Health and Welfare Canada[11] issued nutrition recommendations to increase consumption of complex carbohydrates. Finally, the *Food Pyramid Guide*, the 1992[12] guidepost by which individual consumers evaluate their own diets, reemphasizes the need to increase complex carbohydrates in the diet relative to other foods (i.e., three times as many vegetables, fruits and cereal grains products). Complex carbohydrates was a term also used by the USDA and U.S. Department of Health and Human Services (DHHS) in publications where they recommended eating diets with increasing amounts of complex carbohydrates[13]. Consumers, although aware of the recommendation to increase their consumption of complex carbohydrates, were unable to do so because they were unable to find this information on the food label. Furthermore, the specific benefits of complex carbohydrates is confounded by the fact that diets high in complex carbohydrates are usually mixed diets containing cereal grains, fruits, and vegetables which are high in fiber, vitamins, minerals and low in fat.

The Association of Official Analytical Chemists, International (AOAC) workshop on complex carbohydrates and dietary fiber[14] (a follow-up to the International Life Sciences Institute [ILSI]-North American Workshop held in November 1994 in Washington, D.C.)[15,16], held in September, 1995 sought to resolve issues surrounding the definition of complex carbohydrates for analytical purposes. The participants generally supported the expansion of the definition of complex carbohydrates to include dietary fiber in addition to available starches[16,17]. For the nutrition label, complex carbohydrates could be derived as the sum of analytically measured starch and dietary fiber values or by the calculation total carbohydrates – (sugars + available oligosaccharides). Among the scientists support-

ing the definition of complex carbohydrates as the sum of starch and dietary fiber, about 55% supported the labeling of analytically derived values for dietary fiber and starch and approximately 45% were of the opinion that values calculated by the difference method would be acceptable. It was recommended that the associate referee continue study based on these findings. It was interesting to note that only two persons supported FDA's 1991 definition of complex carbohydrates as total carbohydrates – dietary fiber – simple sugars.

Foremost in the minds of scientists and regulators were three questions that had to be answered before the nutrition labeling of complex carbohydrates could be fact.

1. Can a clear physiological difference be demonstrated for simple as compared to complex carbohydrates?
2. Can analytical methodology be developed which chemically differentiates the simple and complex carbohydrates? and
3. Could the answers to questions (1) and (2) serve as the definition of complex carbohydrates?

The AOAC in response to the final NLEA regulations, in 1992, assembled a task force to review available methodology for all nutrients. A Subcommittee on Carbohydrate Methodology composed of representatives from the food industry, academia, FDA and USDA was specifically charged with recommending and validating appropriate methodology to be used on carbohydrate analysis for nutrient labeling purposes and identifying future needs in the area of carbohydrate analysis. The work of the Subcommittee revealed that FDA was correct in its evaluation of the complex carbohydrate dilemma, that there was no clear definition of complex carbohydrates and there was no validated method for those definitions of complex carbohydrates that were put forth. The FDA concluded and the Subcommittee agreed that there was no professional consensus on the meaning of the term complex carbohydrates to require mandatory nutrition labeling. Could complex carbohydrates be defined from the standpoint of structure, function and contribution to health and, if so, could methodology be developed for the analysis of complex carbohydrates?

To address these issues the AOAC established a General and Associate Refereeship for complex carbohydrates in 1993. An international survey[14] was initiated to ascertain if a definition of complex carbohydrates could be reached and then determine the most appropriate means of measuring it. There was broad consensus that the term complex carbohydrate should encompass both starch and dietary fiber.

The second international survey was conducted and showed ageement with the first that complex carbohydrates are generally defined as polysaccharides and there was support for expanding the definition of complex carbohydrates to include dietary fiber[14,15].

Approximately 200 survey questionnares were distributed to prominent professional in carbohydrate science. The survey yielded results from 114 respondents in 29 different countries.

Respondents were asked to select from a provided list of carbohydrate components those which should be included in the definition of complex carbohydrates for food labeling purposes. The question was presented in a multiple choice format, with the possibility of writing in additional options. The 107 respondents were also asked to explain and justify their choices.

Most respondents felt that available starches (72%) should be included in the definition of complex carbohydrates (Table 1). The majority of respondents also listed the terms non-starch polysaccharides (69%), and unavailable or total starches (58%) to be included in the complex carbohydrate fraction. Approximately one-third of the respondents included both available (33%) and unavailable oligosaccharides (32%), and lignin (29%) in their

Table 1. For nutrition labeling purposes, which should be included in the complex carbohydrate definition? Check all that apply*

Answer	Number	Percent
Available starches	77	72
Non-starch polysaccharides (NSP)	74	69
Total starches	62	58
Unavailable starches (Resistant starches)	62	58
Oligosaccharides, available	35	33
Oligosaccharides, unavailable (resistantoligosaccharides)	34	32
Lignin	31	29

* Number of respondents = 107

definition of complex carbohydrates. Further data analyses showed that 62% of the respondents indicated that both total starches and unavailable polysaccharides were complex carbohydrates.

In the second survey respondents were asked to define complex carbohydrates using their own descriptions. This open ended-question was intended to elicit an unprompted definition of complex caarbohydrates directly from the respondent. Of the 72 persons who responded, 63 provided a definition of complex carbohydrates, while 9 stated they did not like the term and failed to provide a defintion. Of the 63 respondents, 52 (82%) included dietary fiber polysaccharides in addition to available starches in their own definition of complex carbohydrates. Table 2 shows the most common description of complex carbohydrates as provided by the 52 respondents. While 19 (26%) respondents described complex carbohydrates simply as polysaccharides, ten (14%) based their answers on the physiology of digestion, defining complex carbohydrates as glucose polymers that are slowly digested or not digested in the small intestine. Another ten respondents (14%) included all carbohydrates except simple sugars in their definition, while seven respondents (10%) described complex carbohydrates as starch plus dietary fiber. Eight responses referred to complex carbohydrates as starches or available starches, and the remainder used terms such as unavailable starches or oligosaccharides, cell wall carbohydrates or lignin. In summary,

Table 2. By using your own description, please define complex carbohydrates

Definition provided	No. of respondents providing the definition	%
Polysaccharides	19	26
Glucose polymers which are slowly digested or not digested in the small intestine	10	14
Carbohydrates minus simple sugars	10	14
Don't like the term	9	13
Starches and dietary fiber	7	10
Available starches	4	6
Starch + dietary fiber +oligosaccharides	2	3
Glucan with complex structure	2	3
Dietary fiber + unavailable oligosaccharides	1	1
NSP + lignin	1	1
Starch and cell wall carbohydrates	1	1
Unavailable carbohydrates	1	1
Glucans not soluble in 80% EtOH	1	1
Carbohydrates of plant origin (>DP4)	1	1

82% of the respondants included available starch and dietary fiber polysaccharides in the complex carbohydrate complex.

At the same time, on August 8, 1994, a meeting was held between the AOAC chair of the Food and Nutrition Committee, the AOAC general referee for dietary fiber and complex carbohydrates, the AOAC associate referee for complex carbohydrates, and members of the staff of the nutrient data laboratory of the USDA. The purpose of the meeting was to summarize input from the survey on complex carbohydrate and to highlight areas in need of further collaborative effort. The following conclusions were drawn from this meeting:

1. Consumers and health educators are generally familiar with recommendations regarding dietary consumption of complex carbohydrates and improved health. Depending upon the wording of a particular recommendation, complex carbohydrates includes includes digestible complex carbohydrates and may or may not include dietary fiber.
2. A single clear definition of complex carbohydrates is desirable.
3. Based on the results of the recent AOAC International survey, complex carbohydrates should consist of "both dietary fiber and digestible complex carbohydrates fraction of the food."
4. Analytical methods have been developed and proposed which will quantitatively define and determine the food fraction(s), meeting the current scientific consensus for the definition of complex carbohydrates. The proposed methods will still need to be collaboratively studied through the AOAC International.
5. The definition meets both the physiological and chemical basis for complex carbohydrates considered important by the scientists surveyed. Enzyme systems used for analysis mimic the human digestive system, thus providing a physiological basis. Complexity of the carbohydrates is chemically established by the insolubility of the fraction in four part of alcohol and one part of water. This has long been considered by analysts as the standard basis for separating simple compounds from the more complex ones found in food matrices.
6. A potential scheme for listing carbohydrate information on labels and in nutrition data table might be:

    Total carbohydrates
      Complex carbohydrates
        Dietary fiber
        Digestible complex carbohydrates
      Sugars
      Sugar alcohols(optional)
      Other carbohydrates

We think that there is a clear demonstration that the physiological effects and associated analytical methodology lead to a definition of complex carbohydrate. That is, complex carbohydrates is the sum of available starch and dietary fiber. Whether this determination is made chemically by individually determining available starch and total dietary fiber or by difference of total carbohydrates – (sugar + available oligosaccharides), is of little consequence.

At the present time two methods have proven suitable for the determination of complex carbohydrates. They are the HPLC determination of available starch or the enzymatic-UV and colorimetric determination of available starch both coupled with the

enzymatic-gravimetric determination of dietary fiber[18,19]. These methods are currently undergoing collaborative study under the auspices of the AACC and AOAC International.

Now that we have discussed the various aspects of complex carbohydrates inluding definition, analysis, and somewhat on what the consumer expects in the way of an effect, we can get to the classification of these complex carbohydrates. First the physiologic response to complex carbohydrates is much more complex than that implied by the term *simple* as compared to *complex carbohydrate*. Simple meaning rapidly metabolized and absorbed and complex meaning not metabolized or slowly metabolized and slowly absorbed or not absorbed at all, which is not true in all cases. The classification of simple and complex carbohydrates requires good methods for determining the recovery and measurement of all non-digestible carbohydrates and and commercially available substances added to foods for their own purposes. One would also have to determine the degree of polymerization that is acceptable for a carbohydrate to be called complex, balancing this with its physiologic effects of the compound in question.

A determination will have to be made on whether raffinose (trisaccharide) and stachyose (tetrasaccharide) should be included in the complex carbohydrate complex (which they should be by their physiological response). Raffinose is the most important of the trisaccharrides, occuring in sugar beets and concentrated in sugar beet molasses. Cotton seed meal contains about 8% raffinose and is the material generally used for its preparation. Raffinose is also frequently found in higher plants and fungi. It is hydrolyzed by the enzymes of the gastrointestinal bacteria of herbivorous animals and serves as food for these animals. Raffinose is not well utilized as food by man. Stachyose is indigestible and is the only tetrasaccharide known, occurring in the tubers of *Stachys tuberifera*, and the seeds of various leguminous plants.

Wood contains another polymeric substance, lignin, which we also consider as a complex carbohydrate (because it is included in the dietary fiber fraction). It makes up nearly 25% of the dry weight of the wood. Lignin is a polymer of aromatic alcohols although it is defined in Dorland's Illustrated Medical Dictionary, 25th Edition as "a polysaccharide which in connection with cellulose forms the cell wall of plants and thus of wood."

Eastwood, a world renowned expert on the physiological function of fiber, thinks the present dilemma about the effects of fiber is due to the indecision as to what is meant by fiber. He feels the more appropriate term is the umbrella term complex carbohydrates. This would include resistant starch, plant cell wall material, mucilages, gums, and isolates identified by their plant origin[20].

It would appear that if all the factors we have discussed are taken into consideration, complex carbohydrates would include available starches and dietary fiber. The starches could include amylose, amylopectin, modified starches, dextrins and insoluble, digestible or indigestible oligosaccharides. The dietary fiber portion of complex carbohydrates would include soluble and insoluble dietary fiber (including resistant starch) plus resistant oligosaccharides. Simple carbohydrates would be defined and measured as any soluble, digestible, and absorbable sugars and any soluble and digestible oligosaccharides.

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DETERMINATION OF COMPLEX CARBOHYDRATES IN FOODS AS THE SUM OF AVAILABLE STARCH AND DIETARY FIBER

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DEFINITION OF DIETARY FIBER AND COMPLEX CARBOHYDRATES

Three international surveys (1, 2) were conducted to obtain the views of professionals in the field on the definition of complex carbohydrates and dietary fiber. The survey showed that complex carbohydrates are generally defined as polysaccharides and there was support for expanding the definition of complex carbohydrates to include dietary fiber. The survey results also indicated that the term dietary fiber should include resistant oligosaccharides as well as non starch polysaccharides, resistant starches, and lignin. The survey results indicated a need for still greater consensus regarding the proposed inclusion of resistant oligosaccharides and lignin.

THE 1995 AOAC INTERNATIONAL WORKSHOP RESULTS

The AOAC International Workshop was also held in Nashville, Tennessee, on September 15–16, 1995, with 57 participants from the United States and Europe (3). The foremost authorities in the world from industry, academia, and government participated. The Workshop was a follow-up to the International surveys as well as the activities of the Carbohydrates Subcommittee of the Nutrients Labeling Analysis Task Force of 1992–1993 and the International Life Science Institute (ILSI)-North American Workshop held in November 1995 in Washington, D.C. (4).

In the panel discussion, the panelists (moderator, L. Prosky; panelists, J. Slavin, D. Gordon, M. Roberfroid, F. Scott, E. Bartnikowska, and R. Wood) discussed the appropriateness of complex carbohydrates labeling as well as the advantages of the labeling. The

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discussion centered around what complex carbohydrates mean to the lay person, the scientist, and the politician. The panel recommended a vote be taken at the end of the meeting to determine the definition of complex carbohydrates. There was also much discussion of the components that were not fully recovered in the AOAC dietary fiber fraction (resistant oligosaccharides). These resistant oligosaccharides (oligofructans, polydextrose) have many of physiological properties of dietary fiber. It was suggested that a method be collaborated for determination by the AOAC.

There was a general agreement among the Workshop participants that dietary fiber should be included in the definition of complex carbohydrates and that resistant oligosaccharides are part of dietary fiber. Twenty-nine persons, representing 85% of the 34 people providing a definition for complex carbohydrates, agreed that the definition is the sum of dietary fiber and starch (technically speaking, available or enzyme available starch). For the nutrition label, complex carbohydrates could be derived as the sum of analytically measured starch and dietary fiber values or by the calculation as total carbohydrates – (sugar + available oligosaccharides). Among the 29 scientists supporting the definition of complex carbohydrates as the sum of starch and dietary fiber, sixteen persons (corresponding to 55%) supported the labeling of analytically derived values (total dietary fiber and available starch) and 13 persons (45%) were of the opinion that the values calculated by the difference method would be acceptable. It was recommended that the associate referee continue study based on this definition. An interesting point to note is only two persons supported the FDA's 1991 definition of complex carbohydrates as total carbohydrates – dietary fiber – simple sugars. Other definitions considered were the sum of starch and non-starch saccharides (3 persons) and the sum of starch and non-starch polysaccharides (which correspond to polysaccharides; 0 persons). Strong interest was shown in analytical methods for resistant oligosaccharides. It was recommended to establish new associate refereeships in this area. Methods for determining resistant oligosaccharides need to be collaboratively validated.

The workshop participants unanimously supported the inclusion of RO in the dietary fiber definition. The participants also supported the expansion of the complex carbohydrates definition to include the newly defined dietary fiber in addition to available starches. This new definition would not include available oligosaccharides present in candies.

An analytical method for complex carbohydrates needs to be developed and collaboratively validated based on the definition as the sum of (available) starch and dietary fiber.

The addition of dietary fiber, including resistant oligosaccharides, in the complex carbohydrates definition would also contribute to more active research in the nutritional and food technology areas. A common understanding of complex carbohydrates, including resistant oligosaccharides, should be developed. Existing dietary fiber methods should be fine tuned to fully recover these resistant oligosaccharides as well as resistant starches. We want to emphasize that this new definition would not affect the complex carbohydrates and dietary fiber values of most food products.

ANALYSIS

Complex carbohydrates are determined as the sum of available starch and dietary fiber, as shown in figure 1. Duplicate samples of dried foods, fat-extracted if containing >10% fat, undergo sequential enzymatic treatments by heat-stable α-amylase, protease, and amyloglucosidase to digest starch and protein. Enzymatic digestion steps have been adopted from AOAC official final action methods 991.43 (5) and 985.29 (6) for dietary fiber determination.

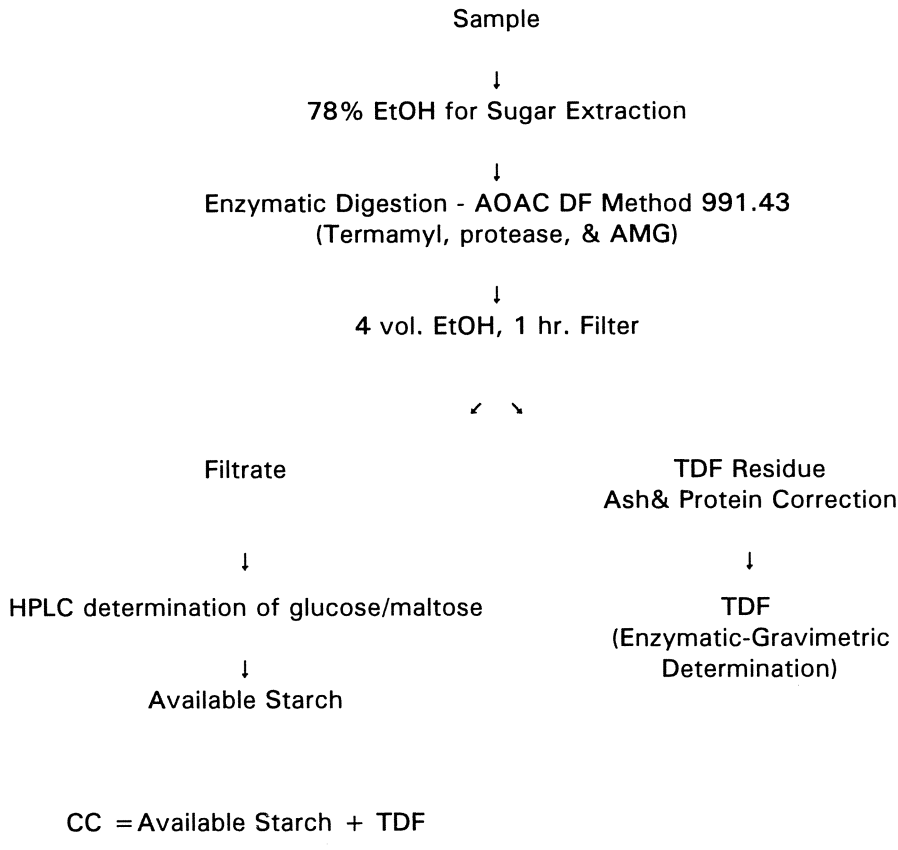

Figure 1. Analysis of complex carbohydrates (CC).

For available starch, the enzyme digestate, containing mostly monosaccharides and some disaccharides, is analyzed by HPLC. Measured glucose and maltose are converted into a starch unit and reported as available starch. HPLC conditions have been adopted from AOAC official final action method 982.14 (7) for sugar determination.

Chromatographic Conditions

Analytical Column:	Zorbax NH_2, 4.6 mm I.D. × 25 cm
Guard Column:	Supelco, 10 cm, aminopropylsilyl
Mobile Phase:	70% acetonitrile, 30% water (v/v)
Flow Rate:	1.0 mL/min.
Injection Temperature:	Room temp.
Elution Mode:	Isocratic
Detector:	Refractive index
Detector Sensitivity:	0.020×10^{-3}
Response Time:	2.0 min.
Run Time:	25 min.
Injection Volume:	15 μL

Table 1. Determination of available starch in foods by HPLC

Sample	% available starch	% complex CHO
Corn cereal	76.2 ± 2.59	78.8 ± 2.59
Rice cereal	72.5 ± 0.27	73.5 ± 0.27
Wheat cereal	22.6 ± 0.86	53.5 ± 0.86
Wheat bran cereal	49.3 ± 0.50	67.0 ± 1.10
Cracker	71.4 ± 0.59	74.6 ± 0.37
Bread	57.7 ± 1.01	64.9 ± 0.89
Green bean	16.6 ± 0.30	46.7 ± 0.30
Wheat bran	19.6 ± 1.03	62.8 ± 0.14

N=4

$$\text{Available starch} = ((\text{glucose (g/100g)} \times 0.9) + (\text{maltose (g/100g)} \times 0.95) \times \text{Desugaring factor}$$

(To reflect the weight change of sample after desugaring pretreatment)

$$\text{Desugaring factor} = \frac{\text{original sample wt (g)}}{\text{sample wt after desugaring}}$$

For Complex Carbohydrates

$$\text{Complex Carbohydrates (CC, g/100g)} = \text{Available Starch (g/100g)} + \text{Dietary Fiber (g/100g)}$$

ANALYTICAL RESULTS

The HPLC method described in this paper is a quantitative method for available starch in foods. A variety of samples, such as grains, ready-to-eat breakfast cereals, breads, crackers, fruits, vegetables, and mixed diets can be analyzed by using this method. The elution orders of sugars in this HPLC method, using an aminobonded silica column packing and acetonitrile-water mobile phase, follow the expected saccharides polarity: fructose, glucose, sucrose, and maltose. The enzymatic digestate of the desugared sample showed mostly glucose peaks and negligible peaks from maltose and sucrose. The sample chromatograms demonstrated that the starch digestion steps using three enzymes, heat stable alpha-amylase, protease, and amyloglucosidase, were near completion, producing mostly glucose. The glucose values in the enzyme hydrolysate was multiplied by the factor 0.9 to convert into starch values.

The HPLC approach offers the most accurate and specific determinations of mono- and di-saccharides. Thus, the HPLC technique can give accurate figures for available starch, which is derived from glucose and maltose values of the starch hydrolysates. However, the method requires a high capital investment and trained personnel for troubleshooting. The presence of salt, especially in the hydrolysate of processed foods, interferes with the analysis because chloride ions elute closely after glucose. It is important to wash the column for 2 hours at 1.5 mL/min with a solution of 0.1% tetraethylenepentamine (TEPA) (pH approximately 7 with acetic acid) in 78% acetonitrile in water and finally with normal mobile phase (7). The starch digestion in our method was done by heat stable alpha-amylase and amyloglu-

cosidase, which are more specific to glucose-glucose bonds. Thus, most of the sucrose in the sample (99%) was recovered as sucrose in the enzyme hydrolysate.

In Table 1 are the reported available starch and complex carbohydrates data generated by the HPLC method. The HPLC method generated the values in excellent precision for all the samples tested. Based on the results, we suggest that this HPLC method for analysis of available starches be collaboratively validated by different laboratories.

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9

APPLICATION OF COMPLEX CARBOHYDRATES IN THE FOOD INDUSTRY

The Consumer Perspective

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The consumer perspective is, after all, the most important reason for researching the importance of complex carbohydrates and fiber in the diet. Our job, be it industry or government, is to help the consumer to eat more foods rich in these nutrients because we so fervently believe they will improve the quality of life.

You know the pros and cons of labeling complex carbohydrates, the difficulty of defining them and analyzing them. A bigger challenging is to communicate the importance of complex carbohydrates to the consumer. First, I think we all agree that the consumer is totally confused with our "nutrition-speak." They do not fully comprehend complex carbohydrates, let alone resistant starch, pectins, soluble and insoluble fiber.

But we also know that consumers want to do the right thing. Nutrition is the second most important factor when buying foods according to Food Marketing Institute's (FMI) 1995 Consumer Trends[2] study, ranking even above price[1] (figure 1). In FMI's 1994 Trends survey, 64 percent said they are "very concerned" about the nutritional content of their foods. We also know from consumer research that consumers want to be healthy, but there are too many barriers in their minds, with confusion topping the list.

In the midst of the confusion, consumers are getting the message about fat. In fact, it may be the only message they are getting. In the 1989–91 USDA Diet and Health Knowledge Survey[3] just released, 54 percent thought they should lower the fat content of their diets (figure 2). In FMI's 1995 Shopping for Health[1] survey, 70 percent of the "very health conscious" shoppers said their diets were very low in fat intake, while only 39 percent of average shoppers thought their diets were very low in fat intake (figure 3).

Consumers are also starting to get the message about fiber. In USDA's Diet and Health Knowledge[3] survey, 39 percent of consumers thought they needed more fiber (figure 4). Actually, very few Americans get enough fiber. As you know, we are averaging about 12 grams per day and should be eating 20 to 35 grams. In the same FMI Shopping for Health[4] study, only 9 percent of the "very health conscious" shoppers said they had a high intake of fiber; 5 percent

Dietary Fiber in Health and Disease, edited by Kritchevsky and Bonfield
Plenum Press, New York, 1997

Source: FMI, 1995 Consumer Trends

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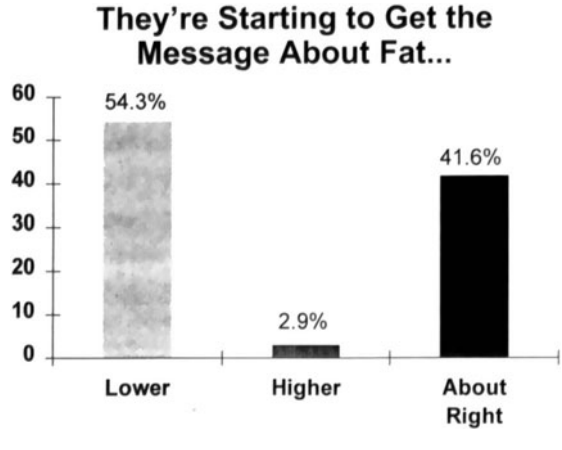
Figure 1

of the average shoppers said they had a high intake (figure 5). Unfortunately, at one time, Americans knew they should be eating more fiber, but just one article in a professional journal which said oat bran didn't perform as first thought, changed consumers' perceptions about fiber. In 1995, however, the media once again started to emphasize the importance of fiber in the diet. This fiasco was a perfect example of how premature, flawed studies contribute to consumer confusion.

Fortunately, consumers are more often looking to the food label for answers to "nutrition-speak." In 1992, only 52 percent "almost always" looked at a food label when they were buying a food for the first time, but by 1995, 61 percent looked at the label[1] (figure 6). Read-



Source: USDA, 1989-91 Diet and Health Knowledge Survey

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Figure 2

Amount of Fat in Shoppers' Diets

Fat Intake	1995 %	Very Health Conscious %
Very low	39	70
Moderate	15	5
Very high	11	3

Source: FMI/Prevention, Shopping for Health 1995

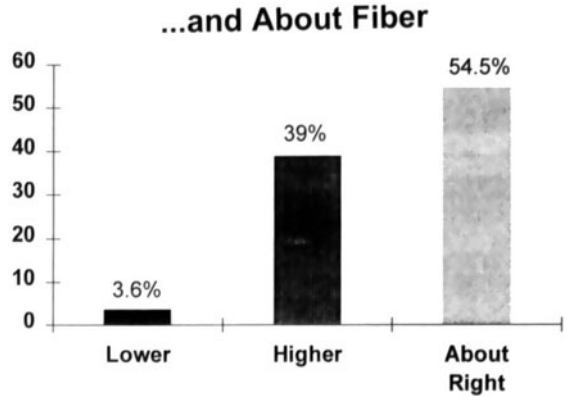
Wheat Foods Council

Figure 3

ing labels does lead to action. Twenty-two percent of shoppers in FMI's[1] survey started buying a new food product specifically because of something on the label (figure 7). The FMI study also shows that shoppers who are very health conscious are more aware of the food label. That awareness appears to help them make healthful diet changes.

While we know Americans are obsessed with fat, we also are aware of their lack of concern about complex carbohydrates. USDA's 1995 Healthy Eating Index[3] reported that less than 10 percent of Americans scored a perfect "10" on the amount of grains they ate (figure 8). Even fruits and vegetables scored higher than grains. In fact, the average American scored a 6.1 out of 10 on the amount of grains in their diet. When I went to school 61 percent was a low D, almost an F.

There are numerous other studies that confirm USDA's research. An actual intake study by MRCA[5] in 1994 showed Americans were averaging 5.1 servings of the grain group daily, less than the minimum recommended. In a 1995 survey[6] the Wheat Foods Council conducted, 91 percent of Americans said they only averaged three servings a day.



Source: USDA, 1989-91 Diet and Health Knowledge Survey

Wheat Foods Council

Figure 4

Amount of Fiber in Shoppers' Diets

Fiber Intake	1995 %	Very Health Conscious %
High fiber	5	9
Moderate	60	75
Low fiber	35	16

Source: FMI/Prevention, Shopping for Health 1995

Wheat Foods Council

Figure 5

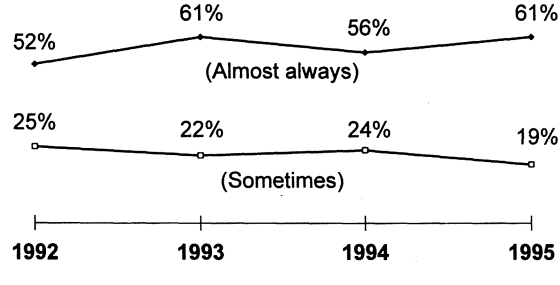
In that same study, we also discovered that most Americans don't realize they are not consuming enough grains. They recognize that Americans need more complex carbohydrates, but think that they personally eat enough. Seventy-four percent say they are eating the right amount and ten percent think they are eating too much.

Fruits and vegetables continue to enjoy a reputation for being healthful — a message boosted by the industry's "5 A Day" campaign. When asked which food group should people be eating the most of, both vegetables and fruits were named more than grains — even though the recommended servings of the grain group are more than fruits and vegetables combined. These studies show that neither grains, complex carbohydrates or fiber are top-of-mind to the consumer. When asked by FMI[12] in 1994 what they are doing to improve their diets, only seven percent of consumers mentioned eating more fiber and starches (figure 9).

Part of the problem is that consumers don't know what "grains" are, let alone complex carbohydrates and starch. In 1995 USDA conducted focus groups[7] in order to make the Dietary Guidelines more consumer-friendly. One man thought grains were wood

And They're Looking to the Food Label for Answers

How often do you check the nutrition label when buying a food for the first time?



Source: FMI/Prevention 1995

Wheat Foods Council

Figure 6

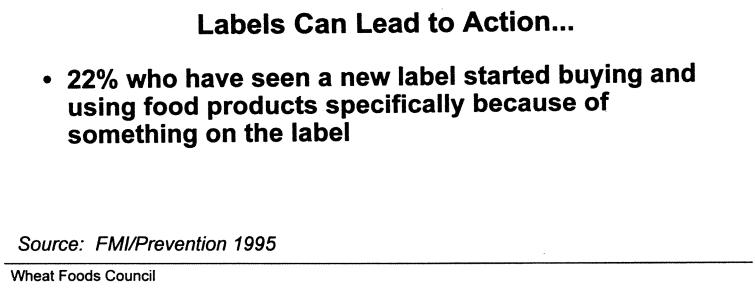


Figure 7

chips. An over-weight woman thought grains were foods that taste bad, but are good for you. However, African-American women associated grains with "healthy," as did the women in four focus groups[8] conducted by the Wheat Foods Council.

To emphasize how confused consumers are, a 1991 Gallup survey[9] conducted by the Wheat Foods Council reported that forty-nine percent of American adults did not know white bread was made from wheat and 49 percent thought oatmeal was made from wheat.

The good news is, in the past two years, the number of American adults who think bread is fattening has dropped 10 percentage points from 50 to 40 percent[6]. The bad news is, four out of every 10 Americans still think bread is fattening.

Articles such as the one in a February 1995 New York Times which informed Americans that "Yes, Pasta Makes You Fat," do not help our mission to inform the public about the importance of increased complex carbohydrates. Finally, after a year of reputable nutritionists working to debunk the story, we are again making progress with science-based information.

With this as a backdrop, what can industry do to help Americans understand the importance of complex carbohydrates and fiber in the diet and encourage them to eat more

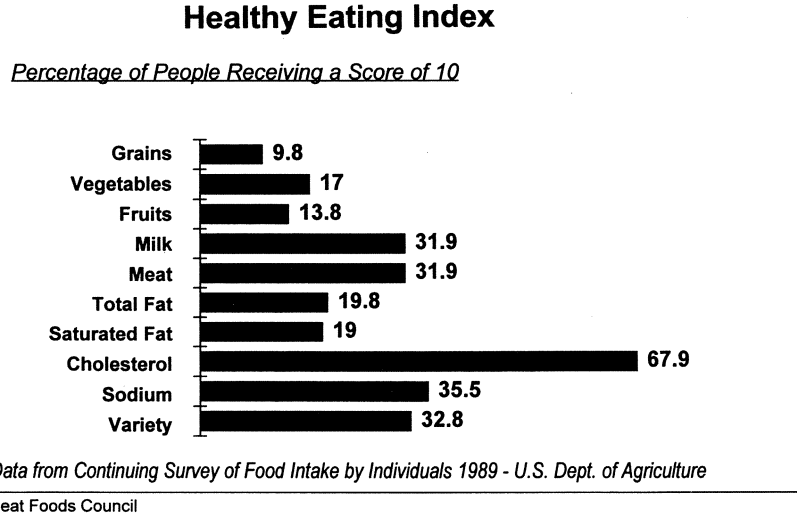


Figure 8

Dietary Changes to Insure Health % Making Change

	1990	1992	1994	1996
More fruits/vegetables	57	60	63	77
Less fats/oils	27	28	32	42
More fiber	16	8	7	7
More starch, rice potato, pasta	—	5	7	6
More whole grain	—	2	1	4

Source: FMI, 1996 Consumer Trends

Wheat Foods Council

Figure 9

foods rich in these nutrients? All other food groups have major retail campaigns to promote their products to the consumer: Pork. The Other White Meat; Beef. It's What's for Dinner; Got Milk?; 5 A Day for Better Health. There's an obvious grain foods gap. By filling this gap, we would be doing the consumer a valuable service. The grain group is the top source of complex carbohydrates and fiber in the diet. Grains are generally low in fat, convenient, inexpensive and found in a wide variety of products.

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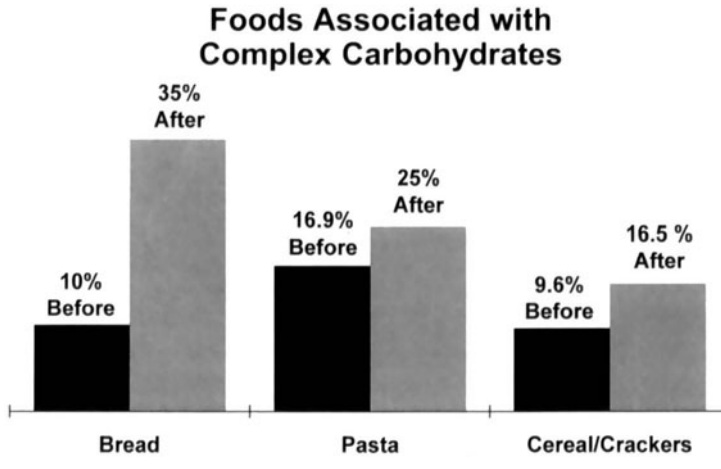
Figure 10

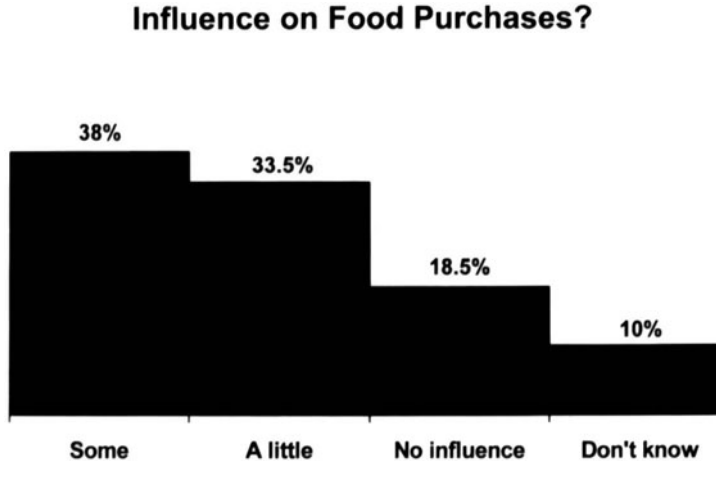


Source: Shopper Intercept Tests, April 1995

Wheat Foods Council

Figure 11

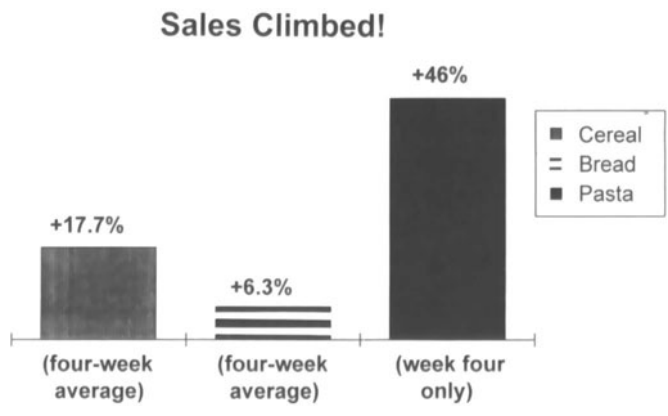
In April of 1995, the Wheat Foods Council developed a retail pilot project[10] with three objectives: introduce a grains logo; educate the consumer about the nutritional benefits of grains; and measure sales. Food sampling, coupons, bag stuffers, a "grain shopping list;" a dietitian in the store; and a "scavenger hunt" grains contest were utilized throughout the month. The logo was developed through focus group research and store intercepts to help consumers link the positive perceptions of grains with specific foods (figure 10).



Source: Shopper Intercept Tests, April 1995

Wheat Foods Council

Figure 12



Wheat Foods Council

Figure 13

Familiarity with the term "complex carbohydrates" more than doubled from 25 percent to 56 percent. Consumer understanding of what foods are complex carbohydrates also increased substantially. Recognition of bread went from 10 to 35 percent; pasta from 17 to 25 percent; and cereal and crackers from 10 to 16.5 percent (figure 11).

Over 70 percent of the shoppers said it would have at least a little influence on their shopping behavior (figure 12). And it obviously did. Bread increased in sales 6.3 percent; cereal almost 18 percent and pasta was flat until the fourth week and then it rose 46 percent (figure 13). The sudden rise in pasta purchases may be related to "stocking up" on pasta, but not knowing just emphasizes the need to conduct more and longer projects to see if the sales increases can be maintained after the promotion is over. Our take-home message is education at the point-of-sale can make a difference.

Where does the industry go from here? The industry must be consistent in their messages; avoid sending conflicting signals and must be positive. Americans are tired of the "diet police." Most Americans like grains — let's let them know it's OK to enjoy more. And industry should focus on "how." How do people get more complex carbohydrates in their diet? What are some painless, small steps they can take?

An excellent starting point we can all use for credibility is the newly revised Dietary Guidelines that put "grains" in front of fruits and vegetables. This is the government and highly reputable nutritionists telling people to eat more grains — not the industry.

What else can industry do? Define complex carbohydrates so they can be put on the food label, if so desired. Be practical in that definition and what you expect the consumer to understand — or care about. Label foods, if and when approval is given. Give "how to" information in your ads and promotional materials. And finally, differentiate brands responsibly.

There are some positive happenings at this time:

A Dietary Guideline Alliance has been formed to develop consumer friendly messages for the entire nutrition community to employ. This alliance includes representatives of all five food groups, the U.S. Department of Agriculture, Food and Drug Administra-

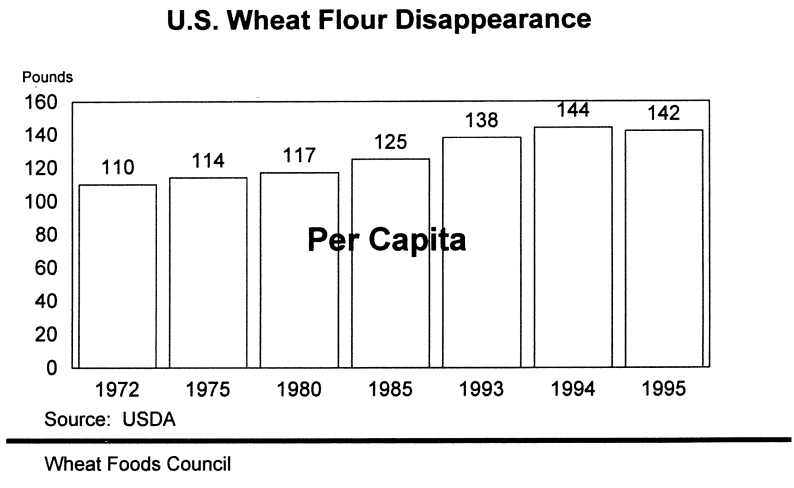


Figure 14

tion, American Dietetics Association, International Food Information Council, FMI and many others.

The proposed revision for the "Healthy" definition, if approved, will allow high complex carbohydrate foods, such as white bread, to carry the "healthy" claim on the packages.

The oat bran proposal — the first health claim which talks about a food rather than a nutrient — if approved, should help the consumer identify high fiber oat and oat bran foods.

The Wheat Foods Council is researching the possibility of putting a retail logo on packages to help the consumer identify grains. There would be a minimal charge to use the logo and the only criteria would be that the formula contain at least 50 percent of grains by weight.

In the meantime, one of the best things this industry can continue to do is promote the Food Guide Pyramid. When people know about the Pyramid, they are more likely to consume more grains. The Pyramid is consumer-friendly and it gives concrete, measurable goals in laymen terms.

We are making progress in increasing consumption of the grain group. We have steadily been increasing wheat flour consumption since the low of 110 pounds in the early 70's to 143 pounds per person in 1995[11] (figure 14). More consumer education about the importance of fiber and complex carbohydrates in the diet will help consumption to continue to grow.

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10

SOLUBLE FIBER AND HYPERTENSION

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1. INTRODUCTION

Hypertension is the most prevalent cardiovascular disorder in the United States. As many as 50 million people in this country have had elevated blood pressure diagnosed.[1–3] Elevated blood pressure has been linked to an increased incidence of stroke and coronary heart disease.[4,5] Treatment of elevated blood pressure decreases the associated mortality and morbidity, and control of hypertension has contributed substantially to a 57% decline in mortality from stroke and 50% in that from coronary heart disease from 1972 to 1990.[1] A reduction in average population diastolic blood pressure of 5 mmHg would be expected to reduce the incidence of stroke by about one-third, resulting in the prevention of 100,000 strokes in the U.S. each year.[6]

In the battle against hypertension, recent epidemiological and intervention studies have renewed interest in the role of nutrition in essential hypertension, especially for mild to moderate elevations. In particular, individual dietary modifications including a high fiber intake have been shown to reduce blood pressure. Although the effects appear modest in some cases, such nutritional interventions generally do not result in significant side effects and they are relatively inexpensive.

2. DEVELOPMENT OF THE DIETARY FIBER AND BLOOD PRESSURE HYPOTHESIS

A relationship between dietary fiber intake and blood pressure was first proposed 21 years ago as a possible explanation for the observation of lower mean blood pressures among vegetarians when compared with omnivores from the same population group.[7] Mean blood pressure of lacto-ovo vegetarians, a group whose intake of plant-based foods

Dietary Fiber in Health and Disease, edited by Kritchevsky and Bonfield
Plenum Press, New York, 1997

is somewhat lower than that of vegans but higher than that of omnivores, lies between the mean blood pressures of vegans and omnivores.[8] The vegetarian diet is higher in total dietary fiber than the omnivorous diet, but also differs in levels of intakes of a number of other nutrients also associated with blood pressure, including composition of fatty acids, potassium, and magnesium[9] Consequently, the research which followed after the reports of these findings were published was focused primarily on fat and mineral influences on blood pressure.

Nevertheless, two separate but parallel tracks of research reignited interest in the possibility of a protective effect of dietary fiber on blood pressure again in the mid to late 1980's. From one of these tracks of research has emerged convincing evidence of a strong relationship between insulin resistance and blood pressure in both men and women which is independent of age, body weight, and glucose intolerance[10,11] Although a causal relationship between insulin resistance and hypertension has not been proven to date, this possibility exists and has certainly not been excluded.[12]

The second track of research involved a number of studies examining the importance of dietary fiber in regulating plasma glucose and insulin levels.[13,14] These studies demonstrated an attenuation of postprandial insulin and glucose responses to carbohydrate when ingested with dietary fiber. These studies also suggested that soluble fiber was more effective than insoluble fiber in achieving this attenuation because of its viscosity.[15,16] The viscous mass formed in the digestive tract with soluble fiber is believed to be responsible for slowing down digestive and absorptive processes, thus modulating the rate of postprandial glucose release into the blood.

3. DIETARY FIBER AND BLOOD PRESSURE: A REVIEW OF PUBLISHED STUDIES

3.1. Observational Studies

An inverse relationship between fiber intake and blood pressure has been reported by researchers based on large scale observational studies. When cross sectional data from the Caerphilly Heart Study in South Wales, a survey of 2,512 men aged 45–49 years, were examined by Lichtenstein and co-workers,[17] daily cereal fiber intake (g/day) obtained from a food frequency questionnaire was found to be associated with both systolic pressure (SP) ($r=-0.053$, $p<0.01$) and diastolic pressure (DP) ($r=-0.057$, $p<0.01$). Moving from the lowest quintile of daily cereal fiber intake (≤ 4 g/d) to the highest (≥ 10.5 g/d), mean SP decreased 3.3 mmHg and mean DP decreased 2.1 mmHg. After adjustment for age, body mass index, prevalent ischemic heart disease, and heart rate, the effect attributable to fiber was less but still significant, -0.186 mmHg SP (95% CI=-0.363, -0.009) and -0.111 mmHg DP (95% CI=-0.228, 0.005) for each gram of cereal fiber eaten daily in employed men. Elliott et al,[18] reported that analysis of seven-day weighed dietary records completed by 387 men in the same Caerphilly study showed a tendency for low intakes of cereal fiber in men with higher blood pressure, although the differences were small.

When fiber intake was estimated by seven-day diet record in 845 community-dwelling men of the Baltimore Longitudinal Study of Aging, Hallfrisch and co-workers,[19] reported that the quintile of men consuming an average of 6 g fiber/day had DP approximately 3 mmHg lower than those consuming 2 g/day ($p<0.05$). There was also a negative association between fiber intake and SP ($p<0.005$), and the difference between highest and lowest quintiles of fiber intake was about 5 mmHg.

In a large prospective study, the Health Professionals Follow-Up Study, Ascherio et al,[20] found an independent inverse association between dietary fiber and the risk of developing hypertension among 30,681 US normotensive males, aged 40–75 years. For those with a fiber intake of <12 g/day the relative risk of developing hypertension was 1.57 (95% confidence interval, 1.20–2.05) compared with an intake of >24 g/day. These associations remained after adjustment for age, body mass index, alcohol consumption, waist-to-hip ratio, physical activity and smoking. The inverse associations between nutrient intake and hypertension were stronger in men younger than 50 years old. Also, blood pressure levels were inversely associated with the intake of both fruit and fruit fiber for SP and DP, but this association was not evident when fiber from other sources was studied independently,[20,21] In a parallel prospective study involving 58,000 nurses the relative risk of developing hypertension was reduced by 24% in women consuming more than 24 g of fiber a day compared with women consuming less than 10 g/day.[22]

Another cross-sectional study examining the relationship between dietary fiber and blood pressure looked at consumption of fiber from both oats and buckwheat in 857 Chinese men and women.[23] Although buckwheat is not as concentrated a source of soluble fiber as oats, the average consumption of this grain among 19% of these people was considerable (>200 g/day), which made a substantial contribution to their total intake of soluble fiber. In multivariate regression analysis controlling for other possible confounders, oats intake was found to be significantly inversely associated with both SP and DP in this cohort despite very low mean blood pressures. More specifically, this association was attributed to intake of soluble fiber and was not related to total dietary fiber intake.

3.2. Clinical Trials

The relationship between habitual dietary fiber intake and blood pressure was first explored in 1979 by Wright et al,[24] This study found that when 94 healthy adult men and women were classified according to their usual intake of total fiber, mean blood pressure of those in the group with the highest fiber intake (24.5 g/d) was significantly lower than those in the group with the lowest fiber intake (16.1 g/d). In addition, when the group with the usual high intake of fiber consumed an experimental diet containing the level of fiber of the group with the lowest intake, mean blood pressure increased over a four week period, Likewise, mean blood pressure significantly decreased in the low fiber group when they consumed the fiber intake of the high fiber group.

From the late seventies to the present, many studies have employed dietary fiber as an intervention with blood pressure as a primary endpoint a representative group of 15 is listed in the bibliography.[24,25] These studies have been conducted in normotensives and hypertensives, in the obese and non-obese, in men and women, in younger and older adults, in blacks and whites, and in diabetics as well as non-diabetics with durations lasting from one month to one year or longer. All but three of these studies were clinical intervention studies designed to test the blood pressure lowering effect of dietary fiber intake from natural fiber sources such as fruits and vegetables. Three of the intervention studies used double-blinded, placebo-controlled designs testing food-derived fiber supplements.[26,27]

Of these studies listed, blood pressure was significantly reduced in 11.[24,25,27–33] Significant reductions were observed for both SP and DP in eight of these 11 studies.[24–26,28,30–32] In the other three studies, a significant reduction was noted only for DP.[27,29,33] One of the double-blinded, placebo-controlled trials demonstrated significant reductions in both SP and DP,[26] and the other two in DP only.[27,33] One of these placebo-controlled trials included nor-

motensives who were moderately obese.[33] No significant effect on either SP or DP was observed in four studies; the authors of one suggested the possibility of confounding by drug treatment,[34] and the authors of another suggested that the emphasis placed on increasing intake of apricots as the source of increased fiber intake may have resulted in the inadvertent failure to include the "critical blood-pressure-lowering component of fiber" in their experimental diet.[32]

The reductions in blood pressure observed in response to increasing fiber intake were independent of age, degree of obesity, heart rate, and baseline blood pressure,[35] and were also not associated with weight change.[26,29,33] Among diabetics the reduction in blood pressure was associated with improvement in glucose control, and was also independent of weight.[30] In the double-blinded, placebo-controlled study of 62 moderately obese but normotensive women conducted by Rossner et al,[33] a statistically significant weight loss was observed in both the fiber-treated group and the placebo group. However, the change in DP from baseline was significant at both 4 and 10 weeks only in the group consuming the fiber supplement. At 10 weeks, the difference in DP between the fiber supplemented group and the control was borderline statistically significant ($p=0.07$).

Of the six intervention studies which included normotensives,[24,32,33,35–37] three showed significant reductions in blood pressure.[24,33,35] All of the other studies involved hypertensives,[25–31] but only one did not report significant blood pressure reductions in response to increased fiber intake.[34] Most of the hypertensives in these studies were receiving antihypertensive medications to control their blood pressures. In one study,[25] 145 hypertensives were taken off their medications for 4 weeks prior to the experimental period. Significant reductions in both SP and DP were reported after 4 weeks only among those receiving the experimental high fiber diet compared with controls, without significant differences in weight changes between the two groups.

A decrease in the number of subjects needing medication to control blood pressure or a decrease in the dosage of medication required to control blood pressure in those still needing medication were outcomes of several of the studies involving treated hypertensives.[31,38] This is a particularly important finding because antihypertensive medications do not appear to affect the attendant insulin resistance of hypertension and some of these drugs may actually worsen it.[39] Because insulin resistance is a well-established risk factor for coronary artery disease (CAD),[40,41] the absence of a treatment effect on this condition is believed to explain why control of blood pressure by pharmacologic means has not resulted in a decrease in risk of CAD among hypertensives.[42,43] In addition, the number of complaints associated with hypertension was reduced to almost none after one year on a vegan diet suggesting that the quality of life for these hypertensives was also considerably improved by increasing dietary fiber intake.[38]

The level of total fiber intake consumed by subjects in the intervention studies ranged from 30 g to 50 g. The three placebo-controlled trials added 6.5–7 g of fiber in a supplement to the usual diets.[26,27] Only one of these studies reported total fiber intake at 8.4 g/1000 kcal,[33] and one determined that the fiber supplement would contribute about 50% of the total fiber in the typical Scandinavian diet.[27]

Many of the studies, particularly those involving hypertensives, also included changes in intakes of fat, potassium or sodium or a combination of these factors. However, the majority of these studies concluded that fiber was involved in the blood pressure response. One study which did not show a blood pressure lowering effect did not rule out the possibility that fiber may play a role in lowering blood pressure, but suggested that this effect may not have been demonstrated because subjects did not selectively increase their soluble fiber intake on the high fiber diet.[34] In addition, Rouse et al,[35] demonstrated

that the significant blood pressure reductions observed in healthy adults consuming a vegetarian diet were not related to changes in urinary sodium and potassium excretion or the urinary sodium/potassium ratio.

Two clinical trials used a soluble fiber source as their predominant treatment. Singh et al,[25] withdrew 70 hypertensive patients from pharmaceutical treatment and used a dietary intervention with daily 12.2 grams of fiber from cereal and 47.8 grams of fiber from fruit (mainly from guava, a fruit rich in soluble fiber) for 4 weeks. Systolic blood pressure dropped 12.0 mmHg from 162.5 ± 8.5 mmHg and DP decreased 10.2 mmHg from 104.5 ± 6.5. Fasting blood sugar was lowered from 110 mg/mL to 100 mg/mL while lipid profile was improved. All above changes were statistically significant when compared with those of 72 matched patients in a control group.[25] Krotkiewski reported a study on the effect of gel-forming fiber, guar gum, on the blood pressure.[44] Forty-two hypertensive participants received daily 21 grams of guar gum for 2 weeks after diet stabilization. Those whose weight decreased were dropped from the study. Systolic blood pressure decreased by 9.8% and DP decreased by 9% after the treatment and did not revert to pre-treatment levels until 3 weeks after the washout. It was concluded that fiber supplementation can lower blood pressure in hypertensive patients without co-existence of weight changes.

4. THE RELATIONSHIP BETWEEN BLOOD PRESSURE AND INSULIN RESISTANCE

A relationship between abnormalities in carbohydrate metabolism and hypertension was first reported in 1996 by Welborn et al.[10] Since this time, a number of other studies have documented high insulin levels in patients with essential hypertension in the presence of normal or only moderately elevated glucose levels, indicating that these patients were insulin resistant.[39,45–48] Also during this period a number of observations were made regarding the close association between the insulin resistant states of obesity and noninsulin-dependent diabetes mellitus and hypertension.[12,49–52] Increased risk of CAD is shared by hypertensives, non insulin-dependent diabetics and the obese, and insulin resistance is an established risk factor for CAD.[40,53] The clustering of these conditions along with elevated triglyceride levels has been give the label of "the deadly quartet" or "syndrome X".[54,55] It has been estimated that 85% of diabetics are hypertensive and obese, 80% of obese subjects have abnormal glucose tolerance and are hypertensive, and 67% of hypertensives are both diabetic and obese.[56] Moreover, the type of obesity most strongly related to development of hypertension is the central body or android pattern, which is also associate with insulin resistance and increased risk of noninsulin dependent diabetes mellitus.[54]

Hypertensives have a significantly higher plasma insulin response to an oral glucose load than do normotensives.[39,45,47–49] This observation has been made in both lean,[39,47] and obese individuals.[49] This abnormality is secondary to the ability of insulin to stimulate glucose uptake and is the hallmark of insulin resistance.[52] Hypertensives treated with antihypertensive medications have higher glucose and insulin responses after an oral challenge than do untreated hypertensives, suggesting that these medications may lower blood pressure without correcting insulin abnormalities,[57] and that some conventional treatments may even aggravate them.[58,59] To identify the insulin resistance in these studies, the euglycemic insulin-clamp method was performed to overcome the difficulties associated with accurately measuring insulin responsiveness through superimposing steady-state conditions with exogenously infused insulin and glucose.[46] Consequently, the findings from

these studies have helped to establish a convincing body of evidence that hypertension is an insulin-resistant state.

Blood pressure has also been significantly and positively associated with insulin response in both normotensive and hypertensive men after a glucose load and after ingesting a meal.[45] Other studies have also found positive associations between SP and DP and insulin response following an oral glucose challenge in both lean and obese hypertensives,[47,49] but not among lean normotensives.[47,50] Fasting insulin levels have been found to be an independent predictor of development of hypertension and blood lipid abnormalities in a cohort of young adult blacks and whites in the Coronary Artery Risk Development in Young Adults (CARDIA) longitudinal observational study.[60] These associations between blood pressure and blood lipids, insulin and glucose have been observed in hypertensives and normotensives despite the absence of significant differences in mean levels of blood lipids,[47] or postprandial blood glucose and glucose tolerance, between these groups.[45]

Several plausible mechanisms have been proposed to explain the association between insulin resistance and blood pressure and evidence in accumulating to suggest that any one or a combination of any of these mechanisms may be involved. These include the enhancement of sympathetic nervous system activity by insulin, which in turn increases insulin levels further and results in enhanced peripheral resistance[54]; increased renal reabsorption of sodium either directly by insulin or through insulin-mediated increases in the renin-angiotensin-aldosterone system which shift the pressure/natriuresis curve to a higher level[54,61–63]; mitogenic effects of insulin on vascular smooth muscle cells resulting in capillary hypertrophy[64]; and increased activity of specific membrane cation transport systems which increase extracellular sodium and intracellular calcium concentrations impacting plasm volume and peripheral resistance.[12,51,65]

5. DIETARY FIBER AND INSULIN/INSULIN SENSITIVITY

Over the same time period that the relationship between hypertension and insulin resistance has been under study, a parallel area of research has investigated the relationship between dietary fiber intake and carbohydrate metabolism.

A diet rich in beta-glucan (e.g., oat bran) has been reported of benefit in the regulation of postprandial blood glucose and insulin levels in non-insulin dependent diabetic and non-diabetic individuals. Wood and co-authors[66] studied the effects of oat bran (containing 14.5% beta-glucan) and oat gum (containing 80% beta-glucan) on postprandial glycemia in nine healthy subjects. They found that both treatments reduced postprandial peak blood glucose and insulin levels. The similar effects were also demonstrated by Braaten et al,[67] among non-insulin dependent diabetic and non-diabetic subjects. In this study, oat bran and oat gum reduced postprandial plasma glucose excursions and insulin levels when compared with the control wheat farina meal in both groups. Various investigators have reported improved glucose disposal[68,69] increased insulin receptor binding on fat cells[70] and improved diabetic control[71] with increased dietary fiber intake.

The mechanism of action by which dietary fiber, especially soluble fiber, effects glucose metabolism is somewhat speculative but it clearly has been shown to slow carbohydrate absorption from the gut.[66] This results in a flattened post-prandial glycemic curve and consequently, a lower insulin response is demanded. Lower insulin demand and a flatter glycemic curve may over time up-regulate insulin receptors on target tissues and increase insulin sensitivity. How this occurs is not clear, but it has been postulated that the fermentation products, short chained fatty acids (e.g., propionate) that are produced in the

colon by the action of bacteria on partially digested complex carbohydrates, may directly effect glucose and insulin metabolism. Venter[72] demonstrated improved glucose tolerance, insulin sensitivity and lipid metabolism (high-density lipoprotein) with a dietary supplement of sodium propionate (7.5 g daily × 7 weeks) in 10 healthy female volunteers. The fact that it took approximately 3 weeks to wash out the beneficial blood pressure effect of a guar gum intervention on a group of hypertensive patients suggests that it was more than simply the immediate effect of delayed absorption of carbohydrate from the gut.[44] More research is needed to elucidate these metabolic effects and mechanisms of action.

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11

SOLUBLE FIBER AND ENERGY REGULATION

Current Knowledge and Future Directions

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1. INTRODUCTION

Americans are becoming fatter. Recent longitudinal data from the National Health and Nutrition Examination Survey[1] shows that the median body mass index of adult men and women increased from 25.3 to 26.3 kg/m^2 between the periods 1960–1962 and 1988–1991, corresponding to a body weight gain of 8 lb. While it is clear that the immediate underlying cause of this increase can be traced to an energy intake greater than energy expenditure, the underlying causes of the imbalance remain unclear. Has metabolizable energy intake increased, or has energy expenditure fallen, or has a combination of these two events occurred? If metabolizable energy intake has increased, why has this happened? In this brief review we focus on the potential role of soluble fiber in influencing energy intake and hence body weight regulation, and on some experimental considerations for future investigational studies in this area.

2. SHORT-TERM EFFECTS OF SOLUBLE FIBER ON DIGESTION AND METABOLISM OF ENERGY

Investigations of the physiological effects of dietary fiber, particularly soluble fiber, in human digestion and metabolism have suggested multiple mechanisms which theoretically could influence short term body energy regulation. The mechanisms can be subdivided into two classes; a) those that effect a decrease in energy intake, and b) those that promote energy excretion. The mechanisms most likely to be effective in influencing body energy regulalation are briefly described below.

2.1. Effect of Soluble Fiber Intake on Energy Intake

Consumption of soluble fiber and soluble fiber-containing foods result in gel formation in the stomach, which may increase gastric distention and in addition reduces the rate

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of gastric emptying. This gastric distention due to gel formation, while difficult to characterize experimentally, has been proposed as the mechanism for observed increases in perceived fullness and decreases in hunger following consumption of soluble fiber[2–5]. In addition, delayed access of macronutrients to the digestive processes and to absorptive surfaces of the small intestine may decrease subsequent hunger by blunting glycemic, insulinemic, and resultant counteregulatory responses following feeding.

After passage from the stomach, digestion and absorption of macronutrients are delayed or modestly diminished in the small intestine by the presence of soluble fiber. Prolongation of carbohydrate absorption has been found to favorably influence insulin dynamics following a single fiber-containing meal and after longer-term interventions[5–7]. In addition to benefiting disorders characterized by hypersinsulinemia, such as obesity, Type II diabetes and some types of hypertension, alterations in insulin peak concentration and total secretion in response to a meal (ie. area under the curve) may also enhance satiety and prolong the latency until the next meal.

These effects of soluble fiber on digestion and absorption translate into increased luminal concentrations of carbohydrate and fat in the distal small intestine. Subsequent absorption of the fatty acids by the ileum may result in enhanced post-absorptive satiety, the "second meal effect" noted after consumption of soluble fiber[8].

2.2. Effects of Soluble Fiber on Energy Excretion

In the colon, bacteria ferment a significant proportion of soluble fiber to short chain fatty acids (SCFA). These SCFA are utilized by colonocytes for fuel, absorbed for hepatic or systemic utilization, or are excreted. Despite fermentation of some soluble fiber to metabolizable forms of energy, fecal energy loss persists, sometimes exceeding the energy value of the fiber itself[9]. Indeed, an increase of 100 to 200 kcal/day of fecal energy excretion noted in some previous investigations can explain much of the observed effects on body energy described below[10].

The combination of gastric, intestinal, and excretory mechanisms suggest that fiber intake could have salutory effects on body weight regulation. However, longer-term epidemiologic and metabolic trials are required to confirm that these or other physiological phenoma are operative over time or in free-living persons

3. LONGER-TERM EFFECTS OF SOLUBLE FIBER ON ENERGY REGULATION: INFORMATION FROM EPIDEMIOLOGIC AND METABOLIC STUDIES

In cross-sectional studies, total fiber intake and soluble fiber intake have been negatively associated with both energy intake and body fat content. Data from Miller et al. 1994[11] are given in Figure 1 showing that obese men and women report very similar energy intakes to normal-weight individuals but consume significantly less total fiber. These data are consistent with the view that low total dietary fiber and low soluble dietary fiber intakes may be important factors contributing to the maintenance of obesity, and perhaps its development also.

However, a more complex picture is seen in the data from clinical studies that have examined the effects of total and soluble fiber on weight regulation. In particular, a number of clinical studies have been conducted to examine whether a diet high in soluble fiber content will promote weight loss when consumed ad libitum, as summarized in Table 1[2,3,12–22]. It is

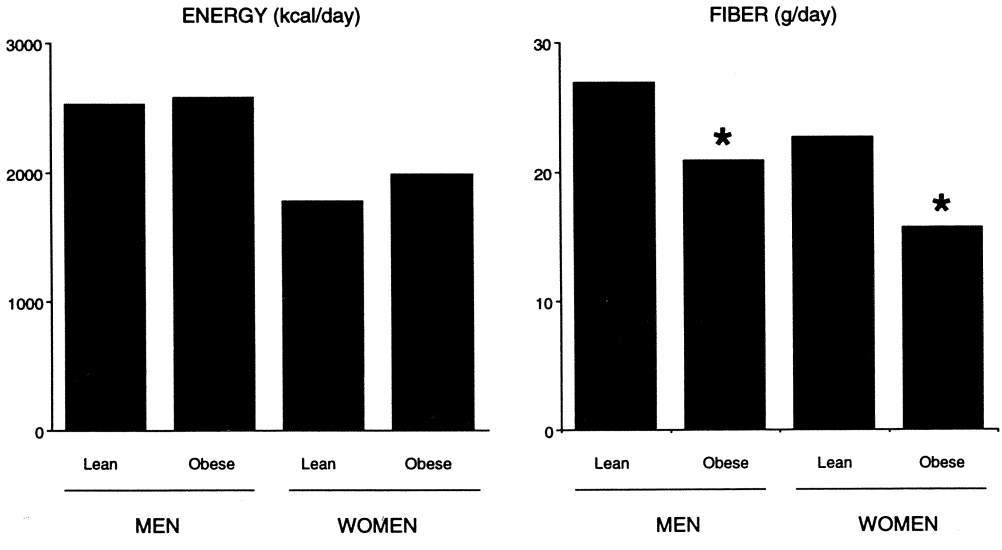

Figure 1. Redrawn from Miller et al. 1994,[11] showing that obese men and women report similar total energy intakes but lower fiber intakes compared to non-obese men and women. Values are means. Significantly different from lean subjects: *P<0.05.

clear that substantial controversy over this issue exists since the number of investigations reporting that soluble fiber induces weight loss are comparable to the number reporting no effect of soluble fiber on body weight.

In part, this controversy may be due to factors such as study duration, amount of fiber, number of subjects and food type, all of which may affect the ability of a particular study to detect an effect. In addition, however, total dietary fiber and soluble fiber in the diet tend to parallel energy density, palatability, sodium content and fat content. Thus, a diet low in total and soluble fiber will tend to be very energy dense, very palatable, and containing high levels of sodium and fat. Conversely, a diet high in total and soluble fiber will tend to have a low energy density, low sodium content and low total fat and may be less palatable than the low fiber diet. All of these non-fiber constituents and attributes have the potential to themselves influence energy intake in the short-term and the long-term. Therefore, to understand the

Table 1. Is there significant or appreciable weight loss in clinical studies of soluble fiber? (Bonfield 1995)[2,3,12-22,33]

Yes	No
Yudkin 1959	Duncan 1960
Evans 1975	Jenkins1979
Jenkins 1979	Hylander 1983
Valle-Jones 1980	Stevens 1987
Tuomilehto 1980	Dodson 1981
Walsh 1984	Krotkieswki 1985
Krotkieswki 1984	
Krotkieswki 1985	

Methods for Assessment of Dietary Compliance

1. Assessment based on using general principals of energy balance

2. Assessment based on direct measurements of energy balance

3. Use of novel methods to assess compliance

Figure 2. A summary of the three methods available for monitoring dietary compliance in metabolic studies.

effects of fiber alone, information is needed from metabolic studies in which great care has been taken to control other aspects of the diet. Both the epidemiological and clinical studies on fiber have not been able to control for all of these factors and this may have had an important influence on the results obtained in some of the previously reported studies.

In addition, we have speculated that the continuing controversy over the effects of soluble fiber on energy regulation may exist, in part, because of the difficulty of exactly controlling all foods that subjects eat — even when they are provided in a metabolic diet. Since subjects participating in studies of several weeks duration usually have free time when they are away from their investigators, it is possible for them to consume additional "illegal" food without the knowledge of the investigators. As well, if meals are provided for consumption at home or work they may not be completely consumed and other foods may be consumed instead. In our experience, it is far more difficult for subjects to consume a diet different from their normal diet (in terms of amount and composition) than they anticipate before they start a metabolic study. This inherent difficulty provides sufficient incentive for a proportion of subjects in metabolic studies to contemplate either dropping out of the study or alternatively remaining in the study but being non-compliant with the dietary requirements. The number of subjects that remain in the study but are non-compliant will depend on exactly how difficult the dietary regimen is that they are expected to maintain.

4. MONITORING COMPLIANCE IN METABOLIC STUDIES

As summarized in Figure 2, there are three main approaches to monitoring compliance in metabolic studies. Which method is most appropriate for use will depend both on the resources available for compliance monitoring and the exact nature and purpose of the study.

4.1. Assessment of Compliance Using General Principles of Energy Balance

The first approach is to retrospectively judge whether the energy balance characteristics of the study, taken as a whole, are biologically possible. This requires knowledge of basic facts and figures on energy regulation and the first Law of Thermodynamics as it applies to energy regulation:

$$\text{Energy Intake} = \text{Energy Expenditure} + \text{Body Energy Stored} \quad (1)$$

For example, it is possible to predict what change in body energy stores can be expected by knowing the extent of undereating. If a group of subjects maintains weight on

2,500 kcal/day and then their intake over 100 days when consuming a diet high in soluble fiber is 1500 kcal/day, they are supposedly undereating by 1,000 kcal/day or 100,000 kcal per study. This 100,000 kcal/study undereating can be predicted to result in a body energy loss of approximately 66,700 kcal/study, since an estimated third of an energy deficit is typically accommodated by a decrease in energy expenditure[23]. Since the average energy content of weight loss is known to be approximately 7.2 kcal/g[24], the expected weight loss in our example group of subjects should be 66,700/7.2 g, or 9.3 kg per study. If weight loss of the group as a whole is appreciably different from this amount (for example, outside the range 4–16 kg) non compliance can be suspected.

The important advantage of this approach is that it is simple and does not require any additional measurements to be made. For this reason, it has potential utility in all metabolic studies in which exact consumption of provided foods is of concern. However, it has the significant disadvantage that the estimate of error is very crude and, thus, only substantial degrees of dietary non-compliance can be detected. Moreover, assessment of study validity is retrospective and thus the results of a whole study may be questioned after there is any possibility of fixing the problem. Finally, this method cannot be used for individual subjects, since the assumptions are too liable to error in individual cases and as well can only be used for studies in which expected weight gain or loss is relatively large. For these reasons, this method remains valuable when no other compliance check is possible, but should be recognized as not giving the best possible results.

4.2. Assessment of Compliance from Measured Energy Balance

The second approach to assessing compliance in metabolic studies is to actually measure energy balance in subjects during the investigation, as we have done in several investigations[25–27]. In this case, repeated measurements of energy parameters are made during a baseline period and throughout the consumption of the experimental diet. For example, energy intake can be determined from exact records of dietary consumption, total energy expenditure can be determined using the doubly labeled water technique[28,29] and body energy stores are determined using a body composition technique such as densitometry[30]. These variables are then related using the equation:

$$\Delta\text{Energy Stores} = \Delta\text{Energy Intake} - \Delta\text{Energy Expenditure} \quad (2)$$

When subjects are accurately consuming the provided diet, there will be a close agreement between changes in energy stores, energy intake and energy expenditure. Thus, this method has the advantage over using general principles of energy regulation to assess compliance that it can detect smaller degrees of non-compliance in groups of subjects and in addition can be used to detect moderate levels of non-compliance in individual subjects also. A disadvantage is that the measurements of energy expenditure and body composition can be time consuming and expensive.

4.3. Use of Novel Methods to Assess Compliance

Dietary non-compliance in metabolic studies can take two forms: i) failure to completely consume all of a provided diet, and ii) consumption of foods than are not provided. Both of these aspects of compliance can be reduced by confining subjects to a metabolic ward during investigations. However, this type of restriction is not practical in all metabolic protocols, particularly those which last for several weeks or longer.

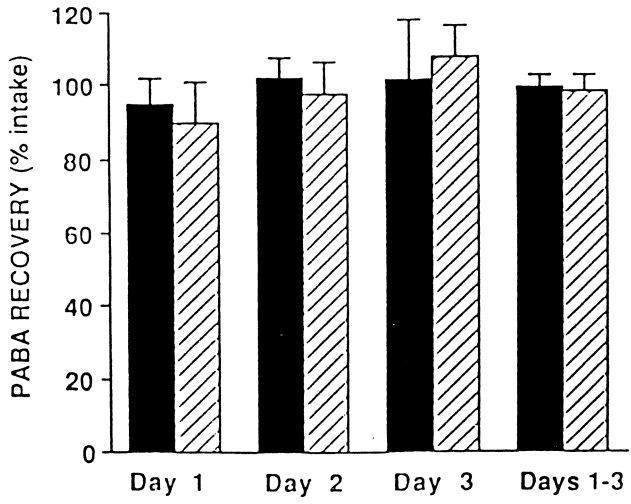

Figure 3. Recovery of PABA in urine over 3 days after consumption of four test foods per day that had each been supplemented with 72 mg PABA.[31] Group 1 (solid bars) received PABA from foods supplemented with PABA after preparation; group 2 (striped bars) received PABA from cookies supplemented with PABA prior to baking. Values are means±SD for 10 subjects. Reprinted with permission from the American Journal of Clinical Nutrition.

We recently developed two new methods for measuring both of these aspects of non-compliance. The PABA dietary compliance technique was developed to monitor non-consumption of provided foods[31]. It involves incorporation of the potassium salt of *p*-aminobenzoic acid (PABA) into the provided diet and measurement of the recovery of PABA and its metabolites in complete 24-hour urine collections. In a validation study of 10 individuals, 288 mg/day PABA was incorporated into food components of a metabolic diet over 3 days and complete 24-hour urine collections were made during a baseline day (to correct for small background levels of PABA excretion) and also during the three PABA supplementation days[31]. The food items used in the study included milk, sandwiches, entrees, cream cheese, orange juice, rice, yogurt, soup, ice cream and salad. PABA concentrations in the complete urine collections were measured using a standard colorimetric assay[31]. Results of the study are shown in Figure 3. The mean recovery of PABA in the complete urine collections was 98.7±3.7 (SD)%. It can be predicted from this result that, in future metabolic studies, 95% of subjects consuming foods marked with PABA and making complete urine collections will have a recovery rate of >92.6% of a PABA supplement (i.e. the lower 5th percentile for PABA recovery) and lower values than this can be taken as indicating dietary non-compliance. This method has the advantage that it can directly detect non-compliance resulting in non-compliance of provided foods and as well is extremely sensitive for this purpose. A potential disadvantage is that urine collections must be complete for the method to be valid, and some subjects may find this hard. However, many metabolic investigations require complete urine collections for other purposes and in these cases will not be an additional burden.

Although the PABA technique is sensitive for detecting non-consumption of provided foods, it does not detect consumption of foods that are not supposed to be consumed. To assess this aspect of non-compliance, which is a particular concern in many long-term metabolic studies, we developed a technique based on measuring urine osmolal-

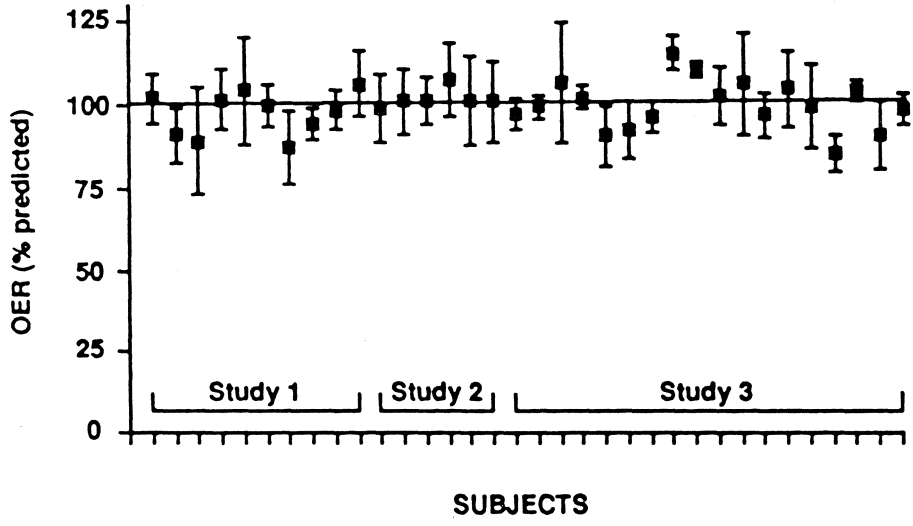

Figure 4. The measured osmolar excretion rate (OER) expressed as a percent of OER predicted from dietary constituents.[32] Values are means±SD for six daily measurements in 34 subjects. Reprinted with permission from the American Journal of Clinical Nutrition.

ity[32]. The basis of this method, which was tested in 35 subjects, is measurement of the osmolality of repeated 24-hour urine collections. The urinary osmol load (equal to the product of the 24-h urine volume and urine osmolality) consists primarily of compounds derived from consumed foods, and in particular nitrogen-containing compounds and sodium and potassium salts. Thus, the osmol load can be predicted from knowing dietary protein, sodium and potassium and taking into account obligatory non-urinary losses of these nutrients. A measured osmol load significantly greater than predicted indicates the consumption of illegal foods. In addition, a measured osmol load significantly less than predicted suggests that provided foods are not being consumed. Urine osmolality is measured quickly and simply using a standard urine osmometer, giving this technique the potential for widespread application.

Data from a 6-day study in 34 healthy adults aged 18–78 years is shown in Figure 4. It can be seen that, in 33 of 35 subjects believed to be compliant since they were under strict dietary supervision, there was no significant difference between the measured mean urine osmolality (based on 6 days of measurements) and that calculated from dietary intake. The coefficients of variation for equations relating dietary intake to the measured urinary osmolar load was 6.94 for 6-day mean measurements. Thus, in most individual subjects, measurement of urine osmolality in 6 complete 24-hour urine collections can detect non-compliance in individual subjects when it results in a change in urinary osmolar load of greater than 13.9% (i.e., >2SD). Thus this method has the potential to provide relatively sensitive direct proof of dietary non-compliance in individual subjects. The potential disadvantage of the technique is that, while it is good at detecting the illegal consumption of foods with high levels of protein, sodium or potassium, there are some foods that could be consumed that would not change urine osmolality — for example, alcohol and some types of candy. In addition, the method is not suitable for use in diabetics since any glucose in the urine would interfere with the results. Thus, investigators using the method should be aware of its potential weaknesses as well as its strengths.

5. SUMMARY

Soluble dietary fiber is a potentially important factor determining our ability to maintain a stable energy balance and avoid obesity. However, clinical metabolic investigations have given conflicting results on the effects of soluble fiber on energy regulation, perhaps for the reasons that a) other dietary components that affect energy regulation have not always be controlled adequately and b) in the past it has been hard to ensure that subjects are consistently compliant with the dietary requirements of soluble fiber protocols. The recent development of techniques for assessing dietary compliance in metabolic studies, combined with increased control of non-fiber dietary variables that influence energy intake, should help ensure more consistent findings in future investigations.

ACKNOWLEDGMENTS

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12

SOLUBLE FIBERS AND DIETARY LIPIDS

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1. INTRODUCTION

Several epidemiological observations have suggested that a high dietary fibre intake is associated to a lower incidence of coronary heart disease[1,2]. In that line, several sources of soluble dietary fibers such as oat bran, guar gum, pectins and psyllium have been shown to lower plasma and LDL cholesterol in human subjects, as recently reviewed[3,7]. Dietary fibers, and especially soluble fibers, can alter several physiological and metabolic processes which are involved in the reported cholesterol-lowering effect of dietary fibers. These include nutrient intake, stomach emptying, carbohydrate and fat digestion and absorption, fecal excretion, postprandial blood response, peripheral tissues sensitivity, fermentation in the colon and liver metabolism[7–9].

The present report is specifically dedicated to the effects of soluble dietary fibers on the handling of dietary lipids.

2. FIBERS AND DIETARY LIPID INTAKE

Ingesting a diet basically composed of fiber-rich food items is expected to reduce the energy density of the diet and especially, the amount of fat and cholesterol ingested due to the predominance of vegetable foodstuffs. Reductions in energy of about 100–200 kcal/d and in lipid intake have been reported. Moreover, an increased satiety and a reduced food intake has been reported following a fiber-rich meal[10].

3. FIBERS AND FAT DIGESTION

Slowing down the rate of gastric emptying and thus absorption is a key mechanism involved in the effect of viscous gums on the postprandial blood glucose rise. This

Dietary Fiber in Health and Disease, edited by Kritchevsky and Bonfield
Plenum Press, New York, 1997

remains to be fully documented in the case of dietary lipids. For instance, during a recent study in humans (Dubois, Thouvenot and Lairon, unpublished observations), we observed that 40 g oat bran significantly reduced the rate of gastric emptying of carbohydrate (pasta) whereas that of dietary fat (olive oil) was increased.

It is now recognized that digestion of dietary fat begins within the stomach where dietary fat is coarsely emulsified and hydrolyzed up to about 10–15%[11]. After dietary fat and cholesterol have been expelled into the duodenum, no marked further emulsification occurs but colipase-pancreatic lipase very efficiently hydrolyzes triglyceride molecules[12]. Subsequently, phospholipids and cholesterol esters are cleaved under the action of pancreatic phospholipase A2 and carboxyl ester hydrolase, respectively. As a result, lipolytic products (mainly monoglycerides and free fatty acids) and free cholesterol are efficiently absorbed by the small intestine mucosa where they are quickly used as substrates for de novo triglyceride, phospholipid and cholesterol ester synthesis. After the intracellular packaging of lipid moities and apoproteins, they are secreted in the form of chylomicrons into the lymph and subsequently into the blood circulation.

3.1. Pancreatic Lipase Activity

That several sources of soluble dietary fibers such as pectins, oat bran, psyllium or sugar-beet fiber reduce the hydrolytic activity of pancreatic lipase *in vitro* has already been documented[13–15]. Part of this loss in enzyme activity could result from some binding of the enzyme to fibers[13,16,17]. But, unfortunately, very limited data are available regarding inhibitory effects *in vivo* in humans. In healthy subjects, adding 15 g pectin to a meal was reported to reduce the pancreatic lipase output by 49%[18] whereas 5–15 g pectin did not display any marked effect in another study[19]. In pancreatectomized patients receiving enzyme supplement, 5 g pectin reduced the lipase activity by 63%[20].

3.2. Lipid Emulsification *in Vitro*

It appears that the mechanisms whereby soluble fibers might alter the lipolysis process are only poorly understood. We aimed at investigating some physico-chemical aspects involved. Emulsification is a key step in fat digestion because gastric and pancreatic lipases act at the fat globule/water interface. Moreover, we recently showed that the size of the fat globule per se is a parameter governing the activity of gastric and pancreatic lipases [21,22]. Thus, we hypothesized that dietary fiber could interfere with the normal process of dietary fat emulsification. With that aim we designed *in vitro* studies to determine the effects, and some mechanisms behind, of soluble fibers on fat emulsification and subsequent lipolysis in gastric and duodenal conditions[23,24]. *In vitro* experimental conditions were developped in order to mimick as closely as possible the physiological conditions; low shear forces applied during a sufficiently long period of time were used (shaking at 200 strokes/min for 2 hours at 37°C). Five sources of dietary fibers were used including charged gum arabic (not viscous), two differently charged pectins (low viscosity) and three uncharged guar gums (with low, medium and high viscosity, respectively).

In the gastric conditions at pH 5.4, the amount of emulsified lipid was not affected whereas the size of the emulsified droplets (measured by using a particle-size analyser) was increased by raising the concentration of viscous fibers only. As shown in Figure 1A, at a given low fiber concentration (0.3%), only viscous fibers significantly increased droplet size and reduced droplet surface area. On the whole, the droplet size was positively correlated and the overall droplet surface area was negatively correlated with the medium

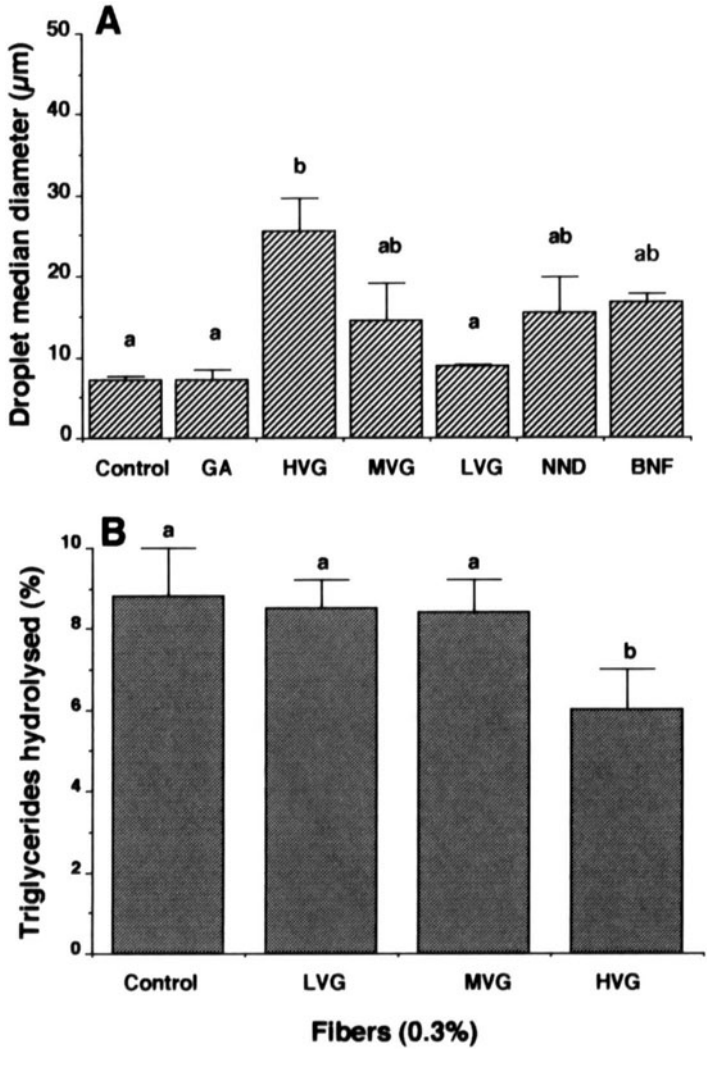

Figure 1. Effects of soluble fibers (0.3% w/v) on the median diameter (μm) of emulsified droplets generated in reconstituted gastric medium at pH 4.5. G.A.: Gum Arabic; HVG: High Viscosity Guar gum; MVG: Medium Viscosity Guar gum; LVG: Low Viscosity Guar gum. NAND: 42.0% methylated pectin; BNF: 73.6% methylated pectin. Values are mean ± SEM of three determinations. Different superscript letters indicate significant differences (ANOVA; $P < 0.05$).

viscosity in the range 0–20 mPa.s. When emulsions prepared in the presence of fibres (i.e. with different droplet sizes) were incubated with excess gastric lipase for 30 min (Figure 1B), the high-viscosity guar gum significantly reduced by 32% the extent of triglyceride lipolysis as compared with control and low- or medium viscosity fibers.

In the duodenal conditions, ie at pH 7.5 and in the presence of bile, the amount of emulsified lipids was reduced and the size of emulsified droplets was increased by raising the concentration of viscous fibers only. As shown in Figure 2A and 2B, the extent of emulsification ($r = -0.79$), the droplet size ($r = 0.88$) and consequently, the overall droplet surface area ($r = -0.59$) were strongly correlated to the medium viscosity in the range 0–4

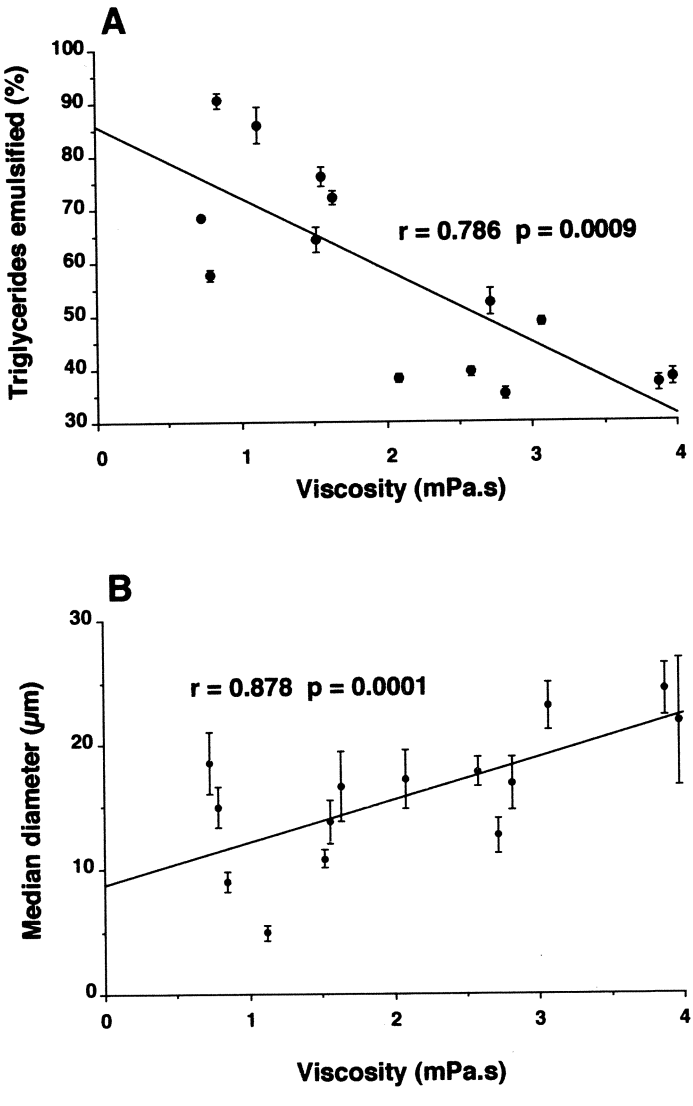

Figure 2. Regression analyses between (A) the amount of triglyceride emulsified (% of lipid present) or (B) the droplet median diameter (μm) and the apparent viscosities in the range 0–4 mPa.s of the reconstituted duodenal medium elicited by the control and fiber solutions used.

mPa.s (as well as in the range 0–20 mPa.s). When incubating emulsions prepared in the presence of fibers (ie with different droplet sizes) with excess enzyme for 2 hours, the high- and medium-viscosity guar gums and one type of pectin significantly reduced the extent of triglyceride lipolysis only (Figure 3A). The extent of triglyceride lipolysis catalyzed by colipase-dependant pancreatic lipase was negatively correlated ($r = -0.97$) to the viscosity of the reconstituted duodenal medium (Figure 3B).

Reducing emulsification of dietary lipids, and subsequently lipolysis, in the stomach and the duodenum is therefore a mechanism by which soluble highly viscous fibers can alter lipid assimilation.

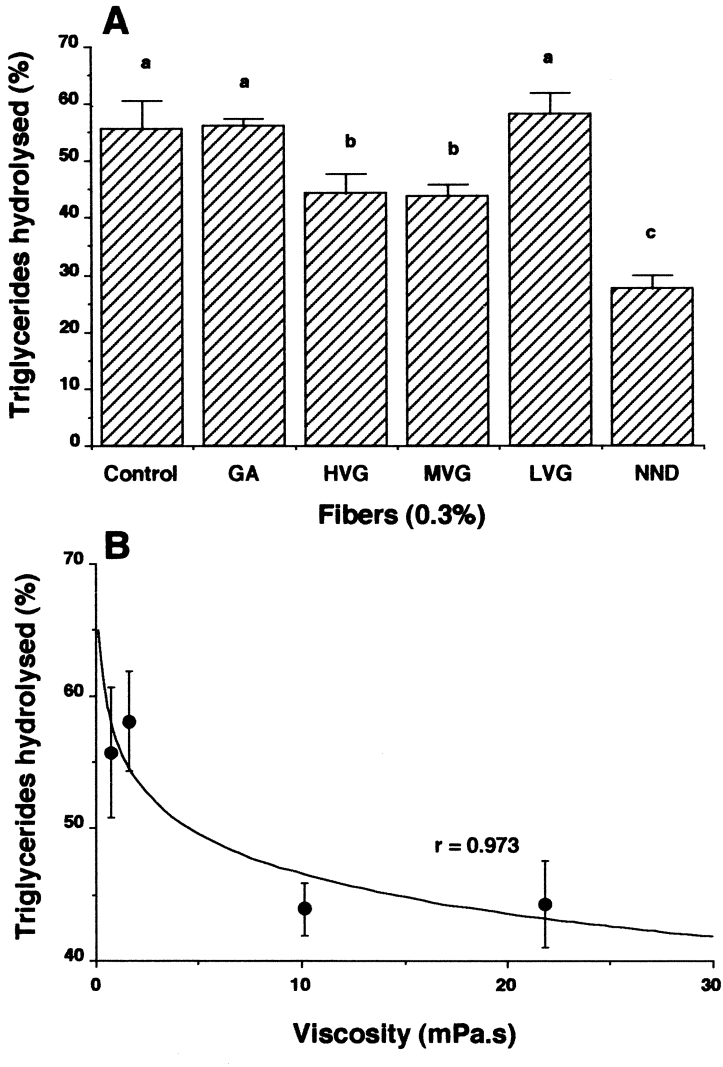

Figure 3. Effects of soluble fibers (0.3% w/v) on (A) the extent of triglyceride lipolysis (%) after 2 hrs incubation with excess pancreatic colipase-lipase in reconstituted duodenal medium and (B) regression analyses between the extent of triglyceride hydrolysis (%) and the apparent viscosities in the range 0–25 mPa.s elicited by the control and fiber solutions used.

3.3. Lipid Emulsification and Lipolysis *in Vivo*

In order to evaluate the existence of such a mechanism *in vivo*, we subsequently designed an experiment in adult rats. Two groups of 6 fasted rats were intragastrically intubated a coarsely emulsified lipid mixture containing radiolabelled triolein with 0.3% high-viscosity guar gum added or not in the aqueous phase and were sacrificed after 30 min digestion. As shown in Figure 4A, the median diameter of droplets in the two emulsions intubated were nearly identical while after 30 min digestion in the stomach, the droplet

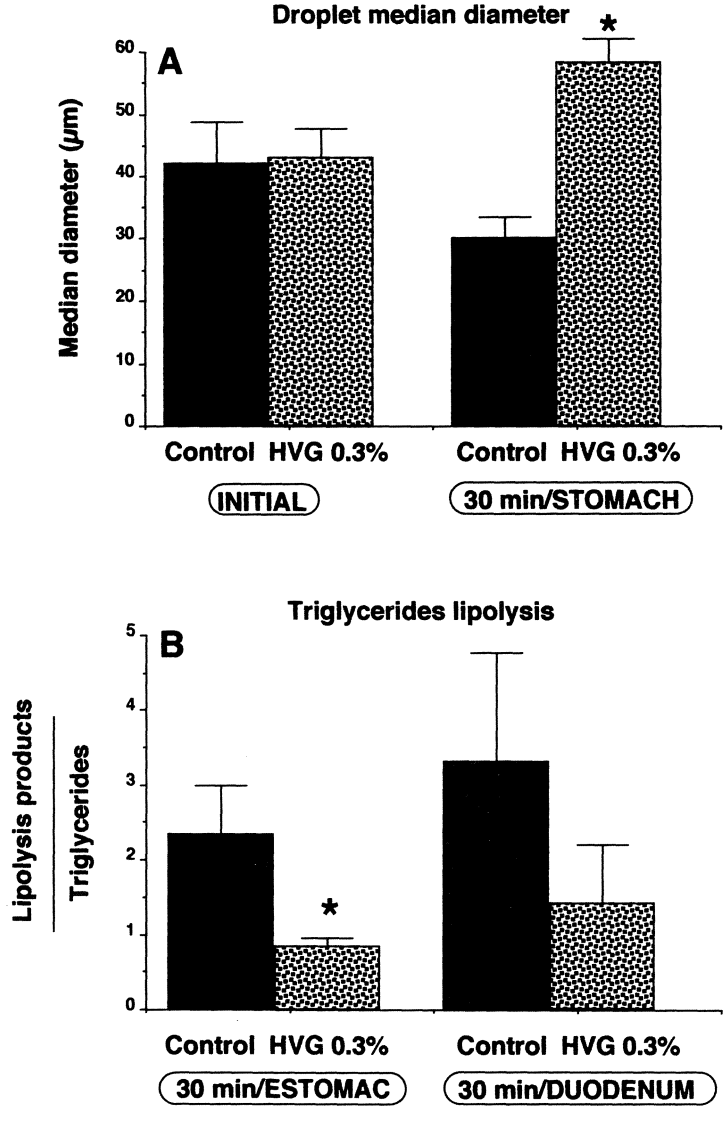

Figure 4. Effects of adding 0.3% high-viscosity guar gum (HVGuar) or not (Control) to an emulsified lipid mixture before and after 30 min digestion in rats. A: median droplet diameter (μm) of emulsions; B: extent of triglyceride lipolysis (lipolytic product/triglyceride ratio) in the stomach and duodenum content after 30 min digestion. Values are mean ± SEM of 6 animals. Asterisks indicate significant differences (ANOVA; $P < 0.05$) between two groups.

median diameter of the emulsion was almost two times higher in the presence of 0.3% high-viscosity guar gum. Consequently, the specific surface area displayed by the emulsion in the gastric content in the presence of guar gum was about half that of the control. At the same time, as shown in Figure 4B and 4C, the extent of triglyceride lipolysis, as determined by the lipolytic products (monoglycerides + free fatty acids) / triglycerides ratio, was 2.8 fold lower in the stomach content and 2.2 fold lower in the duodenum content, in the case of the emul-

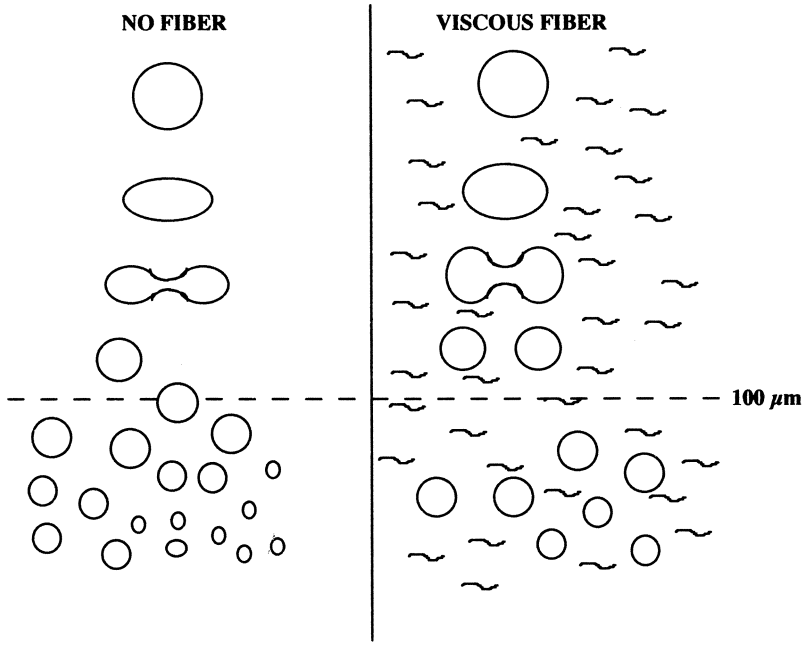

Figure 5. Schematic representation of the effect of viscous soluble fibers on lipid emulsification in the digestive tract. Given a sufficiently increased viscosity of stomach and small intestine contents is reached, the energy efficiency resulting from gut mechanical activity is reduced thus limiting the disruption of large droplet into small ones. As a result, the extent of emulsification is lowered (arbitrary limit set at 100 μm) and a reduced number of droplets with a larger size are generated.

sion with 0.3% high-viscosity guar gum as compared to the control. Thus, these data obtained in the rat show that those dietary fiber that noticeably rise the medium viscosity in the stomach as well as in the duodenum content reduce the extent of fat emulsification and further lipolysis. As illustrated in Figure 5, these observations agree with the concept that increasing the viscosity of the intestinal content to a sufficient extent alters organ motility and potentially decreases intraluminal mixing by increasing resistance to the convective effects of gut contractions 25. Such an effect induced by viscous fibers is expected to slow the rate of lipid absorption by the small intestine and to increase the fecal excretion of fat and cholesterol.

3.4. Lipid Absorption

In addition to the effects reported above on lipid emulsification, other mechanisms can contribute to alterations in lipid absorption. The building of vesicles and mixed micelles in the aqueous phase of the small intestine content can be counteracted by the partial binding or entrapment of lipolytic products as well as cholesterol, phospholipids or bile salts in the presence of viscous fibers like oat bran, guar gum and glucomanan but not wheat bran or cellulose[13,26,27]. Finally, the limiting step in the process of lipid absorption per se is the transport across the unstirred water layer associated to the enterocyte brush-border membrane. Increasing the thickness of the unstrirred water-layer[28] and reducing the rates of diffusion and absorption of cholesterol and free fatty acids have been observed during acute experiments in the presence of highly-viscous fibres[29] but not slightly viscous soluble fibres

(hydrolyzed guar gum or chitosan) or insoluble fibres such as cellulose or some hemi-celluloses[30]. As a result of these alterations induced by viscous soluble fibers, the recovery of oleic acid and cholesterol in the lymph was significantly reduced following infusion of a test-meal in the rat[31,32].

To summarize, viscous soluble fibers can reduce and delay emulsification and triglyceride hydrolysis, alter the building of vesicles and mixed micelles in the intestinal content, alter mucosal morphology, slow the rate of intestinal absorption and reduce and/or delay the lymphatic secretion of lipids from dietary origin.

4. LIPID ILEAL EXCRETION

A direct consequence of the processes described above should likely be an increased excretion of lipid moities at the end of the small intestine. Such exaggerated ileal excretions of lipids have been repeatedly shown in ileostomized human subjects after supplementation of meals with citrus pectin, alginate, oat bran or beta-glucans and barley fiber[33–36]. Earlier studies also reported increased fecal lipid excretions after fiber enrichment of the diet.

5. POSTPRANDIAL LIPEMIA AND LIPOPROTEINS

In the blood circulation, the posprandial state directly reflects the process of digestion-assimilation of lipid nutrients to which we are exposed several times a day and most of the time. Because some previous studies performed in human subjects showed that supplementing test meals with sources of soluble fibers such as guar gum[37,38] or oat bran[39] reduced the postprandial changes in plasma or chylomicron triglycerides and cholesterol, we aimed to directly evaluate the relationship between absorption of dietary lipids from the small intestine and the postprandial response. This was recently studied in 6 ileostomized human subjects who ingested on separate days a standardized test meal providing about 40 g fat and 130 mg cholesterol, and either 2.9 g fiber as wheat bran or 12g fiber as oat bran (A. Lia, D. Lairon and H. Andersson, submitted for publication). After the oat bran meal, and as compared to the low fiber wheat meal, the ileal excretion of triglycerides plus diglycerides increased by 610% and that of monoglycerides plus free fatty acids increased by 400%. These data therefore demonstrate that viscous soluble fibers reduce triglyceride lipolysis as well as absorption of lipolytic products in humans. Concomitantly, the 7 h-postprandial rise in chylomicron triglycerides was lowered by about 40% after the oat bran meal, as were chylomicron phospholipids and cholesterol. As previously shown, blunting the postprandial chylomicron rise would reduce the extent of variation in lipoprotein lipid composition postprandially[40,41].These data therefore show the relation existing between a slowed and/or reduced rate of fat digestion and assimilation and postprandial lipemia as altered by a source of viscous soluble fiber. It is noteworthy that reductions in postprandial triglyceridemia have already been observed in the pig after supplementing meals with high- or medium-viscosity guar gums, but not with a low-viscosity gum (C. Leclere, D. Lairon and M. Champ, submitted for publication).

6. CONCLUSION

In conclusion, soluble dietary fibers act in the upper digestive tract mainly through their effects on the physicochemical properties of the stomach and small intestine media, and essentially viscosity. Almost all steps involved in lipid ingestion, digestion and

assimilation can be altered by soluble fibers given they exert sufficient changes in the functional properties of the digestive tract. Modifications in handling of dietary lipids and cholesterol, as well as bile salts, are thus regarded as important mechanisms of action of soluble dietary fibers leading to their documented cholesterol-lowering effect[7,35,42].

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13

SITES AND MECHANISMS FOR THE HYPOCHOLESTEROLEMIC ACTIONS OF SOLUBLE DIETARY FIBER SOURCES

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1. INTRODUCTION

Earlier research summarized in previous Vahouny Symposia (1–4) is the foundation for the generally accepted concept that soluble, but not insoluble, dietary fibers modulate upper gastrointestinal physiology. This concept was lucidly illustrated by Jenkins et al (5) at an earlier conference. In his diagram, more digesta is in the stomach and is distributed over a larger portion of the small intestine when more fiber is consumed. Implicit in this diagram is that not only is digestion slower but so is absorption, which results in a more physiological load of nutrients entering the liver.

Studies of physiological responses and possible mechanisms of action have used two types of soluble dietary fiber: concentrates of soluble fiber such as gums, pectins and mucilages, and food sources of soluble dietary fiber, e.g. oats, oat bran, barley. Although fiber concentrates provide useful data about the effects of soluble fiber, they are not usually major fiber sources in the food supply. In contrast, studies using food sources of fiber are confounded by the presence of non-fiber components, such as protein and tocotrienols. Not all of the modulation of upper gut physiology can be attributed solely to the soluble fiber fraction (Table 1). Nonetheless, soluble dietary fibers appear to be more effective modulators than insoluble fibers of the processes involved in nutrient assimilation, possibly because they are more concentrated forms of the mediators.

This chapter focuses on one physiological response attributed to soluble fiber and summarizes studies that elaborate on mechanisms by which soluble fiber sources may lower blood cholesterol levels. Hypocholesterolemic action of soluble fibers was selected, because it is one of the few physiological responses for which sufficient data have been generated to develop a likely scenario for a mechanism of action. Fiber and non-fiber constituents of the fiber sources are considered in summarizing the fates of ingested cholesterol, bile acids in the gut lumen, and the extra-lumenal activities that affect sterol homeostasis. Data from a

Dietary Fiber in Health and Disease, edited by Kritchevsky and Bonfield
Plenum Press, New York, 1997

Table 1. Dietary fiber modulates upper gastrointestinal physiology

• Adds lumenal viscosity
• Adds insoluble residue
• Is part of the plant matrix
• Contributes nonfiber components
• Participates in chelation
• May be rapidly fermented

variety of different experiments that manipulated specific variables have helped to distinguish roles for viscosity, fermentation and its primary products — short chain fatty acids (SCFA), lipid soluble components (tocotrienols) and protein in the blood cholesterol-lowering response observed when soluble fiber sources are ingested.

2. CHOLESTEROL-LOWERING MEDIATORS IN SOLUBLE FIBER SOURCES

2.1. Viscosity

Three types of experimental approaches have been used to distinguish a role for viscosity versus one for SCFA in lowering blood cholesterol levels: use of viscous but nonfermentable materials, incorporation into the diet of a fermentable product that has been modified to reduce viscosity, and use of germ-free animals to eliminate SCFA production. Gallaher et al (6) demonstrated that the viscosity of the diet or of small bowel digesta was inversely proportional to blood cholesterol levels in hamsters fed diets for 3 wk containing (g/kg): 1.2 cholesterol, 100 palm oil, and 40 dietary fiber, as cellulose or one of 3-hydroxypropyl methylcelluloses differing in viscosity. Although Gallaher et al (6) did not assess colonic fermentation in this experiment, they cite other data (7) which suggest that this cellulose ether is not fermented. More recently, the same animal model was used to show that lumenal viscosity also correlated inversely with cholesterol absorption (8).

In contrast, methylcelluloses of different viscosities had no effect on blood cholesterol levels, although absorption and blood levels of glucose and triglyceride were altered when rats were fed the more viscous diet (9). Cholesterol synthesis from tritiated water also did not change with the viscosity of the diet. The failure to observe an effect of the viscosity on blood cholesterol levels is not unexpected, given the short duration of this experiment (10 d) and the frequently-encountered resistance of rat blood cholesterol levels to dietary manipulation unless they are first artificially elevated (10). Concentrations of SCFA were low in both the cecum and portal vein (9), compared to literature data (11), suggesting that these cellulose esters were not fermented.

Retaining fermentability, but reducing the viscosity by partial hydrolysis or removal of the viscous polysaccharide from the plant matrix, is another way to study potential effects on blood cholesterol levels of viscosity vs fermentation products. Partially hydrolyzed guar gum does not lower blood cholesterol levels as effectively as its unhydrolyzed more viscous counterpart (12). Reducing viscosity by incorporating β-glucanase into an oat bran bread prevented the increased ileal bile acid excretion (one method of depleting blood cholesterol) that occurred when untreated oat bran was consumed by human ileostomates (13) (Table 2).

Table 2. Bile acid (BA) excretion in ileal effluent and feces in humans consuming oat bran (OB)[a]

	%Δ from control		Total BA (mg/d)	
	CDCA	CA	Control	Oat bran
Ileal losses				
Zhang et al, ref 59				
↓ BA output (5)[b]	142[c]	130[c]	422	993[c]
↑ BA output (4)	12	23	2,279	2,708
Lia et al, ref 13 (9)				
untreated OB	72[c]	47	606	851[c]
OB plus β-glucanase	3	−37	606	463
Fecal losses				
Marlett et al, ref 60 (9)	334	223	184	396[c]

[a]CDCA, chenodeoxycholic acid; CA, cholic acid.
[b]number of subjects in parentheses.
[c]test and control significantly different, $p<0.05$.

Fermentation and the primary products of this process, SCFA, also can be eliminated as a variable by using germ-free animals. Alvarez-Leita et al. (14) recently demonstrated that guar gum lowered plasma total cholesterol levels and hepatic total cholesterol concentrations in germ-free rats fed defined-ingredient diets containing (g/kg): 50 guar gum, 8 cholesterol and 50 cellulose, compared to germ-free rats fed diets containing only the cholesterol and cellulose (Table 3). Plasma LDL-cholesterol and hepatic esterified cholesterol levels also were significantly lower. Hepatic cholesterolgenesis from acetate was low in both groups of rats, probably because the exogenous cholesterol suppressed endogenous synthesis.

2.2. Fermentation and Short Chain Fatty Acids

It has been more difficult to identify an independent role for SCFA, although it is reasonable to hypothesize that the large influx of SCFA into the body, which would occur with very rapid fermentation, would affect lipid or carbohydrate metabolism. Surgical removal of the cecum or both the cecum and colon, but not just the colon, negated the cholesterol-lowering effect and increased total bile acid excretion when rats were fed a beet fiber preparation that provided about 8% total dietary fiber, compared to levels obtained when diets containing no fiber or 10% cellulose were used (15). In this study the amount in the cecum of each SCFA and of total SCFA correlated significantly with plasma cholesterol levels, increasing as the blood lipid level decreased. Although surgical

Table 3. Cholesterol (CH) reduction by guar gum in germ-free and heteroxenic rats fed cholesterol[a]

	Germ-free		Heteroxenic	
	Control	Guar	Control	Guar
Plasma CH (mmol/L)	6.72	2.40[b]	4.38	2.53[b]
Hepatic CH (μmol/g)	8.07	3.50[b]	7.31	4.69[b]

[a] adapted from Alvarez-Leite et al, ref 14.
[b] significantly different from control, $p<0.05$.

removal of the cecum and colon in rats does not eliminated the presence of microflora, the microbial population of the ileal excreta from colectomized rats is about 20% of what is counted in rat feces (16). Coprophagy does not alter the hypocholesterolemic effect of oat bran in the rat (17).

Particular emphasis has been placed on propionate as the hypocholesterolemic SCFA. Chen et al (18) demonstrated that propionate inhibited hepatic lipid biosynthesis. Since the interpretation of this work was confounded by the use of the major SCFA (acetate) as the precursor, the experiment was repeated using lower levels of propionate and 3 different precursors (19). Propionate at a concentration of 1.0 mmol inhibited cholesterol biosynthesis in isolated hepatocytes from 1-^{14}C-acetate; only at levels of 2.5 mmol did propionate significantly inhibit cholesterol synthesis from $^{3}H_2O$ and 1-^{14}C-mevalonate. Others (20), also using isolated hepatocytes, reported that physiological concentrations of neither propionate or acetate affected tritiated water incorporation into cholesterol.

In humans, intrarectal infusion of acetate (21, 22) or the ingestion of the rapidly-fermented lactulose (23) *increased* plasma cholesterol levels. However, intrarectal co-infusion of propionate with the ^{13}C acetate reduced label incorporation into plasma cholesterol, but because of the small number of subjects (5), this did not reach statistical significance (Table 4). Propionate co-infusion did significantly decrease the recovery of the label as plasma triglyceride, although the absolute amount of the label in this plasma lipid fraction was very small and only about half of the label could be recovered during the 4 hr experiment (22).

Oral propionate had no effect on plasma cholesterol levels in rats (calcium propionate) (24) or humans (sodium propionate) (25), possibly because disposition of other assimilated nutrients dominated hepatic metabolism. In the rat study, propionate slightly decreased 3-hydroxy-3-methylglutaryl-coenzyme A (HMG CoA) reductase activity (24).

Increasing propionate in the cecum by fermentation of β-cyclodextrins, but not inulin, did decrease plasma cholesterol concentrations in rats, even though weight gain in the responsive rats was significantly less (24). This reduction was accompanied by increased bile acid excretion and possibly increased cholesterol biosynthesis, as suggested by increased microsomal HMG CoA reductase activity which was observed in the β-cyclodextrin — but not the inulin-fed rats (24).

2.3. Plant Protein

When compared to animal protein, several sources of plant protein lower plasma cholesterol levels and lessen the severity of atherosclerotic lesions in animals (26, 27). Soy protein has been the most extensively studied (28) and a recent meta-analysis of data from 38 studies indicates that both blood cholesterol and triglyceride concentrations are lowered in humans when soy protein was consumed (29). The mechanism by which soy protein lowers

Table 4. Increments of ^{13}C in plasma lipids 4 hr after12.4 mmol [1,2-^{13}C] sodium acetate[a]

Route of administration	Cholesterol (μmol/L)	Triglyceride (μmol/L)
Intravenous	16	15
Intrarectal	79	104[b]
Intrarectal + 6mmol propionate	47	44

[a]adapted from Wolever et al, ref 22.
[b]significantly different from other routes, $p<0.05$

Table 5. Atherosclerosis in rabbits fed proteins with different lysine:arginine ratios[a]

	Fish (1.44)[b]	Casein (1.89)	Whole milk (2.44)
Serum cholesterol (mg/dl)	283	530	462
Hepatic cholesterol (g/100g)	0.75	0.90	1.06
Atherosclerosis (arch, graded 0–4)	1.6	2.1	2.6

[a]adapted from Kritchevsky, ref 27.
[b]ratio of lysine to arginine.

blood lipids is not known (28). Extraction of soy estrogens negated the hypolipidemic effect in female primates fed soy protein, suggesting a role for phytoestrogens (30).

Kritchevsky (28) proposed that higher ratios of lysine to arginine were more hypercholesterolemic, and his study in rabbits support this hypothesis (Table 5). Atherosclerotic plaques and hepatic cholesterol levels increased as the proportion of lysine, relative to arginine, increased. Serum cholesterol levels also increased, although their relationship to the three lysine:arginine ratios was not linear. None of the three proteins used in this study are known sources of estrogenic compounds. The hypercholesterolemic effect of casein may be dependent on a high cholesterol diet in rabbits (31).

2.4. Tocotrienols

More recently, attention has been given to the hypocholesterolemic effects of the tocotrienols, which are naturally-occurring farnesylated analogues of tocopherols. Tocotrienols have been found in the lipophilic fractions of several cereal grains, including whole oats, oat bran, barley and rice bran, and in palm oil (32–34). Using HepG2 cells, Parker et al. (35) demonstrated that tocotrienols reduce both HMG CoA reductase activity and protein levels. Additional experiments indicated that tocotrienols influence the mevalonate pathway by post-transcriptional suppression of the enzyme.

In one study, Qureshi et al (36) observed that supplementation of an American Heart Association Step I diet with a toco concentrate isolated from palm oil significantly decreased several measures of plasma lipids in 20 hypercholesterolemic adults (Table 6). Ingestion of toco concentrates, grains containing them or lipid extracts of these grains have not always significantly lowered plasma cholesterol levels (33, 36–38). There are several reasons for these variable results. First, γ-tocotrienol is 30 times more effective at inhibiting cholesterol biosynthesis than the alpha-analogue (39); however, α-tocotrienol is usually the most common toco (32, 40, 41). Second, research in chickens indicates that α-*tocopherol* attenuates the cholesterol-lowering effect of the tocotrienols and the amounts of α-tocopherols in some grains is sufficiently high to produce this attenuation (42).

Table 6. Plasma lipid changes in hypercholesterolemic humans consuming an American Heart Association (AHA) step I diet with and without tocotrienols[a]

	Baseline	AHA	Toco concentrate
Cholesterol, mmol/L	7.03	6.65	5.97[b]
LDL-cholesterol, mmol/L	5.00	4.61	3.99[b]
ApoB, g/L	0.97	0.90[b]	0.84[b]

[a]adapted from Qureshi et al, ref 36; 20 subjects consumed 260 mg/d of tocos in concentrate.
[b]significantly different from data in same row, $p<0.05$.

Third, concentrations of the different tocos in test materials have not been determined in many studies, and results of several studies indicates that toco concentrations may vary greatly among different lots of the same source. There is a twofold range in total tocos and a wide range in the concentrations of the different toco isomers, including α-tocopherol, among different oat and barley genotypes (41). Different growing conditions (41), exposure to air and moisture during storage (43, 44), and processing for human consumption (45) also significantly alter the toco profiles of some grains. Finally, excess dietary cholesterol, such as what is used in many animal studies to elevate blood cholesterol levels, effectively down-regulates endogenous cholesterol biosynthesis and eliminates the site of tocotrienol action.

3. SITES AND MECHANISMS OF ACTION

There are two obvious mechanisms to explore as bases for the cholesterol-lowering effects of soluble dietary fiber sources. One is increased bile acid loss, since bile acid synthesis accounts for 40 to 50% of the daily elimination of cholesterol (46, 47) and plasma lipoprotein cholesterol is quantitatively the most important substrate for bile acid synthesis (48). The other site is endogenous cholesterol biosynthesis, as it accounts for approximately 75–80% of the total body cholesterol pool. These mechanisms highlight two sites of action, the gastrointestinal tract and the liver.

3.1. Gastrointestinal Tract

Soluble dietary fibers usually increase fecal excretion of bile acids (49–54), although the increase is not always significant (49, 51, 53–56). This less-than-expected increase in fecal bile acids may be a reflection of microbial metabolism; it has been estimated that as much as 45% of the total fecal steroids (57, 58) may be lost to unmeasured bile acid derivatives.

Two laboratories (13, 59) have reported that oat bran supplementation significantly increased ileal excretion of bile acids (Table 2). However, Zhang et al (59) observed that when ileal bile acid output was high (>1,000 mg/d) during the low fiber period, incorporation of oat bran had no effect (Table 2). Differences in the amounts of bile acids recovered in ileal effluent vs. feces should reflect microbial metabolism to unmeasured steroid derivatives. Indeed, fecal bile acid losses, of about 400 mg/d, by humans consuming oat bran during constant diet conditions were about half of what was measured in two studies of ileal bile acid loss in humans (60) (Table 2). Although comparisons across studies should be made with caution, subjects in all three of these studies consumed diets of similar energy (2,300–3,000 Kcal), fat (28–35% of Kcal) and oat bran dietary fiber (18–22 g/d) contents (13, 59, 60).

Results from our study, in which bile acid kinetics were directly assessed using stable isotopes, support the insensitivity of fecal bile acids as a measure of decreased ileal absorption of bile acids (60). Bile acid synthesis did increase as fecal losses increased in the oat bran portion of the study (Table 7). However, only about half of the measured bile acid synthesis was detected as fecal losses. Synthesis is a measure of excretion if the bile acid pool is in steady state. Steady state conditions were likely, as bile acid kinetics were measured after four weeks of the constant test diet and fecal bile acid excretion during weeks two and three were comparable to those measured during the fourth week of the study (60).

Table 7. Effect of oat bran (OB) on bile acid metabolism in nine normocholesterolemic men[a]

	Pool size (mg)	Fractional turnover rate/24 hr	Synthesis (mg/day)
Chenodeoxycholic acid			
Basal diet	785	0.25	195
Basal + OB	835	0.40[b]	303[b]
Cholic acid			
Basal diet	1,107	0.31	328
Basal + OB	875[b]	0.70[b]	526[b]
Deoxycholic acid			
Basal diet	1,067		
Basal + OB	2,168[b]		

[a]adapted from Marlett et al, ref 60. Total bile acid pool size did not change and during the basal diet was 5,746 mg, and during the basal + OB diet, 6,411 mg.
[b]significantly different from basal diet period, $p<0.05$.

Although data are limited, not all viscous fibers appear to have the same effect on ileal bile acid excretion as oat bran. Barley supplementation did not affect bile acid content of ileal effluent, but rather increased net cholesterol loss (13). When psyllium seed husk was used, bile acids in ileal effluent were increased by about 50%, whereas when whole psyllium seed was consumed, ileal effluent bile acids increased 25% (55). However, only the latter reached statistical significance, which highlights the common problem of individual variability in response.

Although decreased absorption of dietary cholesterol may occur when viscous fibers are consumed, exogenous cholesterol is quantitatively not a major source for total body cholesterol. However, deoxycholic acid (DCA), compared to chenodeoxycholic acid (CDCA), is an effective inhibitor of cholesterol absorption in healthy human beings (61). We (60) reported that when oat bran was incorporated into a constant low fiber diet, the proportion of total bile acid pool that was DCA substantially increased (79%), even though total pool size did not change (Table 7). This observation would be consistent with the hypothesis that oat bran consumption altered the bile acid pool to a compositional profile inhibitory of cholesterol absorption.

3.2. Hepatic Sterol Synthesis

Enterohepatically circulating primary bile acids and DCA (46, 62) inhibit bile acid synthesis (47) by affecting hepatic cholesterol 7α-hydroxylase activity. The reduction in the flux of bile acid returning to the liver when viscous fibers are consumed may be a stimulus for increased synthesis of both primary bile acids. However, if the proportion of the total pool that was DCA increases, as was observed during oat bran consumption (Table 7), then the synthetic stimulus would be dampened. Such a dampening would explain why cholic acid (CA) synthesis did not maintain original pool size in our study. In other studies involving dietary fiber, increases in biliary DCA have been accompanied by a decreased proportion of the total biliary bile acid pool that was CA when the diet was supplemented with pectin (63) but not psyllium (64).

Cholesterol synthesis also appears to be affected by changes in the relative composition of the bile acid pool. DCA, compared with CA and CDCA, has been shown to be a substantially more effective inhibitor of HMG CoA reductase, the rate-limiting enzyme in the cholesterol biosynthesis pathway (46). Thus, oat bran (60) and pectin (63), when consumed as part of a diet of typical composition, may alter endogenous cholesterol biosyn-

thesis by increasing the proportion of the total bile acid pool that is DCA. However, other reports (64–67) of the effects of these dietary fibers on cholesterol biosynthesis are inconsistent with this hypothesis. Cholesterol biosynthesis increased to offset fecal sterol losses when a very large dose of pectin (40 to 50 g/d) was added to a low cholesterol diet of hyperlipidemic and normolipidemic subjects (66) or when psyllium was added to a very low cholesterol diet (67). Oat bran and barley, compared to other viscous fibers, have the added hypocholesterolemic factors of tocotrienols, which as summarized above, also act by inhibiting HMG CoA reductase.

4. CONCLUSIONS

4.1. Dietary Fiber in the Upper Gastrointestinal Tract

The heterogeneity of the dietary fiber complex and the complexity of the digesta mean it is unlikely that a single unifying mechanism is responsible fiber's action on the assimilation of all diet constituents. The dietary fiber content of most foods, as consumed, is low (<5%) and the majority of it is insoluble fiber (68, 69). Hypocholesterolemic cereal grains also have protein that may contribute to the cholesterol-lowering response through an as yet uncharacterized mechanism.

It is generally accepted that soluble dietary fiber, compared to insoluble fiber, slows the rates of gastric emptying and small bowel transit. However, it is not clear that it has a demonstrable effect on movement of chyme when it is part of a complex meal. The gut responds to the presence of contents, even inert material, and thus, the amount of digesta and the presence of insoluble dietary fiber have an impact on the overall physiological response. Complex communication between the stomach and duodenum regulates gastric emptying. This communication is designed to present the digestion section with materials at a rate at which it can process chyme. The interplay between this multitude of factors and dietary fiber has not been systematically addressed and its role in the responses that we attribute to fiber cannot be underestimated. The net effect of all dietary fiber of slowing the movement of chyme out of the stomach and to a lesser extent throughout the small bowel has been nicely illustrated by Jenkins et al (5). Because of the viscosity of many soluble fibers, this distribution of digesta over a greater length of small bowel is likely to be accentuated when more soluble fiber is consumed.

This movement, in turn, will affect the rate, amount, and composition of gastrointestinal secretions and subsequent absorption. The complexity of the plant material also influences its rate of dissolution and hence, nutrient assimilation rates. Results of most studies indicate that gastrointestinal secretions are adequate, even in the presence of increased fiber (70), although some fiber sources have anti-nutritive factors that specifically inactivate some digestive enzymes. In addition, interference with digestion by high fiber intakes may be significant in situations when secretions are reduced, e.g. aging and pancreatic insufficiency accompanying cystic fibrosis and chronic pancreatitis; such individuals may be particularly vulnerable when mixed diets are supplemented with viscous soluble fiber concentrates.

Although the addition of viscous soluble fibers to chyme undoubtedly slows nutrient-enzyme interactions and micellar formation, and increases the unstirred water layer along the mucosal surface, the gut and its secretions are designed to extract nutrients from complex plant material. However, this overall slowing of nutrient assimilation means that

hormonal secretions/responses are thus tempered and a situation similar to what occurs with nibbling, as opposed to meals, may occur.

4.2 Hypocholesterolemic Actions

Soluble dietary fiber sources that lower blood cholesterol levels differ substantially in the amounts of total dietary fiber and soluble fiber they contain (Table 8). Further, the compositions of the fiber in these hypocholesterolemic materials differ, consisting of varying quantities of pectins, hemicelluloses and mucilages (71). Yet, when amazingly small amounts, e.g. 20 g of dietary fiber added to a typical mixed diet of ~500 g (dry wt), they lower blood cholesterol levels.

Two characteristics common to nearly all hypocholesterolemic fibers are viscosity and the presumed rapid fermentation in the large intestine. Data reviewed herein suggest that *viscosity and not fermentation* is responsible for the cholesterol-lowering. One major drain on blood cholesterol levels is bile acid synthesis, which increases when losses increase. In contrast to most small bowel absorption, bile acid absorption is confined to the distal ileum. Throughout the small bowel, fluid and secretions are re-absorbed as nutrients are assimilated to maintain intraluminal tonicity. Thus, in the distal ileum, the concentration of any undigested material, i.e. dietary fiber, is likely to be relatively high. I hypothesize that this concentration is sufficient to trap bile acids in the fiber matrix, effectively preventing their reabsorption. Even though the same *amount* of fiber is present in the more proximal regions of the gut lumen, sufficient fluid and other digesta components are present to "dilute" the effective viscosity of the fiber source.

This viscosity effect on the body's sterol pool is independent of fermentation and available evidence also suggests it is the more powerful of the two proposed mechanisms. When cholesterol is lowered by SCFA, it appears to occur by propionate inhibition of acetate incorporation into the sterol ring. Fermentation is a continual process throughout the large bowel. Further, SCFA absorption occurs primarily by facilitated diffusion, meaning that only when fermentation is extensive *and* very rapid would it be possible for significant boluses of acetate and propionate to reach the liver and interact. Only rarely is propionate more than 15–25% of the total SCFA in the cecum (11, 72). Thus, it is not surprising that it has been difficult to demonstrate in the whole animal a definitive role for propionate as an inhibitor of endogenous cholesterol biosynthesis. Unabsorbed dietary

Table 8. Soluble dietary fiber (SDF) sources[a]

Source	SDF (% of total)	Total fiber
Barley	31	16
Barley bran	7	29
Oats	40	12
Oat bran	38	18
Rye bran	12	23
Beans	6	24
Citrus fiber	18	58
Gum arabic	100	88
Psyllium hydrophilic mucilloid	83*	101
Soy polysaccharide	6	78

[a]Adapted from Marlett, ref 71. Soluble fraction was recovered with a aqueous solution, except for * in which tertiary butyl alcohol was the solvent.

cholesterol may be similarly trapped in ileal lumenal contents, although most cholesterol absorption probably occurs in the more proximal regions of the small intestine.

Data also are sufficient to hypothesize that at least some fibers, e.g. oat bran and pectin, alter the composition of the bile acid pool by increasing the proportion that is DCA. The increase in the proportion of DCA implies increased colonic bile acid absorption, which may occur as a compensatory response to the drain on the sterol pool created by the decreased ileal absorption. The increased proportion of the total bile acid pool that is DCA has a threefold effect on sterol balance. First, it inhibits cholesterol absorption in the gastrointestinal tract. Second, it decreases HMG CoA reductase activity, a point of control in the cholesterol biosynthetic pathway, and it decreases cholesterol 7α-hydroxylase activity.

The role for vegetable proteins in effecting lower blood cholesterol levels remains unknown, and more research is needed to elucidate any mechanism.

Demonstrating a role for tocotrienols has been muddled by three major problems. First, there has been a failure to measure the punitive active agents in the test materials used in an experiment. Second, it has been only recently demonstrated that tocopherols can inhibit tocotrienols' action. Finally, many of the experiments yielding negative results used animals fed exogenous cholesterol which shuts down endogenous synthesis, the likely site of tocotrienols' action.

Detecting a fiber-induced response in the midst of the complex environments of the liver and gastrointestinal tract highlights the ability of very small amounts of indigestible material to influence these systems. Well-designed experiments using sensitive and specific methods of detection are needed to elucidate specific mechanisms of action.

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14

BUTYRATE AND THE COLONOCYTE

Production, Absorption, Metabolism, and Therapeutic Implications

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Butyrate is one of the three principal short chain fatty acids (SCFA) in humans generated by colonic microbial fermentation of dietary substrates. SCFA are the C2–5 organic fatty acids, also called the volatile fatty acids (VFA), which include acetate, propionate and butyrate. Colonic intraluminal levels of butyrate may play a role in the pathogenesis of a variety of diseases of the large intestine. This chapter reviews the production, absorption, metabolism, and therapeutic implications of butyrate.

1. PRODUCTION, ABSORPTION AND METABOLISM

1.1. Butyrate Production

Butyrate is produced by the microbial fermentation of undigested material reaching the colon. Dietary fiber constitutes the principal substrate for the fermentation of SCFA in humans (Rombeau 1990); however, undigested starch, protein and endogenously formed substrates also contribute to SCFA production (Macfarlane 1995).

SCFA production rates by colonic fermentation are in the range of 300–400 mmol/day assuming the fermentation of 32–42g of carbohydrate per day in the large intestine (Cummings 1994). In an autopsy study (data obtained within 4 hours of death), Cummings et al demonstrated total SCFA concentrations (mmol/Kg intestinal contents) are low in the terminal ileum (13 ± 6) and high in the colon, ranging from 131 ± 1 in the cecum to 80 ± 11 in the descending colon, showing a significant decreasing trend from cecum to descending colon with an associated trend of increasing intraluminal pH from the cecum (5.6 ± .2) to

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the descending colon (6.6 ± .1). Total SCFA concentrations measured in the blood (μmol/L) were 375 ± 70 (portal), 148 ± 42 (hepatic) and 79 ± 22 (peripheral vein) (Cummings 1987). This study demonstrated a constant molar ratio of 57:22:21 acetate:propionate:butyrate in the colon with 72% of butyrate being metabolized by colonic epithelium (Cummings 1994).

1.1.1. Influence of Dietary Substrate on Fermentation Patterns. The many hundreds of species of colonic bacteria generate enzymes that interact in a complex manner to ferment substrates into SCFA. The predominant bacterial species include the bacteroides, bifidobacter, eubacteria, clostridia and many varieties of anaerobic Gram-positive cocci (Macfarlane 1995). One of the mechanisms by which dietary byproducts control colonic fermentation is by introducing certain bacterial populations into the colon. The types of bacterial populations residing in the colonic lumen may in turn influence colonic pathology such as colon cancer risk. In a multi-cultural study comparing fecal flora of polyp patients, it was found that total concentrations of Bacteroides and Bifidobacterium species were positively associated with increased risk of colon cancer while some Lactobacillus species and Eubacterium aerofaciens were associated with a lower risk of colon cancer (Moore 1995).

Orally administered butyrate is completely absorbed in the proximal intestine and therefore does not reach the colon (Young 1994). Tributyrin and butyrate esters taken orally are absorbed slowly and increase the butyrate concentration in the proximal colon of animals. These compounds are expensive, thus limiting their clinical use. A more physiologic approach to increasing the butyrate concentration in the lumen of both the proximal and distal colon is via dietary manipulations that rely on the bacterial fermentation of dietary fiber (Topping 1993). Butyrate is known to inhibit histone deacetylase leading to histone hyperacetylation *in vitro*. In a study which increased the concentration of butyrate in the distal colon in rats *in vivo* by supplementing wheat bran to the diet, a significant correlation between luminal butyrate levels and colonic epithelia cell histone hyperacetylation was found (Boffa 1992). This demonstrates that dietary manipulations can affect intraluminal levels of butyrate which in turn can affect the colonic epithelium at a molecular level.

Fermentation of certain dietary fibers consistently leads to increased proportions of butyrate production. Fermentation of soy fibers leads to higher proportions of butyrate and propionate when compared to citrus pectin, sugarbeet fiber, pea fiber, and oat fiber (Titgemeyer 1991). Gums produces more butyrate and propionate than apple pectin (Titgemeyer 1991). Oat bran enhances butyrate production in the large intestine of pigs; however, similar findings do not occur with a β-glucan enriched oat fraction (Knudsen 1993). Corn starch fermentation by human colonic fecal suspensions yields more butyrate than cabbage fiber fermentation (Weaver 1992). Slowly fermentable fiber such as wheat bran is more effective than completely fermentable fibers such as oat bran in producing high levels of butyrate throughout the proximal and distal colon in rats (McIntyre1991) and humans (Kashtan 1992). Therefore, it may be possible to manipulate fiber sources in the diet, such as to achieve desired amounts of butyrate production in the colon. Moreover, most of the studies indicate that variations in SCFA production are more likely related to the type of substrate fermented than the individual tested.

1.1.2. The Contribution of Proteins as Fermentation Substrates and Variability of Fermentation Patterns. Substrates other than dietary fiber and resistant starch contribute to formation of SCFA. Human fecal homogenates collected within thirty minutes of defecation, from fifty subjects, were used to evaluate the production of SCFA after incubation

with ispaghula, glucose, and albumin (Mortensen 1992). Isobutyrate, valerate, and isovalerate were formed in 100% of the homogenates incubated with albumin, but only in 2–4% of the homogenates incubated with ispaghula or glucose. This study, as well as others (MacFarlane 1995), showed that proteins are fermented to all types of SCFA, including the isoacids and the valeric acids. However, the isoacids were not produced from the fermentation of glucose or ispaghula, confirming that monosaccharides and polysaccharides are converted to various proportions of acetate, propionate, and butyrate, but not to the isoacids.

In the study mentioned above (Mortensen 1992), consistent patterns of fermentation were noted in fifty subjects, which included sixteen healthy persons and thirty-four patients with prior history of colonic adenomas and colonic cancer. Additionally, partial colonic resections did not significantly influence the pattern of SCFA production from fecal samples (Mortensen 1992). Therefore, the evidence suggests that the fermentation substrate is more important than individual variability in determining the nature of the fermentation by-products. However, another study in humans (three patients) suggested that fermentation is influenced not only by substrate and method of fiber preparation but also by inoculum source, suggesting that individual variability in colonic microbial activities also plays some role in the ultimate nature of the fermentation by-product (Bourquin 1992).

1.2. SCFA Absorption and Effects on Water/Electrolytes

SCFA are weak acids with pK_a's of 4.75, 4.87, and 4.81 for acetate, propionate, and butyrate, respectively. More than 90% of the SCFA produced in the hindgut of mammals exist in the anionic, dissociated form. These compounds constitute the major anions in the fluid phase of the colonic lumen (Engelhardt 1983). SCFA are rapidly absorbed by the colonic mucosa *in vivo*, and enhance colonic sodium, chloride, calcium and fluid absorption, and potassium secretion (Sellin 1993, Binder 1989, Lutz 1991). SCFA absorption in association with fluid and electrolyte fluxes are noted in many animal models as well as man (Bowling 1993)

1.2.1. SCFA Absorption. The absorption of SCFA in the proximal colon takes place via three mechanisms: 1) the passive absorption of the lipid soluble, undissociated form, probably coupled to a Na^+-H^+ exchange; 2) a $SCFA^-$-HCO_3^- exchange; and 3) the diffusion of the anionic form through paracellular "leaky spots" (Engelhardt 1995, Binder 1989, Rechkemmer 1988, Umesaki 1979) (See Figure 1). In the proximal colon, 50% of SCFA are transported in the dissociated form and 50% in the undissociated form, according to measurements of diffusion potentials across isolated epithelium of guinea pigs (Ronnaü 1989). In this study, the transport rates of the three major SCFA (acetate, propionate, butyrate) in the proximal colon were found to be similar. In the distal colon, the absorption of SCFA were found to be primarily based on diffusion of the free acids in the lipid soluble, undissociated form, with transport rates increasing with chain length (See Figure 2).

Nonionic diffusion probably takes place after protonation of the SCFA, linked to the Na^+-H^+, K^+-H^+ and Na^+-K^+ exchange mechanisms on the apical and basolateral membranes of the colonocyte (Sellin 1993, Rechkemmer 1988, 1988b, Engelhardt 1983), and a small fraction provided by the bulk luminal solution (See Figures 1 and 2). Hydrogen ions may be generated by converting CO_2 into carbonic acid and subsequent dissociation of carbonic acid into a proton and bicarbonate.

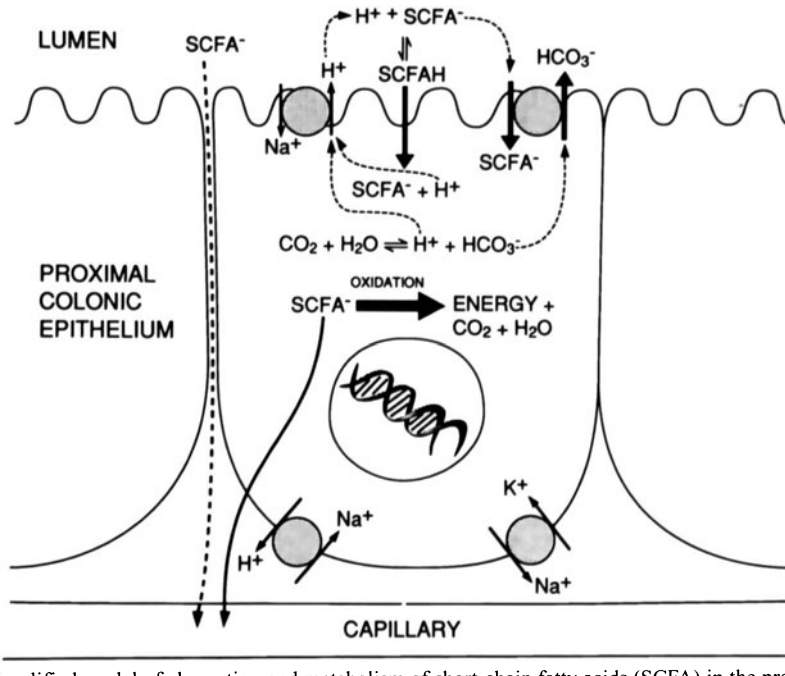

Figure 1. Simplified model of absorption and metabolism of short-chain fatty acids (SCFA) in the proximal colonic epithelium of composite animal species. SCFA is the dissociated form; SCFAH is the undissociated form. Adapted and reproduced with permission (Engelhardt 1995).

1.2.2. SCFA Effects on Water and Electrolytes. In vitro studies using isolated pig mucosa from the proximal and distal colon showed that sodium absorption is enhanced in the proximal colon with the addition of SCFA. This stimulation was dependent on concentration and followed Michaelis-Menten kinetics (Holtug 1992). While both acetate and butyrate stimulates sodium absorption in the distal colon, only butyrate-dependent sodium transport is described by the Michaelis-Menten kinetics. In the distal colon, propionate inhibits sodium absorption. In these studies, acetate and propionate fluxes were equivalent in the mucosa to serosa and serosa to mucosa directions in both the proximal and distal colon. However, the distal colon demonstrated lower permeability to the fluxes of SCFA.

The proximal colon has twice the capacity to absorb sodium when compared to the distal colon and twice the capacity to secrete hydrogen ions than the distal colon in the presence of SCFA (Roediger 1986). These findings support the hypothesis that enhancement of sodium absorption by free fatty acids is coupled to a sodium-hydrogen exchange mechanism in the proximal colon (Engelhardt 1995).

An *in vivo* luminal perfusion technique in rat colon was used to show that butyrate significantly enhances sodium and water absorption, while not influencing calcium absorption, in the proximal colon (Lutz 1991). In the distal colon, however, butyrate and acetate significantly increase calcium absorption, while the absorption of sodium and water is not affected. The authors postulate that a calcium-hydrogen exchanger mechanism exists at the apical membrane of the distal colon which mediates calcium uptake into the epithelial cell. Recently, an *in vivo* human study indicated that the SCFA acetate and

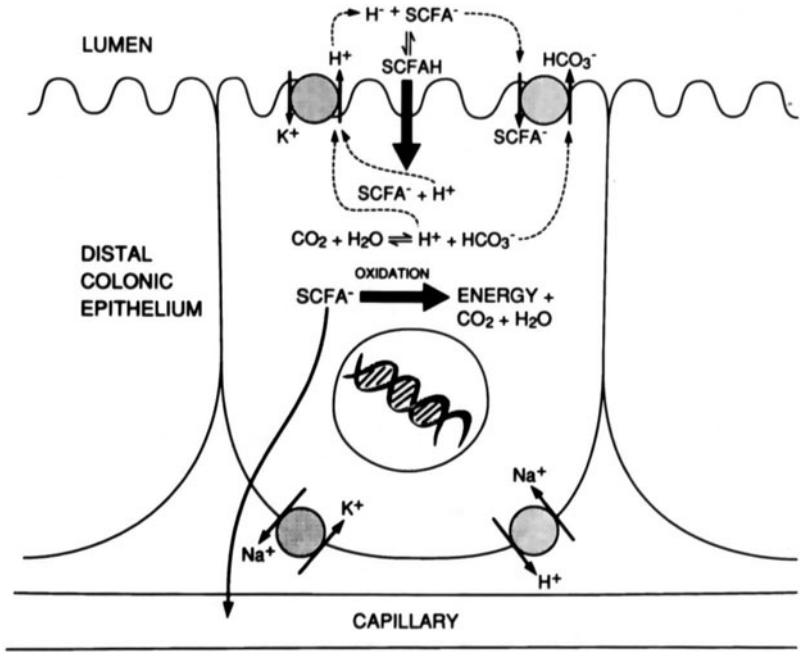

Figure 2. Simplified model of absorption and metabolism of short-chain fatty acid (SCFA) in the distal colonic epithelium of composite animal species. SCFA is the dissociated form; SCFAH is the undissociated form. Adapted and reproduced with permission (Engelhardt 1995).

propionate enhanced calcium absorption from the distal colon (Trinidad 1996). The effects of butyrate were not examined in this aforementioned study.

1.2.3. Possible Mechanisms for the SCFA Effects on Fluid/Electrolytes. The mechanisms by which SCFA exert their effects on fluid and electrolyte fluxes are not fully understood, but are thought to be, at least in part, dependent on CO_2 generation from the oxidation of butyrate (Roediger 1982). Of significance is that butyrate is the preferred oxidizable fuel for the colonic epithelium (Roediger 1982). Other mechanisms probably include the butyrate-induced upregulation of Na^+-H+ transport by inducing the transcription of mRNA for the Na^+-H^+ exchanger (Bishop 1992). Also, the induction of other key genes encoding for membrane ATPase ion exchangers may be involved. For example, induction of normal colonocytes by sodium butyrate resulted in a two-fold increase in the amount of mRNA expression for the gene encoding the Na^+,K^+-ATPase alpha-subunit (Chehab 1987). Hormonal, paracrine, and autocrine factors may also play a role. For example, in isolated rabbit distal colon, n-butyrate (10 mM) causes a stepwise, concentration-dependent increase in Peptide YY (PYY) (Longo 1991). PYY is a gastrointestinal neuroendocrine peptide which inhibits gastric acid and pepsin secretion, and increases gastric emptying and the mouth-to-cecum transit time in humans (Adrian 1985).

1.3. Metabolism

Several studies show that SCFA are the preferred oxidizable fuel for colonocytes (Cherbuy 1995, Topping 1993, Awad 1990, Roediger 1982). Normal colonocytes rapidly

metabolize n-butyrate to CO_2 and ketones (Roediger 1994). *In vitro*, normal rat colonocytes utilize SCFA in the following order of preference: butyrate > acetate > propionate (Awad 1990). The preference of colonocytes for butyrate as an energy substrate appears to be a tissue specific phenomenon. It is most important in the rectum and distal colon and less pronounced in the cecum (Roediger 1994). These findings are interesting due to the predominant anatomic distribution of colon cancer and ulcerative colitis in the distal colon and rectum.

2. POTENTIAL THERAPEUTIC EFFECTS

2.1. Colonic Neoplasia

The variable effects of dietary fiber on colonic tumorigenesis may be a reflection of the varying fermentation patterns according to substrate, colonic region and genetic predisposition. Butyrate concentrations appear to protect against colonic carcinogenesis (Young 1996, McIntyre 1993). Carbohydrate fermentation predominates within the proximal colon (Mackie 1988), leading to the production of high amounts of butyrate. In the distal colon, there is a relative shortage of carbohydrates, and protein fermentation predominates leading to the formation of the isoacids and valeric acids (Mortensen 1992). The preferential production of isoacids and valeric acids from protein and the relative decrease in butyrate production in the distal colon could theoretically play a role in the association between high protein, low fiber diets and increased colon cancer.

Dietary wheat bran generates high distal colonic and fecal butyrate concentrations in the rat (McIntyre 1991). It has been shown that wheat bran is protective against tumors and aberrant crypt formation in a rat colorectal cancer model (Young 1996, McIntyre 1993). However, other factors in the specific type of fiber ingested are probably also important since another colorectal cancer study in rats showed that a resistant starch diet did not inhibit colonic carcinogenesis despite causing an increase in the intraluminal colonic butyrate concentration (Sakamoto 1996).

The shift from aerobic to anaerobic metabolism that occurs with neoplastic transformation might lead to the inability to oxidize n-butyrate. The resulting accumulation of butyrate in the cytoplasm of the neoplastic colonocyte may be the cause of increased sensitivity to butyrate and the paradoxical effect of butyrate on neoplastic (causing growth arrest) versus normal cells (stimulus for proliferation) (Young 1994). The importance of butyrate in the pathogenesis of colon cancer and its effects on normal (trophic effect) versus neoplastic colonocytes (growth inhibitory effect) is discussed further in this text.

2.2. SCFA and Enteral Feeding-Related Diarrhea

Evidence suggests that colonic secretion of water and electrolytes plays an important role in the pathogenesis of enteral feeding-related diarrhea (Bowling 1993). Whether or not fiber should be added to artificial enteral nutrition is a controversial issue (Scheppach 1990). There is much experimental evidence in animals to support the addition of fiber to enteral feedings, however, clinical studies are thus far inconclusive. Initial work on SCFA and colonic fluid and electrolyte movement suggested that SCFA helped cause diarrhea and produced fluid and electrolyte secretion (Bustos-Fernandez 1971). Subsequent work, however, showed that SCFA are not involved in the pathogenesis of diarrhea, but in fact are compensatory and conservatory in some diarrhea disorders. The

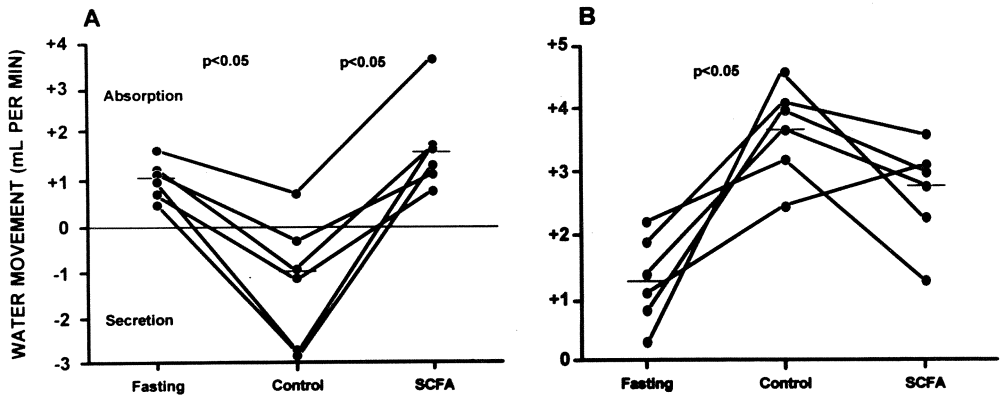

Figure 3. SCFA effects on enteral feeding induced colonic water movement in the ascending colon (A) and descending colon (B). Both the control and the SCFA groups received enteral feedings. Enteral feeding induces a net secretory effect on the ascending colon. The fluid secretion that occurs in the ascending colon during intragastric feeding is reversed by a cecal infusion of SCFA. In the distal colon, there is net absorption of water throughout all study groups. Adapted and reproduced with permission (Bowling 1993).

compensatory effects are mediated in part as the result of their stimulation of sodium, chloride, and colonic fluid absorption (Bowling 1993). Colonic secretion of water and electrolytes (induced by enteral feeding) is inhibited in healthy human volunteers by cecal infusion of SCFA mixtures in physiologic concentrations (acetate 50mmol/L, propionate 20mmol/L, butyrate 20mmol/L) (Bowling 1993) (See Figure 3).

2.3. Ulcerative Colitis

Studies suggest that the inhibition of fatty acid oxidation in ulcerative colitis may be of pathogenic importance (Butzer 1995, Scheppach 1992). The details on the proposed pathogenic model for ulcerative colitis and the relevance for butyrate have been reviewed (Gibson 1992). Lack of SCFA in the colonic lumen leads to a form of "nutritional colitis" (Roediger 1994). In fact, decreased fecal concentrations of SCFA are noted in patients with ulcerative colitis, but not in those with Crohn's colitis (Vernay 1987). The distal colonic epithelium in ulcerative colitis patients exhibit an impaired ability to oxidize butyrate *in vitro* (Roediger 1980) and *in vivo* (Roediger 1984). The evidence suggests that colonic epithelium in ulcerative colitis does not fully compensate for its energy deficiency through use of alternative substrates (Kameyama 1984). In contrast, normal colonocytes isolated from germ free rats (lacking colonic SCFA) demonstrate partial compensation for their artificially induced energy-deprived state by an increased ability to use glutamine as a substrate (Cherbuy 1995).

Histopathologically, the lesions of diversion colitis and ulcerative colitis are indistinguishable (Glotzer 1981), suggesting a connection between ulcerative colitis and a SCFA deprived state. The chemically-induced inhibition of fatty acid oxidation in experimental animals produces mucosal inflammation very similar to that of ulcerative colitis (Glotzer 1981). The predominant dependence of the distal colon on butyrate as its source of energy may be the explanation for the characteristic distribution of ulcerative colitis.

Butyrate enemas (100mmol/L) were used to treat 10 ulcerative colitis patients in a placebo controlled, single blinded, randomized trial (Scheppach 1992) (See Figure 4).

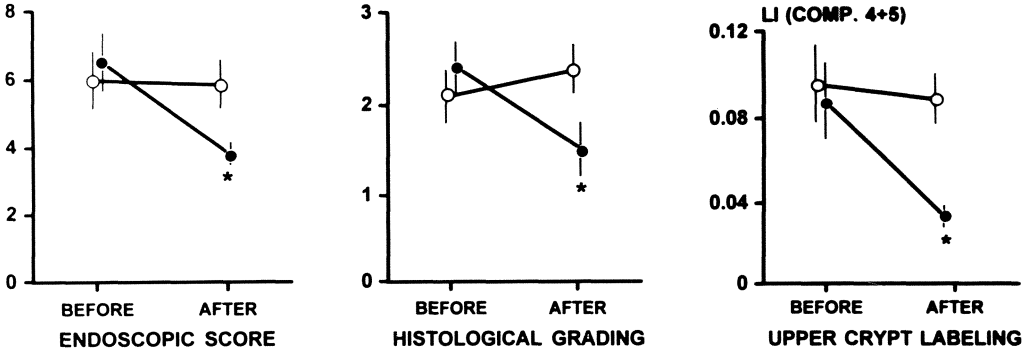

Figure 4. Endoscopic score (n=10), histological grading (n=10), and upper-crypt labeling frequency (n=6) in patients with distal ulceratilve colitis before and after treatment with sodium butyrate (●) or sodium chloride (○) (control) enemas. Vertical bars indicate 1 SEM; *significant differences (endoscopic score, $p<0.01$; histological grading, $p<0.02$; upper-crypt labeling, $p<0.03$; Wilcoxon test) Adapted and reproduced with permission (Scheppach 1992).

Findings from this study support the view that butyrate deficiency may play a role in the pathogenesis of distal ulcerative colitis and that colonic irrigation with butyrate is beneficial in the treatment of this condition. Study variables included stool frequency, discharge of blood per rectum, endoscopic score, histologic degree of inflammation, and upper crypt labeling index, all of which were significantly reduced in patients treated with the butyrate enemas. In an *in vitro* study that incubated human colonic epithelial cells from patients with ulcerative colitis with butyrate, there was a significant increase in protein synthesis when compared to Crohn's colitis and diverticulitis (Frankel 1994). The authors postulated that the therapeutic effects of butyrate in patients with ulcerative colitis may be due to its use as a metabolic fuel to increase protein production and thus promote healing (Frankel 1994).

2.4. Diversion Colitis

SCFA deficiency induced by diverting the fecal stream often results in the development of diversion colitis of varying severity. This colitis may be reversed by the instillation of butyrate and other SCFA into the diverted colon (Harig 1989). Therefore, it seems that butyrate and other SCFA are essential in maintaining the integrity of the colonic mucosa. Four patients with diversion colitis were treated with colonic irrigations of SCFA mixtures (acetate 60mmol/L, propionate 30mmol/L, and butyrate 40mmol/L) for 2–3 weeks. This therapy resulted in macroscopic and histological resolution of inflammation (Harig 1989). However, in a prospective, double-blinded study using rectal enemas twice daily for 14 days, with mixtures of SCFA (acetate 60mmol/L, propionate 30mmol/L, butyrate 40 mmol/L), there was not significant endoscopic or histologic improvement in 14 patients (Guillmot 1991). The authors concluded that the heterogeneity of the bacterial population in different types of excluded colon or inadequate total amount of SCFA infusate may have accounted for these negative findings. The etiology of the colonic diversion may be another reason for conflicting results. Further investigation is needed in order to understand such divergent results.

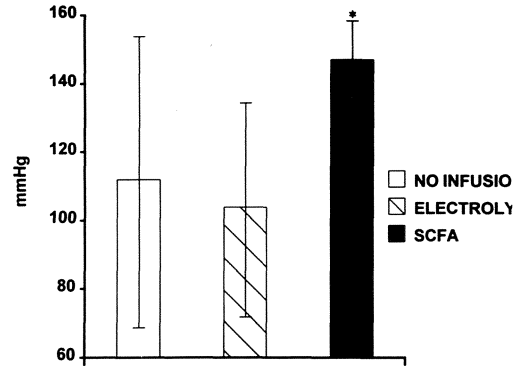

Figure 5. Bar graphs of bursting pressure. The colons for the SCFA group burst at significantly higher pressure than the two control groups. Bursting pressures did not differ significantly between colons of the electrolyte and the no infusion groups Adapted and reproduced with permission (Rolandelli 1986).

2.5. Colonic Anastomosis Healing

SCFA may have a therapeutic effect on colonic injury. Intraluminal infusion of a SCFA mixture (acetate 75mmol/L, propionate 35mmol/L, and butyrate 20 mmol/L) in rats produced significantly stronger colonic anastomoses when compared to infusions of an electrolyte vehicle (Rolandelli 1986) (See Figure 5). This SCFA mediated enhancement of colonic anastomosis healing may result from increased mucosal proliferation (Sakata 1983) and stimulation of colonic blood flow and uptake of oxygen (Kvietys 1981). The effects of butyrate alone on anastomosis healing have not been studied. However, it is likely that intracolonic butyrate infusions may have a role in enhancing the healing of surgically created anastomosis.

2.6. Effects on Colonic Motility and Blood Flow

An *in vitro* study examined the contractile effect of SCFA on isolated proximal, middle, and distal segments of the rat colon. Butyrate was an effective stimulant of colon contraction at 0.1 mM which suggests that butyrate may stimulate colonic contractions via an enteric reflex involving local sensory and cholinergic nerves (Yajima 1985). The middle and distal colon showed a stimulatory effect of butyrate on contractile response. However, the proximal colon lacked any contractile response to either butyrate or other SCFA investigated. The data suggests that butyrate is probably one of the physiologic luminal stimuli regulating colonic motility.

SCFA infused into autoperfused dog colon in physiologic concentrations can induce a 24% increase in colonic blood flow (Kvietys 1981). Dietary manipulations that increase cecal SCFA concentrations lead to an increase in cecal blood flow in rats (Demigne 1985). Acetate is thought to be the primary cause of these blood flow effects in the dog model but direct vasodilatory effects of all SCFA may mediate this stimulation of blood flow (Rombeau 1990).

3. SUMMARY AND CONCLUSIONS

Butyrate, a SCFA generated by microbial fermentation of dietary substrates, is produced in the colon of humans and may influence colonic disease. It is possible to manipulate the diet in order to enhance levels of butyrate in various regions of the large intestine. Butyrate is absorbed by colonocytes in the proximal colon via passive diffusion and by

active transport mechanisms which are linked to various ion exchange transporters. In the distal colon, the main mechanism of absorption is passive diffusion of the lipid-soluble form. Butyrate and other SCFA are important for the absorption of electrolytes by the large intestine and may play a role in preventing certain types of diarrhea. The mechanism by which butyrate and other SCFA exerts control over fluid and electrolyte fluxes in the colon is not well delineated though it may occur through an energy generated fuel effect, the up-regulation of various electrolyte transport systems, as well as possible effects on neuroendocrine factors. Butyrate has been shown to have beneficial effects on some colonic pathologies. This SCFA may be protective against colorectal neoplasia. Butyrate regulates colonic motility, increases colonic blood flow and may enhance colonic anastomosis healing. Butyrate may reduce the symptoms from ulcerative colitis and diversion colitis and it may prevent the progression of colitis in general. Further investigations are needed to confirm these findings in controlled, randomized, double blinded clinical studies.

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15

SHORT CHAIN FATTY ACIDS, INTESTINAL ADAPTATION, AND NUTRIENT UTILIZATION

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1. INTRODUCTION

The gut, composed of epithelial cells, gut associated immune cells, and smooth muscle cells, is a highly dynamic organ recognized for its role in nutrient absorption and the exclusion of foreign substances, ie gut barrier function. Less well understood is its influence on the utilization of nutrients even though > 50% of the post-prandial insulin response is mediated by gut-secreted peptides (the "incretin" effect)[1,2]. Thus, a consideration of the effect of dietary fiber on nutrient utilization must consider two domains: 1) the requirement of the gut for nutrients per se and 2) the secretion of peptides from the gut to potentially influence nutrient utilization in other tissues.

2. INTESTINAL NUTRIENT REQUIREMENTS

2.1. Mucosal Mass and Nutrient Utilization

Intestinal mucosal mass (cell number) is the result of an equilibrium between cell gain (proliferation) and cell loss (apoptosis) for epithelial cells and recruitment vs cell loss for immune cells. Continuous proliferation of epithelial cells occurs only in the lower crypt regions[3], and cells migrate from there onto and along villi to be eventually extruded from the tips into the lumen of the gut. The rate of epithelial cell migration varies among species and with diet and age, but estimates of total protein turnover suggest complete epithelial replacement in 2–3 days for the rat[4] and in 4–6 days in humans[3]. The regulation of mucosal cell proliferation and apoptosis is still poorly understood.

The intestine requires a disproportionate amount of nutrients given its relative mass of <10% of total body mass[6]. For example, the gut, spleen and pancreas represent ~5% of the body weight in 37 kg pigs but account for 25% of whole body oxygen consumption[6].

Dietary Fiber in Health and Disease, edited by Kritchevsky and Bonfield
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Whole-gut protein constitutes ~7% of whole-body protein in growing rats, but the total amount of protein synthesized per day in the stomach, small and large intestine approximates 19% of the total for the whole body[7]. The rate of protein synthesis is higher in the mucosa (140%/d) than the serosa (66%/d), indicative of the higher turnover of epithelial cells in the mucosa[8].

2.2. Physiologic Factors Influencing Nutrient Utilization

A number of physiologic and pathologic conditions, including alterations in route of intake (enteral vs parenteral), dietary intake (quantity and composition), age, physiologic state (pregnancy, lactation), surgery, irradiation, and disease states such as inflammatory bowel disease, starvation, diabetes mellitus, and infection, modulate both intestinal mass and nutrient exchange (uptake minus metabolism) between the gut and other tissues[9]. Nutrient transport capacity of epithelial cells seems to be programmed within the crypts prior to migration up along the villus[10]. Thus, a lag in intestinal glucose uptake is seen upon exposure to new diets until a new population of enterocytes has migrated from the crypts to replace previously-programmed enterocyte populations along the villus[10]. Typically, 2–3 days of dietary exposure are required to change nutrient tranport capacity[10,11].

Cell proliferation rates[12] and migration rates[13] decrease but small intestinal protein synthesis rate does not seem to change during fasting[14] although fasting induces greater protein losses from the small intestine than the body as a whole[15]. Extended periods of elemental diet consumption are also associated with intestinal atrophy[16–18]. Total intestinal length and mucosal weight are significantly reduced in rats that receive total parenteral rather than enteral nutrients[19–20]. Intestinal mass and cell proliferation rates increase with short-term fiber supplementation of elemental diets[21–22]. This effect seems to be attributable to short chain fatty acids (SCFA) production since luminal SCFA infusions also increase colonic mass and cell proliferation[23–26]. SCFA supplementation of total parenteral nutrition solutions also stimulates intestinal hypertrophy following massive small bowel resection[27–28]. Clearly, many factors influence intestinal mass and nutrient requirements. However, the remainder of this paper will focus on the effect microbial fermentation of dietary fiber to short chain fatty acids in the large bowel of monogastrics.

3. DIETARY FIBER AND HUMAN HEALTH

The recognized importance of dietary fiber in human nutrition[29–31] is not a concept which has always been accepted. Dietary fibers found in plants were considered indigestible by all animals until Henneberg (1854–1860) demonstrated that some digestion occurred in ruminants and horses.[32] Human nutritionists and physicians continued to regard dietary fiber as a negative index of diet quality with little, if any, nutritional value except as a natural remedy for constipation. In a classic experiment, Williams and Olmstead[33] challenged this assumption but it was not until the papers of Cleave[34], Burkitt[35], and Trowell[36] that the incidence of large bowel diseases was epidemiologically related to inadequate intakes of dietary fiber. A large body of experimental and epidemiological literature now exists linking the ingestion of dietary fiber with human health using outcome indices such as glucose absorption and homeostasis, hyperlipidemia, cancer incidence, and intestinal disorders such as constipation[37].

3.1. Dietary Fiber and Colonic Fermentation

In 1982, Van Soest mathematically described the competitive nature between digestion rate and transit rate[32]. This model is a function of inherent characteristics of substrates (composition and physicochemical properties) which limit the rate and extent of digestion and transit rate (the inverse of retention time) which limits the duration of exposure to digestive processes. In applying this concept to studies of the effects of dietary fiber in monogastrics, the entire intestinal tract can be partitioned into at least two compartments: the small and large intestines. The amount of fermentable material leaving the distal ileum reflects the amount (and properties) of that ingested and small intestinal transit rates. Changes in total intake, nutrient digestibility, and/or small intestinal transit rates will influence the amount of material reaching the large intestine. For example, anti-diarrheal drugs which increase transit time, e.g. loperamide, decrease the amount of starch reaching the large bowel whereas compounds like magnesium citrate and senna decrease small intestinal transit time and increase small-intestinal starch losses[38–39]. Starch losses from the small intestine of humans can also be increased by blocking digestion with the use of glucosidase inhibitors[40–41]. Physiologic changes in gastrointestinal transit associated with the menstrual cycle also seem to influence the amount of fermentable carbohydrate reaching the colon[42].

Within the fermentative chamber, the relationship between digestion rate and transit rate seems to hold for both nonruminants (including humans) and ruminants[43]. The larger body size of the human allows proportionately more energy absorption from the hindgut of humans than rodents[43] but less than pigs[44] and chimpanzees[45]. Although there are differences among fiber sources in the rates[46] and extents[47–49] of fiber digestion, the net effect of increasing delivery of substrate to the large bowel is increased microbial fermentation and production of short chain fatty acids (SCFA), i.e. acetic, propionic, and butyric acids[50]. Greater than 95% of the SCFA produced in the large intestine are absorbed and there are estimates of 3–13% of the metabolizable energy being absorbed as SCFA[51].

3.2. Short Chain Fatty Acids and Intestinal Metabolism

It has been suggested that SCFA may up-regulate colonic metabolism, particularly cell proliferation[24,56,52]. We have recently conducted a series of experiments to test the hypothesis that enterocytes and colonocytes isolated from animals fed highly fermentable diets would have increased capacity to oxidize SCFA. Rats were fed 0, 15 or 30% fiber elemental diets formulated to have similar nutrient to digestible energy ratios. After 14d, the animals were sacrificed and colonocytes were isolated, purified using a discontinuous Percoll gradient, and incubated in supplemented culture RPMI 1640 medium as described by Marsman and McBurney[53]. Dietary fiber supplementation significantly increased colon weights although energy and nutrient intakes were similar. Dietary fiber consumption did not alter SCFA oxidation rates, anaplerotic flux (the probability that a molecule will be completely oxidized versus leaving as an intermediate product upon entering the TCA cycle), or ATP yield from glucose, propionate or butyrate in isolated colonocytes[54]. These observations are in agreement with other work[55–56], including that conducted with isolated enterocytes[53]. In a subsequent experiment with similar dietary formulations, supplemental fiber (15%) significantly increased in vivo colonic cell proliferation rates but not protein synthesis or oxygen uptake rates[57]. These studies indicate that longterm ingestion of dietary fiber (>14d) does not influence intestinal epithelial cell metabolism per se. Rather, it suggests that in normal animals, intestinal energy and nutrient requirements are a reflection of cell number. This may not be the case in pathologic situations since SCFA have been implicated in many intestinal diseases[58–62].

4. DIET AND PROGLUCAGON-DERIVED PEPTIDES

4.1. Dietary Fiber and Secretion of Proglucagon-Derived Peptides

It is generally, accepted, however, that the addition of fermentable fibers to diets or the supplementation of parenteral diets with SCFA results in significant gains in distal small intestinal and large bowel mass[21–24,2728,53,64]. These changes are associated with increased expression and secretion of gut hormones[64–66]. Proglucagon is a 160 amino acid polypeptide encoded by the glucagon gene[67] of L cells found along the gastrointestinal tract in increasing number at the distal small intestine and colon[68]. The L cells produce several peptides, including glicentin [corresponding to amino acids 1–69; PG-(1–69)], a C-terminal extended form of glucagon [oxyntomodulin; PG-(33–69)], GLP-1 [PG-(78–108], GLP-2 [PG-(126–158)], and intervening peptide-2 [PG-(111–123)]. Many studies have established a strong relationship between cell proliferation and elevated plasma levels of proglucagon-derived peptides[69–71]. Reimer and McBurney[64] reported that high fiber diets stimulated proglucagon mRNA expression in the ileum and colon of rats and these changes were associated with significantly greater plasma concentrations of GLP-1 thirty minutes after an oral glucose tolerance test. Gee et al.[66] reported that fermentable, but not viscous, carbohydrates increased plasma GLP-1 concentrations. Feeding fiber supplemented elemental diets (Table 1) to Sprague-Dawley rats for 14 d resulted in dose-dependent increases in ileal and colonic proglucagon mRNA expression (Figures 1 and 2). These observations are consistent with an effect of SCFA on small

Table 1. Composition of the experimental diets

	g/kg diet			
Ingredient	5%	10%	15%	30%
Amino acid mix[1]	193.1	186.2	179.3	158.6
Fat[2]	96.6	93.1	89.7	79.3
Glucose	402.2	377.2	352.0	276.7
Sucrose	201.1	188.5	176.0	138.4
Alphacel	8.3	16.7	25.0	50.0
Fibrad[3]	41.7	83.3	125.0	250.0
Mineral mix[4]	42.6	41.0	39.5	35.0
Vitamin mix[5]	12.2	11.7	11.3	10.0
Choline chloride	2.4	2.3	2.2	2.0
Digestible energy[6] (MJ/kg)	17.2	16.6	16.0	14.2
Amino acid content (g/MJ)	11.2	11.2	11.2	11.2
Nitrogen content (g/MJ)	1.72	1.72	1.72	1.72

[1]Supplied (g/kg mixture): L-arginine HCl, 67.5; L-histidine HCl.H2O, 22.5; L-isoleucine, 41.0; L-leucine, 67.5 L-lysine HCl, 90.0; L-methionine, 41.0; L-phenylalanine, 58.0; L-threonine, 41.0; L-tryptophan, 13.0; L-valine, 47.5; L-alanine, 17.5; L-aspartic acid, 17.5; L-glutamic acid, 239.5; glycine, 116.5; L-proline, 27.5; L-cystine, 22.5; L-serine, 17.5.; L-tyrosine, 22.5; L-asparagine, 30.0.

[2]Contained (g/kg mixture): hydrogenated beef tallow, 392; linseed oil 20; and safflower oil, 588. Polyunsaturated:saturated fatty acid ratio was 1.0, as determined chromatographically.

[3]Ross Laboratories, Columbus, OH. Contained a mixture of pea fiber, oat fiber, sugar-beet fiber, xanthan gum and soy lecithin.

[4]Supplied (g/kg mixture): calcium phosphate dibasic, 500; potassium citrate monohydrate, 220; sodium chloride, 74; potassium sulfate, 52; magnesium oxide, 24; ferric citrate pentahydrate, 6.0; manganous carbonate, 3.5; zinc carbonate, 1.6; potassium iodate 0.01; cupric carbonate, 0.3; sodium selenite, 0.01; chromium potassium sulfate, 0.55; sucrose, 118.

[5]Supplied (per kg mixture): thiamine hydrochloride, 600 mg; riboflavin, 600 mg; pyridoxine hydrochloride, 700 mg; nicotinic acid, 3.0 g; D-calcium pentothenate, 1.6 g; folic acid, 200 mg; D-biotin, 20 mg; cyanocobalamin, 1.0 mg; retinyl palmitate (250,000IU/g), 1.6 g; DL-μ-tocopherol acetate (250 IU/g), 20 g; cholecalciferol (400,000 IU/g), 250 mg; menaquinone, 5.0 mg; sucrose, 972.9g).

[6]Digestible energy (DE) was calculated from gross energy of diet components and in vivo DE measurements for the fiber sources.

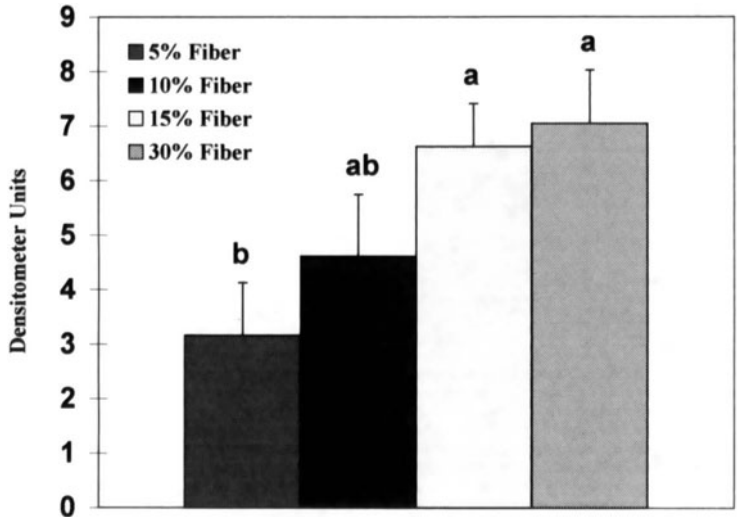

Figure 1. Effect of increasing levels of fiber supplementation on ileal proglucagon mRNA expression in rats. Values are means ± SEM, n = 6 rats/diet treatment. Values with different letters are significantly different ($p < 0.05$).

bowel hypertrophy since the addition of SCFA to intravenous solutions in parenterally-fed rats significantly increased proglucagon mRNA, mucosal mass, DNA and RNA at 3 and 7d following 80% small bowel resection[28].

4.2. Intestinal GLP-1 and Glucose Metabolism

Insulin, a hormone secreted by the pancreas, modulates glucose uptake from the blood into cells, enhances glycogenesis and protein synthesis, and inhibits lipolysis. It

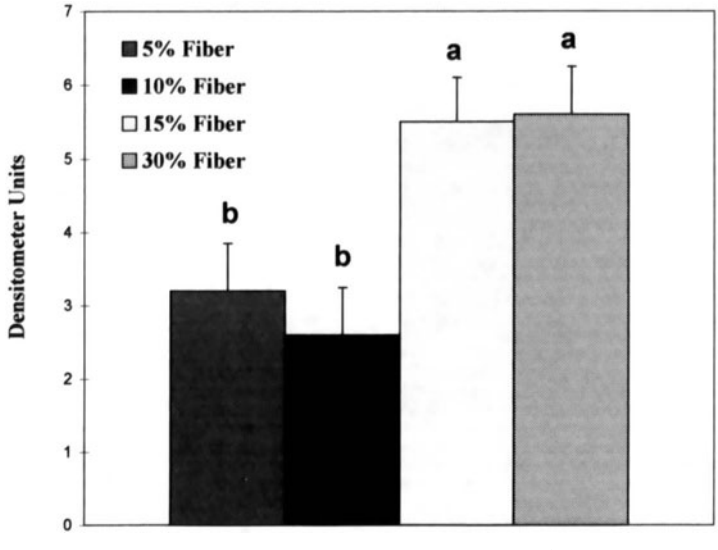

Figure 2. Effect of increasing levels of fiber supplementation on colonic proglucagon mRNA expression in rats. Values are means ± SEM, n = 6 rats/diet treatment. Values with different letters are significantly different ($p < 0.05$).

plays a central role in the regulation of nutrient utilization and partitioning among tissues within the body yet >50% of postprandial insulin secretion is triggered by gut hormones[1–2] and GLP-1 is a physiological important incretin[72–73]. The ingestion of soluble fibers with an oral glucose load improves glucose homeostasis[74] but studies also show that glucose tolerance is improved with longterm ingestion of fiber[75–78]. This is not a direct effect of SCFA on insulin or pancreatic glucagon secretion or glucose uptake since SCFA infusions into the portal vein of pigs do not affect intravenous glucose tolerance[79]. There is an increasing body of evidence suggesting that GLP-1 may also influence glucose homeostasis by delaying gastric emptying and nutrient absorption[80–81]. The relative importance of GLP-1 action on intestinal motility vs its insulinotropic effects remains to be elucidated. Clearly, however, the ingestion of fermentable fiber does increase proglucagon expression and postprandial plasma GLP-1 concentrations[64,66].

5. CONCLUSION

It seems reasonable to speculate that the gastrointestinal tract is capable of modulating pancreatic insulin secretion and therefore nutrient utilization. Indeed, we propose that the ingestion of dietary fiber, long considered to be a dietary constituent with little nutritive value, not only alters events within the lumen of the intestine (ie. digestion, absorption, transit rate) but that it affects gut mass and the secretion of gut peptides to influence nutrient partitioning throughout the body and ultimately human health.

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16

SHORT CHAIN FATTY ACIDS INHIBIT THE EXPRESSION OF THE NEUTROPHIL CHEMOATTRACTANT, INTERLEUKIN 8, IN THE CACO-2 INTESTINAL CELL LINE

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1. INTRODUCTION

The histologic appearance of active ulcerative colitis includes an intense lymphoplasmocytosis limited to the mucosa and submucosa often notable for a neutrophilic infiltrate invading the colonic epithelium (crypt abscess). Multiple investigators have shown that the colonic epithelium is in a hyperproliferative state with expansion of the proliferative compartment from the lower crypt to the upper crypt extending to the surface epithelium (1,2). This hyperproliferative state is independent of the degree of inflammation and the duration of the disease and exists even when the disease is in a quiescent state (1,2). Similar hyperproliferative states have been observed in patients at risk for colonic malignancy such as in familial polyposis coli, sporadic colon adenomas, and familial nonpolyposis colon cancer (3). An intriguing observation made by Scheppach et al. (4) is that the labeling index of colonocytes in the upper crypt of patients with ulcerative colitis fell to that of normal healthy controls after treatment with butyrate enemas. This suggests that butyrate may fundamentally alter the state of proliferation, and perhaps differentiation, of colonic epithelial cells in ulcerative colitis.

Indirect evidence suggests that the state of epithelial cell differentiation plays a role in the immune responsiveness of the intestinal mucosa. For example, only undifferentiated proliferating rat colonic crypt epithelial cells are capable of producing the pro-inflammatory cytokine IL-1β (5). Furthermore, the enteroinvasive bacteria *Yersinia pseudotuberculosis*, which can invade undifferentiated Caco-2 cells, is unable to infect differentiated cells due to the redistribution of a bacterial cell receptor during the process of cellular differentiation (6). Finally, recent studies of transgenic chimeric mice which disrupt the epithelial cell adhesion molecule E-cadherin along the entire crypt-villus axis, but not in the

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villus epithelium alone, produced an inflammatory bowel disease resembling Crohn's disease (7). This suggests that alteration of the intestinal epithelium in the crypt, but not on the villus, leads to intestinal inflammation in immunologically normal mice.

We have been studying the transcriptional regulation of the interleukin 8 (IL-8) gene in intestinal cell line, Caco-2, as a model to elucidate the molecular mechanisms by which the intestinal epithelium responds to an inflammatory stimulus. IL-8, a member of the C-X-C family of chemokines, has been shown to have a diverse spectrum of biological activities including T-cell, neutrophil, and basophil chemotactic properties (13). IL-8 can be produced by a wide variety of cell types and is believed to play a significant role in the initiation of the acute inflammatory response (14). The expression of IL-8 was studied in the colon cancer cell line Caco-2 for several reasons. First, it is the only intestinal epithelial cell model of spontaneous differentiation. In the pre-confluent proliferative state these cells are relatively undifferentiated. However, several days post-confluency Caco-2 cells begin to develop markers of a differentiated phenotype for absorptive enterocytes including the expression of digestive enzymes, tight junctions, a well developed brush border, and a polarized morphology (15). Second, the pattern of inflammatory responsiveness in Caco-2 cells is more similar to that of isolated colonocytes than most other colon cancer cell lines. The inflammatory response in Caco-2 cells, as in isolated colonocytes, can be initiated by IL-1β but not TNF-α (16) in contrast to most other colon cancer cell lines which respond to stimulation with either IL-1β or TNF-α (17). Finally, stimulated cytokine secretion in Caco-2 cells is of a similar order of magnitude to that of normal isolated colonic epithelial cells (17).

Short chain fatty acids (SCFA) are normal products of anaerobic bacterial fermentation of carbohydrates in the colon and are the major source of nutrition for the human colonic epithelium. Butyrate, the SCFA most avidly metabolized by colonocytes, can induce markers of a differentiated phenotype in multiple cell types (8) and has recently been demonstrated to be an effective treatment for distal ulcerative colitis (4,9–11). Three of these reports show that butyrate enemas are effective in patients who have active ulcerative colitis which is refractory to all other modes of treatment (4,10,11). The molecular mechanisms that lead to this response have not been well characterized. One explanation for the efficacy of butyrate in the treatment of ulcerative colitis may be based on its ability to inhibit the production of pro-inflammatory substances by the intestinal epithelium. In this report we show that compounds which induce histone hyperacetylation such as the short chain fatty acids butyrate and propionate as well as the histone deacetylase inhibitor, trichostatin A, each inhibit the ability of the intestinal epithelial cell line Caco-2 to produce the chemoattractive cytokine interleukin 8 (IL-8) in response to stimulation with interleukin 1β (IL-1β). Furthermore, induction of a differentiated phenotype by growth to a post-confluent state also inhibits IL-8 gene expression suggesting that the process of cellular differentiation may be a general mechanism by which some pro-inflammatory substances may be inhibited in Caco-2 cells (12).

2. COMPOUNDS THAT INDUCE HISTONE ACETYLATION INHIBIT IL-8 mRNA EXPRESSION

Figure 1A demonstrates that increasing concentrations of sodium butyrate (NaB) from 0.1 to 2.5 mM lead to a dose dependent reduction in steady state levels of IL-8 mRNA.

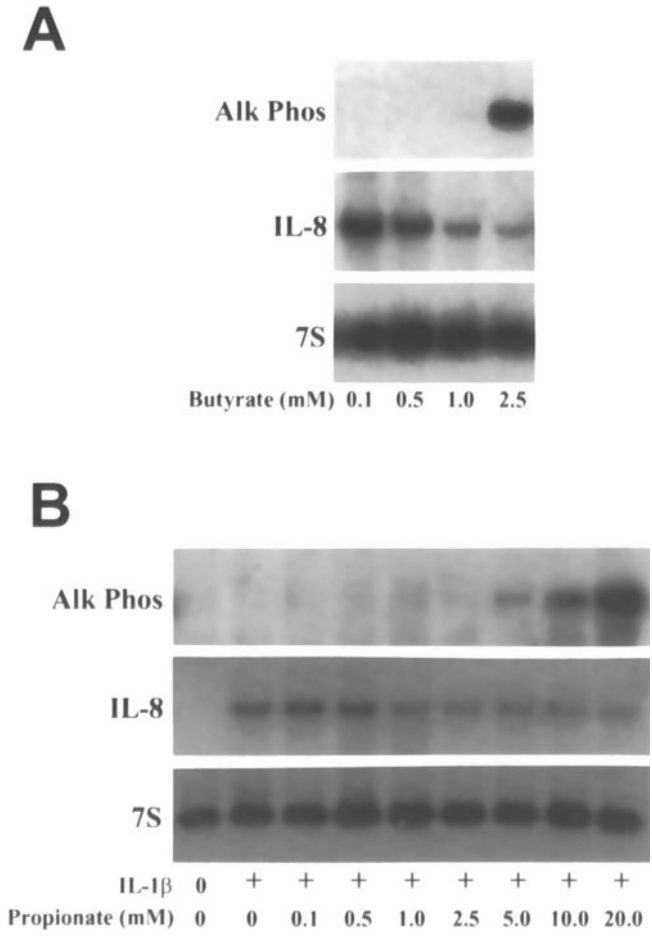

Figure 1. Inhibition of IL-8 mRNA by the SCFAs butyrate (A) and propionate (B) (12).

These concentrations of NaB were well tolerated by Caco-2 cells. IL-8 mRNA expression increased at higher levels of NaB which were toxic to Caco-2 cells and led to significant cell death as measured by trypan blue exclusion (data not shown). Concurrent with the maximal inhibition of IL-8 mRNA expression at 2.5 mM is the induction of mRNA for alkaline phosphatase, a marker for intestinal epithelial differentiation. Figure 1B demonstrates that sodium propionate also inhibits IL-8 mRNA expression in a dose dependent fashion concurrent with the induction of alkaline phosphatase mRNA expression. In contrast to NaB, however, treatment of Caco-2 cells with the maximum concentration of sodium propionate studied, 20 mM, did not lead to cell toxicity or death. Furthermore, the degree to which IL-8 mRNA induction was inhibited was substantially less with sodium propionate compared to that with sodium butyrate. In contrast to sodium butyrate and sodium propionate, sodium acetate neither inhibited IL-8 mRNA induction nor induced the expression of alkaline phosphatase at concentrations up to 20 mM (data not shown). Trichostatin A, specific inhibitor of histone deacetylase activity at nanomolar concentrations (18), also inhibited IL-8 mRNA levels in a dose dependent fashion (Figure 2). Treatment of Caco-2 cells with concentrations less than 10 ng/ml did not inhibit IL-8 gene expression (data not shown).

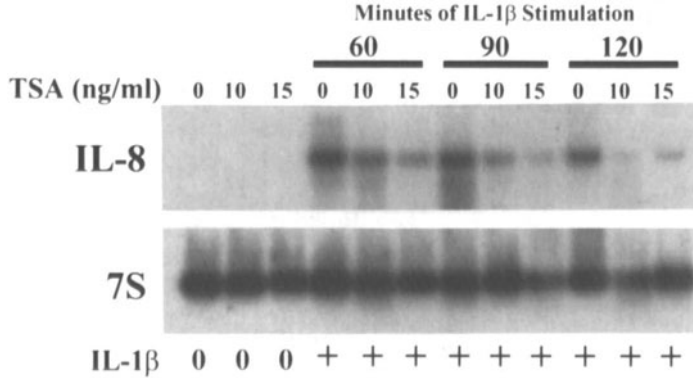

Figure 2. Trichostatin A inhibits IL-8 mRNA expression (12).

Maximal IL-8 mRNA expression occurred between 60 and 120 minutes followed by a rapid decrease thereafter. Trichostatin inhibited IL-8 mRNA expression proportionally at each time point, a finding which was also observed with NaB.

3. HISTONE HYPERACETYLATION CORRELATES WITH THE INHIBITION OF IL-8 GENE EXPRESSION

The most often described mechanism by which SCFAs such as butyrate are able to alter gene expression involves altered chromatin structure secondary to increased histone acetylation. The fundamental subunit of chromatin in all eukaryotes is the nucleosome. It contains approximately 200 bp of DNA wrapped around an octamer of small basic proteins called histones. This octameric complex consists of two dimer histone complexes consisting of histones H2A and H2B along with one tetrameric complex consisting of his-

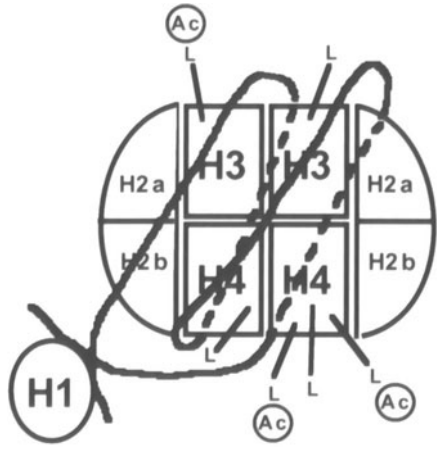

Figure 3. Histone proteins package eukaryotic chromatin into nucleosomes. L = Lysine residue, Ac = Acetyl group.

tones H3 and H4 (Figure 3). A single histone H1 molecule completes the nucleosome structure (19,20).

Each histone protein consists of a globular apolar region and a basic N- or C-terminal region which contain lysine residues that can be reversibly acetylated. The equilibrium between two enzymatic activities, histone acetyltransferase and histone deacetylase, determines the degree to which these histones are acetylated (21). Cousens et al. (22) have shown that NaB leads to histone hyperacetylation by inhibiting histone deacetylase activity. Recently, a cDNA encoding the human histone deacetylase catalytic subunit has been cloned whose predicted protein sequence is very similar to a yeast transcriptional regulator (23). This supports the role for histone deacetylase as an important regulator of eukaryotic transcription. Sodium butyrate has been shown to inhibit histone deacetylase activity leading to the hyperacetylation of certain histone proteins such as histone H4 (22). The Coomassie Blue stained protein gel pictured in Figure 4A shows the effect of various short chain fatty acids on the histone acetylation patterns of primarily histone H4 proteins.

Lanes 1 and 2 were loaded with commercially available H1 and H4 proteins, respectively, isolated from calf thymus (Sigma, St. Louis, MO). Comparison of histones isolated from normal pre-confluent Caco-2 cells in lane 3 to those isolated from Caco-2 cell treated for 3 days with 20 mM sodium acetate, shown in lane 4, demonstrate that acetate does not affect H4 histone acetylation. Unacetylated H4 proteins, marked by the arrow, predominate

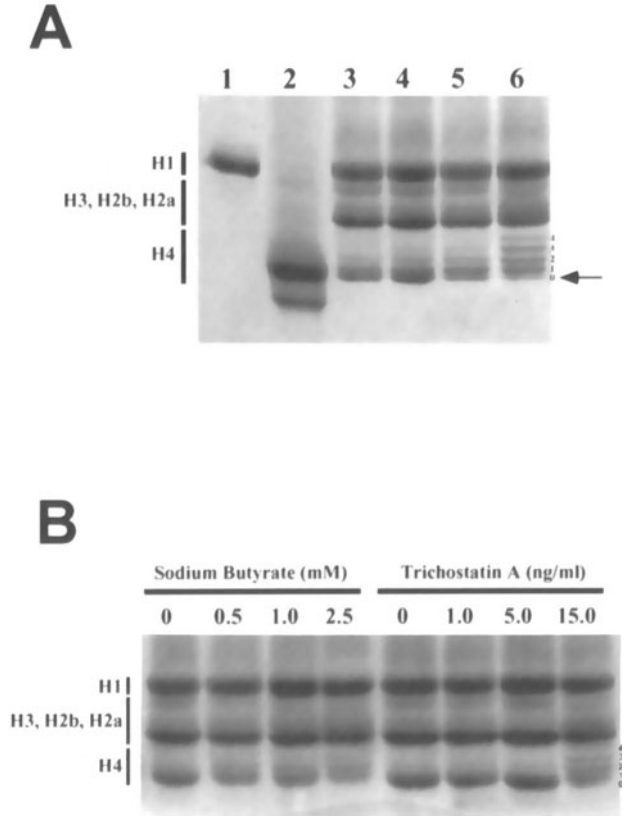

Figure 4. Coomassie Blue stained protein gel of histones isolated from pre-confluent Caco-2 cells treated with various short chain fatty acids (A) or with various concentrations of either sodium butyrate or trichostatin A (B) (12).

in these two lanes. In contrast, histones isolated from Caco-2 cells treated with either 20 mM sodium propionate or 2.5 mM sodium butyrate, shown in lanes 5 and 6 respectively, demonstrate that these two short chain fatty acids are able to induce increased acetylation of H4 histones. There is a decrease in unacetylated H4 proteins with a concurrent increase in mono-acetylated forms with propionate, lane 5, and a increase in di-,tri-, and tetra-acetylated forms with butyrate, lane 6. Figure 4B demonstrates that histones isolated from Caco-2 cells treated with various concentrations of either sodium butyrate or trichostatin A showed a direct correlation between the degree of H4 hyperacetylation and the degree to which the IL-8 gene is inhibited (Figures 1A and 2). Histone acetylation patterns were not altered by treatment of Caco-2 cells with IL-1β (data not shown).

4. SPONTANEOUS DIFFERENTIATION OF CACO-2 CELLS INHIBITS IL-8 GENE EXPRESSION

Figure 5A shows that stimulation of pre-confluent "undifferentiated" Caco-2 cells with IL-1β leads to a dramatic induction of IL-8 mRNA at 2 hours which rapidly decreased at later time points. In contrast, stimulation of post-confluent "differentiated" Caco-2 cells shows very minimal induction of IL-8 mRNA.

In Figure 5B, hybridization of this same RNA using a probe for C/EBP δ, an immediate gene product of the inflammatory response (24), shows that post-confluent Caco-2 cells actually produce greater amounts of this mRNA than pre-confluent cells. This suggests that, although post-confluent Caco-2 cells are still capable of responding to an inflammatory stimulus, the expression of a gene encoding an inflammatory modulating substance such as IL-8 is differentially regulated. Furthermore, the C/EBP δ response of post-confluent cells also suggests that: 1) functional IL-1β is present in the medium of post-confluent Caco-2 cells (post-confluent cells do not produce an inhibitor of IL-1β) and 2) functional IL-1β receptors as well as a signal transduction pathway must still exist in post-confluent Caco-2 cells.

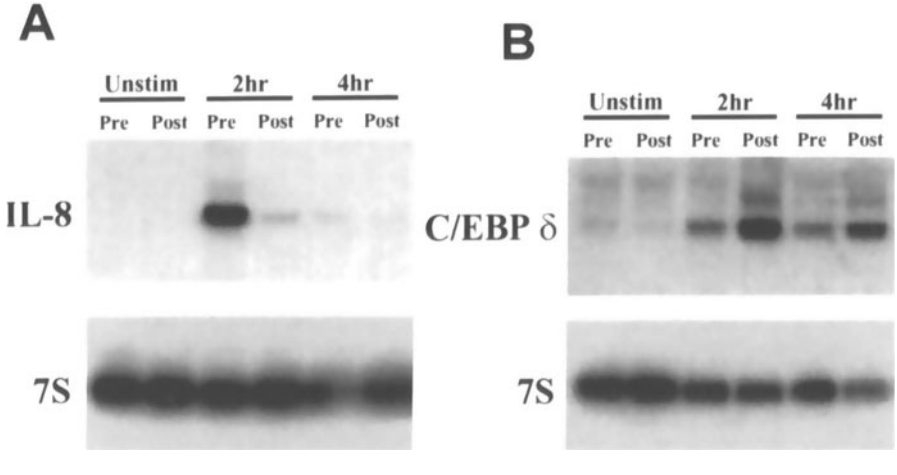

Figure 5. IL-8 (A) or C/EBP δ (B) mRNA expression in pre- and post-confluent Caco-2 cells (12).

5. DISCUSSION

Although short chain fatty enemas have been shown to be an effective treatment for distal ulcerative colitis (4,10,11), the mechanisms by which this response occurs have not been well characterized. A possible mechanism by which butyrate enemas may act to decrease inflammation in patients with distal ulcerative colitis may be through its ability to regulate the expression of immune modulatory substances by the intestinal epithelium. Multiple investigators have shown that intestinal epithelial cells, which are capable of responding to inflammatory stimulation by producing immune modulating substances such as cytokines (16,17,25), immunoreceptors (26), and complement factors (27), may play an active role by participating in the inflammatory response. We have chosen to study the expression of the neutrophil chemoattractive cytokine IL-8 as a marker of the intestinal epithelium's ability to respond to an inflammatory stimulus.

Several lines of evidence suggest that histone hyperacetylation induced by SCFAs may play a role in the inhibition of IL-8 mRNA expression in Caco-2 cells. First, the ability of butyrate and propionate, but not acetate, to induce histone hyperacetylation in Caco-2 cells (Figure 4) is consistent with the observation that the former two SCFAs can inhibit IL-8 expression while the latter cannot. Second, sodium butyrate induces higher degrees of histone acetylation compared to sodium propionate consistent with the greater inhibition of IL-8 mRNA expression by sodium butyrate than sodium propionate (Figure 1). Third, trichostatin A, a specific and potent inhibitor of histone deacetylase activity which is structurally unrelated to SCFAs (18), can also inhibit IL-8 mRNA expression in a dose dependent fashion (Figure 2). Finally, the degree of histone hyperacetylation induced by either sodium butyrate or trichostatin A correlates directly with the degree to which the expression of the IL-8 gene is inhibited (Figure 4).

Spontaneous differentiation of Caco-2 cells also inhibits IL-8 mRNA expression. Pre-confluent Caco-2 cells can be induced by IL-1β to express significant levels of IL-8 mRNA. In contrast, differentiated post-confluent cells express much less IL-8 mRNA in response to IL-1β (Figure 5). This attenuation of IL-8 mRNA expression was reflective of the decrease in IL-8 protein secreted into the medium by cells in the post-confluent state (data not shown). Based on these results, it is tempting to speculate that IL-8 gene expression is inhibited during the process of cellular differentiation induced by histone hyperacetylation. Indeed, it is well recognized that sodium butyrate can inhibit cell proliferation and induce a differentiated phenotype in multiple cell types (8). This effect, which has been demonstrated in multiple colon cancer cell lines (28), is manifested as the induction of brush border membrane associated hydrolases (such as alkaline phosphatase), the development of dome formation indicative of a polarized epithelium, and the inhibition of cellular proliferation (28–30). However, in contrast to treatment with SCFAs, spontaneous differentiation of Caco-2 cells does not alter patterns of histone acetylation (data not shown) suggesting that IL-8 mRNA expression is inhibited through a different mechanism. Nevertheless, we have shown that other agents such as vitamin D [1,25 $(OH)_2D_3$] which induce a partially differentiated phenotype in Caco-2 cells (31) are capable of inhibiting IL-8 gene expression (32). Furthermore, the expression of another pro-inflammatory cytokine, MCP-1, is also inhibited by the growth of Caco-2 cell to a post-confluent state (32). Together, these results suggest that the process of cellular differentiation, regardless of the initiating mechanism, may inhibit the expression of a spectrum of pro-inflammatory substances by Caco-2 cells.

The data presented in our studies would suggest that butyrate enemas may help lead to remission of active ulcerative colitis by inhibiting the expression of inflammatory

mediators, such as IL-8, by the colonic epithelium. The molecular mechanism by which butyrate modulates IL-8 gene expression may be by the induction of histone hyperacetylation. Two recent studies suggest that the observations made in our studies of Caco-2 cells are relevant *in vivo*. First, IL-8 gene expression by isolated colonocytes from patients with inflammatory bowel disease has been shown to be inhibited by incubation with sodium butyrate (33). Second, fecal concentrations of butyrate in rats correlate with increased histone acetylation patterns in colonic epithelial cells (34). Caco-2 cells may ultimately provide a useful model system to elucidate the molecular mechanisms by which SCFAs, histone hyperacetylation, and/or the process of cellular differentiation inhibits IL-8 gene expression.

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17

SHORT CHAIN FATTY ACIDS

Production and Effects on Gut Motility

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1. INTRODUCTION

Short chain fatty acids (SCFA) are produced in the human colon by bacterial fermentation of carbohydrates and amino acids. The physiological importance of these molecules is increasingly recognised. They have been shown to influence cellular turnover not only in the colon, where they are formed, but also at distant sites in the small intestine (Sakata 1989). They provide energy for the colonic enterocyte (Roediger 1980) and peripheral tissues such as muscle (Rémésey et al 1992). SCFA may also influence hepatic metabolism of carbohydrate (Anderson and Bridges 1986) and lipids (Chen et al 1984) and have been used to reduce symptoms of colitis (Breuer 1991).

SCFA have been shown to influence the motility of the small and large intestine (Kamath et al 1988, Yajima 1985) but this action has received little attention and the few studies that have been carried out have not fully characterised this action nor fully explored its role in the control of movement along the gut.

In this chapter, the factors affecting the production of SCFA will be discussed and evidence for the effects of SCFA on motility will be explored.

2. SCFA PRODUCTION

2.1. Bacteria

The colonic microflora of the human adult is very complex with 400 possible species and 10^{12} colony forming units (cfu)/g. The majority of the organisms (99%) are strict anerobes. The main genera in the human colon are bacteroides, bifidobacteria, eubacteria,

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lactobacilli, clostridia, enterobacteria and streptococci. Substrates are passed between bacteria allowing a synergy in the breakdown of complex carbohydrates, production of SCFA, hydrogen and CO_2 and cycling of nitrogen. Thus one bacterial species may split a carbohydrate to small units or sugars and another species may ferment the sugars to acetate, propionate or butyrate, H_2 and CO_2. The end products of fermentation may then be recycled. Hydrogen may be absorbed by the body and excreted in the breath or as flatus but it may be further converted to methane, H_2S or recombined with CO_2 to form acetate (Gibson et al 1990). The exact fate of each carbon, hydrogen or oxygen atom will depend on the bacterial species present, the environmental conditions, for example pH, and the type of substrates available.

The complex colonic microflora and its metabolic capabilities are still not well characterised although it is known that particular substrates tend to produce particular SCFA and that the production of methane and H_2S may depend on the amount of sulphate in colonic content (Gibson et al 1988). There is competition for hydrogen between methanogens and sulphate reducers, but it appears that the methanogens out compete sulphate reducing bacteria when cultures are inoculated with multiple fecal samples containing each as the dominant hydrogen utilisers (Strocchi et al 1994). Knowledge of the competition of bacteria for carbohydrate sources is very limited. Transit time is also important. In a recent study (Oufir et al 1996), where transit was manipulated with cisapride and loperamide, mean whole gut transit time was inversely related to fecal weight, sulphate reducing bacteria, SCFA and hydrogen excretion. Methane excretors had slower transit and lower counts of sulphate reducing bacteria than non-methane excretors.

In general, fermentation studies, whether in vitro or in vivo, tend to treat the colonic microflora as a "black box" measuring the overall production of SCFA and other bacterial metabolites. This approach simply measures the end result of a complex metabolic process, with little knowledge of which bacteria are responsible for each action and product. Every bacterial species has its own characteristic profile of SCFA products and these are often used in species identification. The main groups of bacteria in the colon produce mainly acetic, propionic and lactic acids along with succinic and formic acids. Bacteriodes fragilis group, which form the major bacteroides population in the human colon, produce mainly acetic, propionic and succinic acid. Bifidobacteria and eubacteria produce mainly acetic and lactic acids.

It is not known which bacteria are responsible for each SCFA in the final colonic fermentation mixture and in particular it is not known which species contribute to butyrate production. Butyrate has been identified as a very important bacterial product being the preferred energy source for the colonocyte (Roediger 1980), stimulating both cell proliferation (Sakata 1989) and apoptosis (Hague and Paraskeva 1995) and promoting differentiation of cancer cell lines in vitro (Augeron and Laboisse 1984). However, those bacterial species that are known to produce butyrate, including clostridia and fusobacteria, are not the most numerous species in the colon having 3 to 4 log units less cfu/g feces than the bacteriodes and bifidobacteria (Chadwick et al 1992, Rao 1995). To capitalise on the effects of butyrate on the colonic mucosa it may be useful to identify the organisms responsible, so that their growth can be optimised and butyrate production increased.

2.2. Substrates

Manipulation of the pattern of individual SCFA produced in the colon is possible by feeding large amounts of particular substrates. For example, rats fed ispaghula for four weeks had a much higher propionic acid content of feces compared with those fed a low fibre or wheat bran containing diet (Edwards and Eastwood 1992) and similar results have

been reported in man after 15 days (Marteau et al 1994). Increasing butyrate concentration in the colon would require a carbohydrate that promotes butyrate production, ideally in the distal colon where most disease occurs.

All carbohydrates are fermented to acetate, which is always the predominant SCFA (approx 60% of total), but the proportions of propionate and butyrate vary with the substrates available (Edwards and Parrett 1996). Several studies have attempted to investigate the relationship between the chemical structure of a carbohydrate and the SCFA produced during fermentation. Similar SCFA profiles are produced by carbohydrates containing certain sugars. For example, arabinogalactans and arabinoxylans tend to produce similar proportions of propionic acid (Edwards and Parrett 1996). Mortensen et al (1988) studied the SCFA produced in vitro from mono and disaccharides and found that some sugars, rhamnose, arabinose, xylose, ribose, galacturonic and glucuronic acids, promoted propionic acid production whereas butyrate production was increased by sorbitol, galacturonic, glucuronic acids and to a lesser degree ribose. This agrees in part with the pattern of SCFA produced from arabinoxylans and arabinogalactans but does not explain the higher butyrate produced from starch and oat gum which have glucose as constituent sugar. Salvador et al (1993) compared the disappearance of dietary fibre sugars with SCFA production in vitro. They tested the involvement of each sugar by stepwise linear regression and compared this with the fermentation of the constituent sugars alone. For wheat bran and sugar beet fibre, they found that xylose was the main determining sugar for butyrate production, with glucose having a much lower influence. It is likely that not only the types of sugars present, but also the degree of cross linkage and the presence of side chains will influence the fermentability of a fibre and the pattern of SCFA produced. However, until systematic experiments using oligosaccharides and polysaccharides with known or designed sugar sequences are undertaken, the exact influence of sugar composition and order will not be fully understood.

The reason why some carbohydrates promote propionic acid production whereas others promote butyrate production is unclear. As these SCFA are produced by different bacteria it could be that certain species use different sugar residues as preferred energy source and the presence of such a substrate increases the predominance of that particular organism or group of organisms. On the other hand, environmental conditions may be important. Propionic and lactic acids are more likely to be produced at low pH due to the availibility of hydrogen ions (Edwards et al 1985). However ispaghula, which is characteristically associated with greater propionic acid production, is not rapidly fermented and therefore unlikely to be associated with acid pH (Edwards and Eastwood 1992).

2.3. Development of Fermentation Capacity

Feeding large amounts of individual dietary fibres to increase butyrate production may not be practical in the whole population or in the longterm. Another approach is to try and influence the development and maturation of the colonic microflora in childhood. The colonic microflora of the adult is remarkably stable and changes very little with diet (Bornside 1978). On the other hand the colonic microflora of the infant is directly influenced by diet (Balmer and Wharton 1989). A newborn baby is fed entirely on milk and the colonic microflora is likely to ferment mainly lactose, along with fructo-oligosacchrides in human milk and maltodextrins in infant formula. As weaning commences the infant is exposed to a range of complex carbohydrates for the first time. The weaning process may represent a critical period when longterm bacterial populations and metabolic capabilities are established in response to complex carbohydrates in the diet.

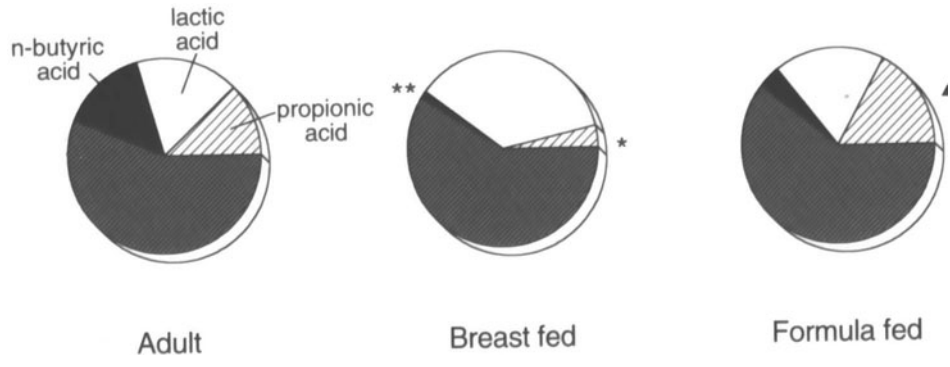

Figure 1. Molar proportions of SCFA produced in vitro in cultures inoculated with the feces of exclusively breast fed or formula fed infants or adults containing 100 mg lactose. * $p<0.05$, ** $p<0.01$ compared with adult, ▲ $p<0.05$ compared with breast fed infants.

The gut is sterile at birth but is very quickly colonised in the first few days of life. The resulting flora is simple in comparison to that of the adult colon but depends on feeding practice (Balmer and Wharton 1989) . The flora of a baby fed exclusively with breast milk has a flora dominated by bifidobacteria and lactobacilli whereas that of babies fed a modern cow's milk based infant formula has more bacteroides and streptococci (Balmer and Wharton 1989). This is reflected in their fermentation products with breast fed babies producing mainly acetate and lactate and formula fed babies producing mainly acetate and propionate. (Edwards et al 1994) Breast fed babies produce very little butyrate. In a recent study (Edwards et al 1994) we could detect butyrate in the feces only 16% breast-fed babies compared with 68% of formula fed-babies. Moreover the fecal microflora of formula-fed babies produced little butyrate compared with adults when cultured with simple and complex carbohydrates in vitro (Parrett et al 1994, Fig 1). The low butyrate production in the infant colon is of great interest in relation to the importance given to butyrate in the adult colon especially with repsects to the action of butyrate on the colonic mucosa. The lack of butyrate in the infant may reflect a different response of the infant colonic mucosa to butyrate or may indicate that the presence of butyrate is not as essential as previously thought.

The reasons for the differences in the fecal flora of the breast fed and formula fed infants are unclear but appear to relate to; 1) the type of protein; whey based formula produces a flora more like that of the breast fed baby (Balmer et al 1989), 2) the availibility of iron which is determined by the amount of iron in infant formula and the presence of lactoferrin in breastmilk. Lactobacilli and bifidobacteria do not need iron whereas bacteroides species and enterobacteria require iron for growth (Archibald 1983), and 3) the buffering capacity of formula milks due mainly to their high calcium and phosphate content. A low pH promotes the growth of bifidobacteria and lactobacilli but inibits other bacteria. There may also be some specific bifidobacteria growth promoters such as oligosaccharides or bifidus factor in breast milk (Stahl et al 1994).

As the weaning process is initiated new complex carbohydrates and unabsorbable oligosaccharides are ingested. The colonic microflora develops in response to these new substrates but the development is gradual. Recent studies have shown that key bacterial metabolic activities such as degradation of mucus, conversion of bilirubin to urobilirubin, metabolism of cholesterol to coprostanol and inactivation of fecal tryptic activity were still increasing by the age of 2 years (Midvedt et al 1988, Midvedt and Midtvedt 1992, 1993, Midtvedt et al 1994).

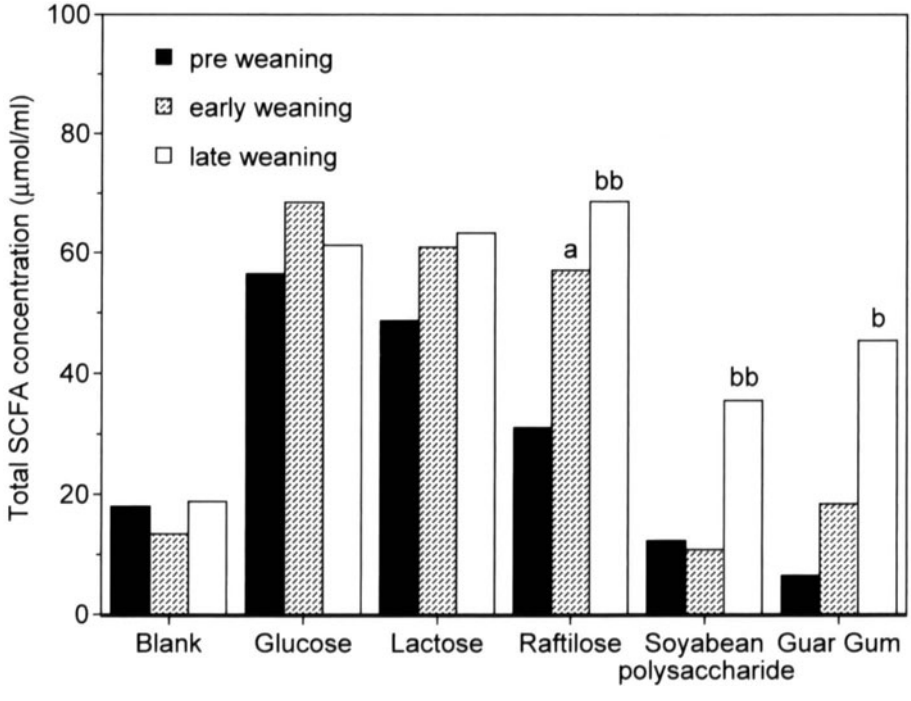

Figure 2. Short chain fatty production in cultures of fecal bacteria from infants before weaning, during early weaning or during late weaning containing 100mg of glucose, lactose, raftilose, soybean polysaccharide or guar gum. a = $p<0.05$ compared with late weaning, b = $p<0.05$, bb = $p<0.01$ compared with early weaning.

We have found that the ability of infants to ferment complex carbohydrates also develops slowly in contrast to the ability to ferment oligosaccharides (Parrett et al 1995). In a recent cross-sectional study, the ability of breast fed and formula fed babies to ferment sugars, oligosaccharides and complex carbohydrates was estimated at 4 weeks, during early weaning (within 6 weeks of first solid food) and during late weaning (after 16 weeks of weaning on chopped/solid food). Fecal bacteria were incubated with substrates in a simple salts media in vitro for 24 hours and total SCFA production estimated. It was found that whilst ability to ferment oligossacharides increased in early weaning, the ability to ferment complex carbohydrates increased only in late weaning and was still much lower than the amount of SCFA produced by bacteria from adult feces under the same conditions (Fig 2). In addition we have detected significant amounts of starch in the feces of children upt 3 years of age Verity and Edwards 1994). This indicates that the development of the colonic flora in response to weaning food is a gradual process and may offer an opportunity to influence long-term bacterial activity by dietary manipulation.

3. ACTIONS OF SCFA ON INTESTINAL MOTILITY

3.1. Actions in the Small Intestine

The main function of the motor patterns of the small intestine are to mix food with digestive enzymes and to move digesta along the intestine towards the colon. The transit

of food along the small intestine takes approximately 5–7 hours and is influenced by several luminal factors such as viscosity, lipid content and liquid/solid properties. It is unlikely that SCFA are present in the small intestine in any appreciable amounts unless they are ingested as a pharmacological agent or are included in certain foods for example acetic acid as vinegar or propionic acid in bread. Thus an action of SCFA on small intestinal motility is likely to be a response to a foreign or noxious stimuli. SCFA may be present in the distal small intestine as a result of bacterial overgrowth or reflux from the colon. Either way the expected response would be that a propulsive motor pattern would be stimulated to clear the contents back into the colon. Studies investigating the action of SCFA on the small intestine have shown that SCFA speed up mouth to cecum transit time in rats (Richardson et al 1991), and induce propulsive motility patterns in the terminal ileum (Kamath et al 1988, Fich et al 1989, Cherbut et al 1996). However, they have little effect on jejunal motility (Masliah et al 1992).

Several studies have examined the effect of SCFA on the motility of the terminal ileum and the ileoceal sphincter in some detail. Using a gamma camera to follow fluid movement Fich et al (1989) showed that SCFA infused into the terminal ileum of dogs stimulated phasic bursts of contraction associated with ileal emptying. SCFA were more effective in the fasting state or shortly after a meal than when the meal was in the ileum. An elegant study in dogs fitted with ileocolonic loops (Kohler et al 1991) allowed investigation of the effects of ileal and colonic infusions of acetic acid and colonic distension on ileocolonic sphincter pressure, ileal motility and colo-ileal reflux. They showed that acetic acid in the ileum increased propagated clustered ileal contractions which increased sphincter pressure when they propagated into the colon. When acetic acid was perfused into the colon, sphincter pressure also increased with less effect on ileal contractions. Acetic acid in the colon also increased the response of the sphincter to colonic distension. These studies also appear to confirm that the ileum responds to SCFA by increasing propulsion to clear colonic reflux and increasing sphincter pressure to prevent further reflux occuring. A recent study of isolated rat ileal segments (Cherbut et al 1996) suggests that this effect is due mainly to the low pH caused by SCFA since other acids such as citric and tartaric produced similar stimulation of ileal contraction whereas the sodium salt of acetate did not. The contractions were not blocked by tetrodotoxin, atropine, hexamethonium, removal of the mucosa or use of a local anaesthetic. However, the calcium channel blocker verapamil completely supressed the effect. Thus the action of SCFA in the terminal ileum appears to be mediated by an acid sensitive calcium dependent action on muscle rather than a specific action of SCFA. This is in contrast to the effect of SCFA on colonic motility as discussed below.

3.2. Colonic Motility

Before considering the action of SCFA on colonic motility and its effects on colonic propulsion, it is important to understand some basic colonic physiology.

The human colon should not be considered as one functional unit. The proximal and distal colon differ in many ways. There are differences in the anatomy, neural and blood supply and the absorption characteristics as the length of the colon is traversed (Edwards 1993). The motility patterns, residence times and properties of luminal contents also differ.

The human colon can be divided arbitrarily into three functional areas:

1. The cecum and proximal colon. These form the main site of bacterial carbohydrate metabolism and therefore act as a fermentation chamber.

2. The transverse colon. Here the predominant motor patterns hold material in the proximal colon for further fermentation or propel it distally emptying the proximal colon. The transverse colon may also be an important site for the absorption of water and the formation of feces.
3. The distal colon and rectum act as a reservoir for fecal material allowing defecation to be delayed until socially convenient.

It is believed that most fermentation occurs in the proximal colon, which may be true for a standard western lower fibre diet. However when fibre intake is increased the fermentability of that fibre will determine whether it is completely fermented in the proximal colon, as is likely with guar gum and gum arabic, or whether fermentation, and therefore SCFA production is spread further around the colon. The site of fermentation will depend not only on the speed of the fermentative processes but also on the control of colonic motility. If propulsion is stimulated before fermentation is completed then SCFA production will be spread distally. The stimuli for proximal colonic emptying may therefore control the exposure of the distal colon to SCFA including butyrate. The action of SCFA themselves on colonic motility is therefore of interest. It seems logical that if SCFA were to slow proximal colonic emptying then there would be more time for fermentation and SCFA absorption, but on the other hand, exposure of the distal colon to SCFA would be reduced. In contrast, if SCFA promoted propulsion then fermentation products would be spread further round the colon, colonic pH would be less and fibre may fermented to a lesser extent.

The emptying rate of the proximal colon may determine the function of the colon overall. A study of regional transit of radio-labelled pellets through the colon of patients with Irritable Bowel Syndrome (Vassallo et al 1992) showed a direct relationship between proximal colonic emptying rate and 24 hour stool weight.

To fulfill the roles of fermentative chamber, propulsion unit and reservoir, each region of the colon has a different predominant motor pattern. Because of the inaccessibility of the human colon, and the necessary invasiveness of the techniques used to study motor patterns, it has been difficult to assess the role of different motility patterns and their relation to flow.

Most colonic contractions consist of ring like contractions of both longitudinal and circular muscle causing the relaxed areas to bulge outwards in haustra. These may move forwards or backwards or remain stationary. The proximal colon has mainly retropusive and mixing movements. The sigmoid colon has a degree of tonic and phasic motor activity and may hold colonic contents back in the descending colon to allow water absorption. The degree of tone or distensibility of the colon will determine the capacity of the colon to retain material after a meal. This tone varies diurnally increasing after meals and decreasing during sleep.

3.2.1. Electrical Basis of Motility. Contraction of the colonic muscles is determined by the electrical activity of the muscle cells (myogenic control). Periodic variations in the resting membrane potential determine the excitability of the muscle and the timing of contractions. When the membrane depolarises beyond a threshold potential the muscle generates an electrical response activity (ERA) which results in muscle contraction. Whether an ERA occurs or not is dependent on a neural or chemical signal occurring at the time of depolarisation (Sarna 1991).

Two types of control activity occur; electrical control activity (ECA) and contractile electric complex (CEC). Electrical control activity, or slow waves, is spontaneous, con-

tinuous (Hardcastle and Mann 1968, Sarna et al 1980) and has a frequency in the range of 2–13 cycles/min (Schang et al 1986). In the proximal colon these oscillations in membrane potential appear to migrate towards the mouth (Huizinaga and Daniel 1986). Those that originate in the mid colon tend to spread towards the rectum. The slow wave can occur at a variety of frequencies depending on the level of stimulation (Schang et al 1986). Recorded slow wave frequency in vivo may be the result of summation of different frequences (Sarna et al 1980).

ERA activity that superimposes on the ECA when stimulated at depolarisation, causing short duration contractions, are known as short spike bursts SSB (Christensen 1974) They last less than 5 seconds and occur in series. These are thought to correspond to non-propagating haustral contractions and are most predominant in the proximal colon.

The second type of control activity, the contractile electrical complex (CEC) is the oscillatory membrane potential which appears to be always associated with long duration contractions (Bueno and Fioramonti 1981). These oscillations are intermittent and may require neural or chemical stimulation. They have a higher frequency than the ECA (slow waves); 24–36 cycles/min (Hardcastle and Mann 1968). If neural or chemical signals occur when the CEC is below threshold a different form of ERA occurs; long spike bursts (LSB) which last more than 10 secs and are associated with long duration contractions that may propel contents along the colon (Bueno and Fioramonti 1981).

3.2.2. Colonic Motor Patterns. Longterm manometric studies of colonic motility, up to 24 hours, have shown that the colon is very quiescient during sleep whether this is at night or during the day. Indeed there was an increase in the motility index of the colon (a measure of the number and amplitude of contrations) when subjects were roused from resting and maintained at a more vigilant state by conversation (Schang et al 1988).

Narducci and colleagues (1987) recorded over 24 hours and reported mainly low amplitude contractions which occurred singly or in bursts but which showed no recognisable pattern. The major simulus for activity was awakening. High amplitude contractions which propagate over long distances at approximately 1cm/sec and are often associated with an urge to defecate have been recorded in several studies. These mass movements are thought to occur by relaxation of the gut in response to descending neural inhibition and the propagation of one ring-like contraction through colonic shortening initiated in the ascending or mid-transverse colon (Kumar et al 1989).

Movement of material towards the rectum can also occur with the long duration contractions associated with the LSB. In contrast, the non-propagating contractions associated with SSB appear to act as baffles slowing transit through the intestine. In diarrhoea SSB activity is virtually absent (Bueno et al 1980) but the motility is very active in constipation (El Sharkway 1983). This presents an apparent paradox in the colon, in that unless propagation is measured, an increase in colonic activity may in fact reflect a slowing of transit, and where there is an inhibition of motility and hence a decrease in motility index. This may be related to an increased flow and decreased transit time. This paradox may explain some of the conflict in the reported effects of SCFA on colonic motility and transit time.

3.2.3. Major Stimuli of Colonic Motility. To assess the possible impact of the action of SCFA on colonic motility it is important to identify the major stimuli of propulsion. Various extrinsic stimulants of colonic motility have been reported. The most powerful is awakening from sleep or rest (Narducci et al 1987, Bassotti and Gaburri 1988, Kumar et al 1989) as described above. The colonic motility index is also increased after ingestion of a high calorie meal (1000Kcal) (Christensen 1985). This is often called the gastro colic

reponse. Very little stimulation is seen after a 350 Kcal meal. "Mass movement" contractions are not stimulated by a meal.

It has been suggested that, like other intestinal responses to food, there is a cephalic. gastric and intestinal phase (Christensen 1985) of the gastocolic reflex , with colonic motility increasing at the sight and smell of food, the presence of food in the stomach and the presence of food in the intestine, both small and large.

Colonic motility increases soon after a meal and this represents the gastric and small intestinal phase of the gastrocolic reflex. There is a second increase in colonic motility which occurs about 70–90 minutes after the meal (Snape et al 1978). This late response may be related to the entry of the meal into the colon. SCFA could modulate either of these phases of the colonic response to ingestion of food but this has not yet been studied.

3.2.4. SCFA and Colonic Motility. The intraluminal factors which influence colonic motility are particularly poorly understood. Distension by the luminal contents or gas produced by bacterial fermentation may induce propulsive activity (Chauve et al 1976, Narducci et al 1985). Bile acids and hydroxy fatty acids have also been shown to stimulate propulsion (Kirwan et al 1975, Edwards et al 1989, Spiller et al 1986).

The fermentative function of the proximal colon has encouraged speculation that SCFA may influence motility perhaps to prevent movement of material from the fermentation chamber allowing bacteria to maintain adequate populations. However, SCFA have been shown both to stimulate (Yajima 1985) and inhibit (Squires et al 1992) colonic motility in the rat in vitro and had no effect on proximal colonic capacitance in the human (Kamath et al 1990).

The reason for these differences in reported effects of SCFA on colonic motility probably relate to differences in experimental design, the complex control of colonic motility and the relationship between motility patterns and propulsion of material along the colon.

Let us consider these studies in more detail. In the original studies of Yajima (1985) and Svenson (1972) there appeared to be a conflict of results. Svenson was examining the effects of SCFA on the motility of the proximal colon of sheep in vivo and found that SCFA decreased flow from the proximal colon. Yajima, however, reported stimulation of contractions by SCFA. Yajima isolated muscle strips from the proximal, mid and distal colon of the rat and investigated the effects of individual SCFA as well as a mixture of Acetic, propionic and butyric acids on contractile activity. He reported no response in the proximal colon but stimulation of contractions in the longitudinal muscle in the mid and distal colon by propionic , butyric and valeric acids. Acetic and lactic acids had no effect. In addition pretreatment of the material with one SCFA inhibited the action of further exposure to other SCFA. Effects of propionic acid were inhibited by atropine and tetrodotoxin but not by hexamethonium and were enhanced by eserine in contrast to the effects of SCFA on ileal muscle strips described above (Cherbut et al 1996). This suggested an enteric reflex involving local sensory and cholinergic nerves.

Studies on muscle strips (Yajima 1985) do not allow for the effect of adjacent regions of the gut and any action of the mucosa. This is the only study to report stimulation of motility by SCFA and the lowest concentrations of SCFA were used (1 mmol/l in contrast to 10mmol/l and 100mmol/l used by Svenson). All other studies have indicated an inhibition of contractile activity by SCFA. Cherbut et al (1991) showed that antibiotics increased contractile activity of the rat colon in vivo and this effect was inhibited when SCFA were infused in the colon. The SCFA produced contrations during infusion but caused inhibition of contractility thereafter. The mechanism of this action was explored in an in vitro study of the isolated rat colon (Squires et al 1992). This study used strain

gauges to measure contractions of the cecum, proximal, mid and distal colon as well as fluid flow, and found that motility was decreased in all areas of the colon by SCFA mixtures and individual SCFA. This effect was dose related with 10mmol/l having much less effect than 100mmol/l SCFA. Propionic acid and butyric acid were more potent inhibitors than acetic acid or SCFA mixtures in the mid and distal colon. Acetic acid was the least effective inhibitor. The inhibition of contractions was seen only at pH 4. Sodium salts of SCFA had no effect. However, unlike in the small intestine other acids had no effect on motility indicating a specific action of SCFA. In this particular experiment the inhibition of contractions was associated with decreased luminal flow. The isolated colon was filled with fluid, however and was under slight luminal pressure. These results may not translate to the human colon. If most of the contractions inhibited are stationary contractions which normal act as baffles to flow then movement trhough the colon would be increased by SCFA. It is also possible that the action of SCFA is to modulate the major stimuli of colonic propulsion, eating and awakening, rather than to have a direct action on propulsion and transit themselves.

3.3. Colonic Motility and Transit Time

If SCFA do influence colonic motility, it is difficult to assess their action on transit time and stool output. Inhibition of motility may hold up material to allow more fermentation to occur but this does not coincide with the actions of dietary fibre on colonic transit time and stool output. A low residue diet is associated with slow transit and small fecal output (Spiller et al 1980). A high mixed fibre diet or a diet supplemented with poorly fermented dietary fibres such as wheat bran or ispaghula decreases the transit time and increases stool output (Cummings et al 1976, Eastwood et al 1978). This may be associated with an increase in LSB and a decrease in SSB (Brodribb et al 1979). However the best stool bulkers are the least fermented. The effect of rapidly fermented dietary fibres on colonic transit is not known. Tomlin and Read (1988), however, compared the fermentation of different dietary fibres in vitro and compared this with transit measurements in humans who had digested the same fibres, and who provided the inoculum for the in vitro studies. They showed that dietary fibres that were fermented speeded transit without increasing stool output which would appear suggest that if SCFA do affect motility they accelerate transit rather than slow it. In a more recent study in the rat (Edwards and Eastwood 1995), rapidly fermented dietary fibres had no effect on stool output, but slowly fermented fibres, which increased fecal SCFA, increased stool water and stool output. These fibres were probably fermented in distal colon. SCFA produced here may have a different effect on motor patterns and flow than those produced in the proximal colon from rapidly fermented fibres. Careful studies of segmental transit and colonic motor patterns are needed before the action of SCFA on motility, colonic transit and stool output can be fully elucidated.

4. CONCLUSIONS

In light of the growing interest in the actions of SCFA on different body systems and diseases, it may become worthwhile to manipulate SCFA in the colon for specific clincial situations such as the treatment of colitis and the prevention of colonic disease. To achieve the ideal SCFA profile for each situation and need, it is necessary to learn more about the bacteria responsible for production of individual SCFA, their preferred sub-

strates and the critical periods for their establishment in the colon. It would be useful to be able to predict the SCFA that an individual carbohydrate will produce so that new carbohydrates sources can be identified and developed. The targeting of the distal colon, where most colonic disease occurs, may be achieved by understanding the factors determining site of fermentation, and therefore SCFA accumulation, and also the efects of SCFA and other luminal contents on motility and colonic segmental transit.

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18

BUTYRATE

Potential Role in Colon Cancer Prevention and Treatment

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1. INTRODUCTION

Butyrate is produced in the colon of mammals as a result of microbial fermentation of dietary fiber, undigested starch, and proteins (Rombeau 1990). Butyrate may be an important protective agent in colonic carcinogenesis (Young 1994, Velázquez 1996a). Trophic effects on normal colonocytes *in vitro* (Scheppach 1992a) and *in vivo* (Velázquez 1996a) are induced by butyrate. In contrast, butyrate arrests the growth of neoplastic colonocytes and inhibits the preneoplastic hyperproliferation induced by some tumor promoters *in vitro* (Bartram 1994). Butyrate induces differentiation of colon cancer cell lines in culture (Young 1994). It also regulates the expression of molecules involved in colonocyte growth and adhesion, and inhibits the expression of several proto-oncogenes relevant to colorectal carcinogenesis, *in vitro* (Young 1994). Recent studies in our laboratory show that intraluminal butyrate treatments in normal rat colon results in proliferation patterns consistent with a potential protective role for butyrate in colon carcinogenesis (Velázquez 1996a). Associated with these effects, butyrate increased c-Jun whereas the secondary bile acid and tumor promoter, deoxycholate increased c-Fos, demonstrating for the first time, that these diet by-products can specifically affect colonic proto-oncogene expression *in vivo*. Moreover, we have recently observed that intravenous infusion of butyrate has significant anti-tumor effects, in an *in vivo* murine model of colon cancer metastatic to the liver (Velázquez 1996b). Preliminary work indicates that butyrate's growth inhibitory effects in neoplastic colonocytes may be linked to inhibition of mevalonate-mediated cell growth. Additional studies are needed to fully evaluate butyrate's anti-neoplastic effects *in vivo,* and to understand its mechanism(s) of action.

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2. DIET AND COLON CANCER PREVENTION: BUTYRATE VERSUS DEOXYCHOLATE

Colon cancer incidence is strongly associated with dietary practices (Howe 1992, Yu 1990). The short-chain fatty acid butyrate (McIntyre 1993, DeCosse 1989), and the secondary bile acid deoxycholate (Reddy 1978), are dietary by-products that have been implicated in colon cancer prevention and promotion, respectively.

Diets high in fiber (Howe 1992) are thought to play a protective role possibly as a result of increased colonic butyrate levels (McIntyre 1993, DeCosse 1989). Experimentally induced high fecal butyrate levels (by a high concentration wheat fiber diet) correlate with decreased tumor incidence and decreased tumor growth in dimethylhydrazine-treated rats (McIntyre 1993) and in human subjects with familial adenomatous polyposis (DeCosse 1989). Butyrate has pronounced anti-neoplastic effects *in vitro* and arrests growth, induces differentiation, and modulates proto-oncogene expression in many human colorectal carcinoma cell lines *in vitro* (Young 1994).

Diets high in fat (Wynder 1987) may promote colon carcinogenesis as a result of increased fecal secondary bile acids such as deoxycholate (Hill 1991). Rectally administered deoxycholate is a co-carcinogen and tumor promoter in rat models of colon carcinogenesis (Reddy 1978). Serum deoxycholate levels are significantly increased in patients with colorectal adenomas compared to healthy gender-matched controls (Bayerdörffer 1993). Increased fecal levels of bile acids correlate strongly with the incidence of colon carcinoma and severe dysplasia in ulcerative colitis patients followed prospectively for 10 years (Hill 1987).

Total crypt and crypt surface hyperproliferation occur in the colonic mucosa of humans at increased risk of colon cancer (Lipkin 1983, Lipkin 1987, Pandey 1995). A shift in the proliferative zone from the base to the surface of the crypt as assessed by increased ϕh value is the best histologic predictor of cancer risk in humans (Lipkin 1983). In animal models, similar cytokinetic abnormalities are observed with chemically induced carcinogenesis (Deschner 1982). Associated with this typical hyperproliferative pattern, aberrant expression of proto-oncogenes c-fos and c-jun occur in the flat, "grossly normal" colonic mucosa adjacent to carcinomas and adenomas in human subjects (Pandey 1995). Similar aberrant expression of c-fos and c-jun is seen within large tubulovillous adenomas and adenocarcinomas in humans (Magrisso 1993, Pandey 1995). The jun and fos gene families encode for DNA binding proteins which dimerize to form the AP-1 transcription factor (de Groot 1990). AP-1 DNA binding regulates multiple genes involved in cellular growth and differentiation (Angel 1988, de Groot 1990). While both c-fos (Sugio 1988, Souleimani 1993, Tang 1995) and c-jun are associated with regulation of growth and induction of differentiation, most reports identify c-jun (but not c-fos) as the key component of the AP-1 complex which mediates terminal differentiation (Yang-Yen 1990, de Groot 1990, Angel 1988, Iwai 1993). Terminal differentiation is usually associated with growth arrest (Colony 1989).

Butyrate and deoxycholate are normally present within the colonic lumen and are known to modulate human colonic crypt proliferation in vitro in an interactive and compartment-specific manner (Bartram 1994). While butyrate enhances crypt base proliferation, deoxycholate increases total crypt and crypt surface proliferation (Bartram 1993). Moreover, co-incubation with butyrate and deoxycholate reverses the deoxycholate-induced surface hyperproliferation in proximal and distal human colon in vitro (Bartram 1994). Until recently, it had been unknown whether these effects occur in vivo (Velázquez 1996a).

In a recent study we tested the hypothesis that butyrate decreases and deoxycholate increases crypt surface proliferation *in vivo* and that these effects are mediated by changes

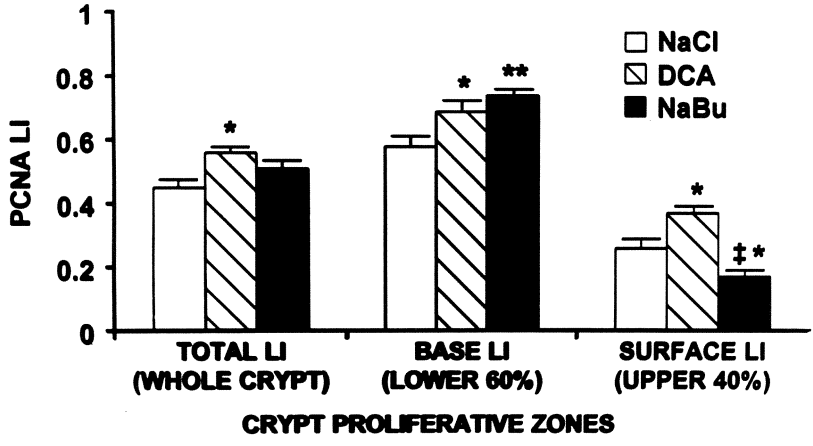

Figure 1. *In vivo* proliferation effects of butyrate (NaBu) and deoxycholate (DCA) measured by Proliferating Cell Nuclear Antigen (PCNA) immunohistochemistry. Total, base, and surface labeling indices (LI) expressed as mean ±SEM. N ≥ 8 for each group; *P ≤ 0.05 versus sodium chloride (NaCl), **P≤ 0.001 versus NaCl, ‡P≤ 0.05 versus butyrate, by ANOVA. Adapted and reproduced with permission (Velázquez 1996 a).

in the expression of the proto-oncogenes c-fos and c-jun which are known to regulate proliferation and differentiation (Velázquez 1996a). Twenty-five adult Sprague Dawley rats underwent colonic isolation and 24hr intraluminal instillation of 10mM sodium chloride, 10mM sodium butyrate, or 10mM sodium deoxycholate. Proliferation of the whole crypt and 5 crypt compartments from base to surface was assessed by Proliferating Cell Nuclear Antigen immunohistochemistry. The ϕh value, an index of "pre-malignant" hyperproliferation (Lipkin 1983), was calculated as the ratio of labeled cells in the two surface compartments divided by the labeled cells in the entire crypt. Expression of c-fos and c-jun was evaluated by Western blot. Crypt surface proliferation and the ϕh value were significantly decreased by butyrate and increased by deoxycholate (Figure 1). Butyrate increased colonic expression of c-jun whereas deoxycholate significantly induced c-fos (Figures 2–3). The dividing cells within the normal colonic mucosa are located in the mid to lower portion of the crypt (Colony 1989). The products of these dividing cells then migrate towards the surface of the crypt and mature or fully differentiate (Colony 1989). Therefore the colonic crypt consists of a spectrum of colonocytes at different stages of the proliferation/differentiation continuum from undifferentiated, pluripotent, rapidly proliferating cells at the base to fully differentiated, growth arrested cells, at the surface. Butyrate consistently increases crypt base proliferation and significantly decreases crypt surface proliferation, thereby demonstrating a specificity to its effects. It is likely that this specificity is based on the proliferation/differentiation stages in the colonocyte along the length of the crypt. In contrast to butyrate's effects, deoxycholate significantly increases total crypt and upper 40% surface proliferation, and the ϕh value. These findings are consistent with studies on colon carcinogenesis which indicate that butyrate may be protective (DeCosse 1989, Young 1994) whereas deoxycholate acts as a tumor promoter (Hill 1991). Our *in vivo* results, in normal rat colon, are consistent with *in vitro* findings in normal human colon biopsies (Bartram 1994). Butyrate exerts a proliferative effect that is confined to the base (lower 60%) compartments of the human colonic crypt *in vitro* (Scheppach 1992a). Co-incubation of butyrate and deoxycholate in this *in vitro* model inhibits deoxycholate induced surface hyperproliferation (Bartram 1994). Also consistent with our findings, are

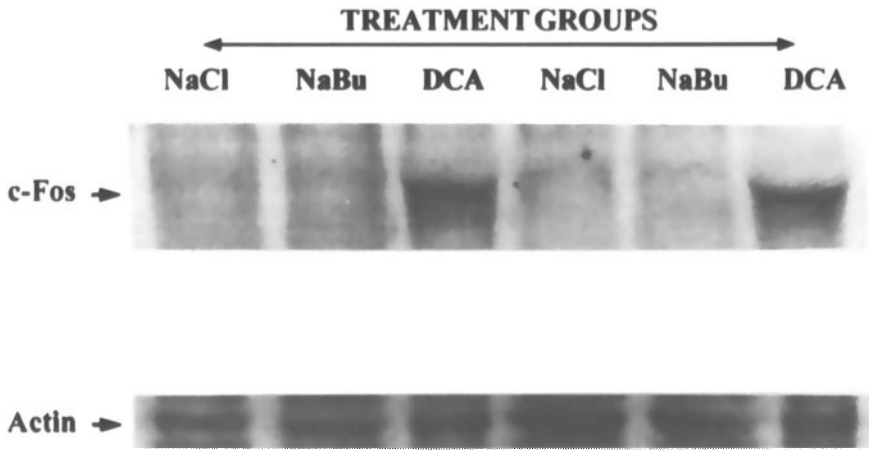

Figure 2. *In vivo* effects of butyrate and deoxycholate on c-fos (62 KD) expression by Western blot. Actin (43 KD) expression in the same study animals is shown. These are the results for six representative study animals (two in each of the treatment groups). Treatment groups: sodium chloride (NaCl), butyrate (NaBu), deoxycholate (DCA). Adapted and reproduced with permission (Velázquez 1996 a).

the *in vivo* effects in ulcerative colitis patients receiving butyrate enemas (Scheppach 1992b). Ulcerative colitis represents a state of abnormal colonic hyperproliferation that leads to an increased risk of developing colon cancer. Treatment with butyrate enemas (100 mmol/L) for two weeks decreases upper 40% surface labeling index to normal levels (Scheppach 1992b). Therefore, results observed in our *in vivo* rat model (Velázquez 1996a) are consistent with those observed in human colon *in vitro* and *in vivo*. These data indicate that the *in vivo* effects on surface proliferation are consistent with a potential protective role for butyrate and a promotive role for deoxycholate in colon carcinogenesis. The observed effects on colonic c-jun and c-fos expression (Velázquez 1996a) represent a novel finding and suggest that direct or indirect modulation of proto-oncogene expression may be the mechanism by which these dietary by-products regulate proliferation *in vivo*.

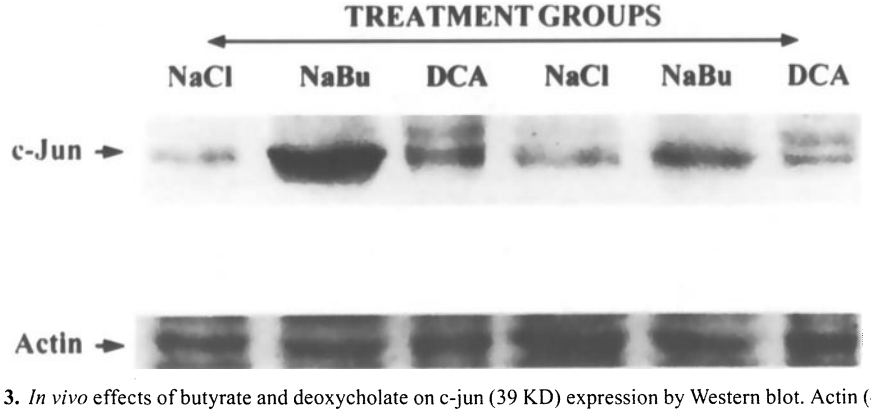

Figure 3. *In vivo* effects of butyrate and deoxycholate on c-jun (39 KD) expression by Western blot. Actin (43 KD) expression in the same study animals is shown. These are the results for six representative study animals (two in each of the treatment groups). Treatment groups: sodium chloride (NaCl), butyrate (NaBu), deoxycholate (DCA). Adapted and reproduced with permission (Velázquez 1996 a).

These findings may have clinical importance since certain dietary fibers such as wheat bran can increase colon levels of butyrate while decreasing deoxycholate levels (Boffa 1992, Reddy 1992). Many epidemiologic studies suggest that dietary fat, which increases fecal bile acids, promotes colonic carcinogenesis whereas increased intake of fiber, which increases fecal butyrate and decreases fecal bile acids, may protect against colon cancer (Hill 1991, Howe 1992). However, reports of the potential anti-neoplastic role of fiber fermentation by-products such as butyrate are inconsistent (Jacobs 1983, Freeman 1986, Wargovich 1992). One study reports a promotive effect of wheat bran on colonic tumorigenesis (Jacobs 1983), whereas two studies in which butyrate was orally supplemented in rats report no butyrate-induced tumor protection (Wargovich 1992). Moreover, one study showed increased colon tumors in the butyrate treated group (Freeman 1986). Our experimental model (Velázquez 1996a) eliminates many confounding variables such as differences in luminal pH, bulk, transit time, washout effect, endogenous short-chain fatty acids and secondary bile acids, and presence of other luminal substances from succus entericus such as calcium and sodium ions that may affect proliferation. These possible confounding variables are difficult to control in diet studies and have all been implicated as possible factors in colon carcinogenesis (Folino 1995, Bartram 1993). Further studies with carefully controlled surgical experimental models are required to delineate whether the compartment-specific proliferation pattern induced by butyrate, in fact protects against tumor carcinogenesis.

The mechanisms by which butyrate and deoxycholate modulate *in vivo* colon crypt proliferation in the observed compartment-specific manner are not known but are probably ultimately mediated by direct or indirect effects on gene expression. *In vitro* studies in human malignant colonocytes show that butyrate inhibited growth is associated with arrest in the G1 phase of the cell cycle (Toscani 1988), induction of morphologic, histologic and biochemical differentiation (Whitehead 1986), and modulation of oncogene expression (Souleimani 1993a, Foss 1989, Stoddart 1989). Some of these effects may be mediated by butyrate-induced inhibition of histone deacetylase (Boffa 1992) which affects chromatin structure and indirectly affects gene expression (Kruh 1994). In CaCo-2 human adenocarcinoma cells, butyrate modulates gene expression *in vitro* by directly activating the placental-type alkaline phosphatase promoter (Deng 1992). Because of these observations, some investigators have hypothesized that butyrate acts as a cofactor of the regulatory transcription proteins which are directly involved in gene expression (Kruh 1994). *In vitro* work in transformed colonocytes and other transformed cell lines demonstrates that butyrate affects both c-fos and c-jun expression and thereby modulates proliferation and differentiation (Souleimani 1993, Tang 1995, Nishina 1993). In some transformed cells butyrate induces c-fos (Souleimani 1993, Tang 1995) whereas in other transformed cells butyrate induces c-jun expression (Rabizadeh 1993, Nishina 1993, Iwai 1993). In these *in vitro* studies the induction of either c-fos or c-jun by butyrate is associated with growth inhibition and differentiation.

Deoxycholate and other secondary bile acids are thought to promote abnormal hyperproliferation and tumor growth by directly or indirectly activating protein kinase C which is a key component in growth signal transduction and amplification (DeRubertis 1987). Effects on protein kinase C are known to indirectly modulate proto-oncogene expression by mechanisms which are not well understood (Borner 1993). The observation that deoxycholate induces c-fos under conditions of concurrent crypt compartment-specific proliferation (Velázquez 1996a) is the first reported evidence that deoxycholate affects colonic proto-oncogene expression. Proto-oncogenes may directly or indirectly mediate proliferation *in vivo*. The possible significance of the differential induction of c-fos but not c-jun is discussed subsequently.

Reports of complex derangement in c-fos and c-jun expression in human colon adenomas and carcinomas (Sugio 1988, Magrisso 1993, Pandey 1995) as well as in the "grossly normal" flat mucosa adjacent to the adenomas and carcinomas (Pandey 1995) suggest that the study of these proto-oncogenes may be important in understanding colon cancer. Interestingly, the altered pattern of proto-oncogene expression in the mucosa adjacent to the tumors is associated with abnormal surface hyperproliferation restricted to that same mucosal region (Pandey 1995).

The expression of nuclear proto-oncogenes such as c-fos and c-jun is thought to be important in regulating proliferation and differentiation in many cell types (de Groot 1990, Sugio 1988, Souleimani 1993, Tang 1995, Yang-Yen 1990, Nishina 1993). C-Jun encodes for one of the components of the transcription factor AP-1 (Angel 1988), which binds to regulatory sequences upstream of many cellular promoters and is important in regulating the transcriptional activation of these genes (Angel 1988, Halazonetis 1988). The c-jun/AP-1 homodimer binds weakly to the DNA regulatory sequences, but the binding affinity of AP-1 for its cognate sequence is dramatically increased when heterodimerization with c-fos occurs (Halazonetis 1988, Busch 1990). Thus, the c-fos/c-jun heterodimer has a much higher affinity to the AP-1 DNA enhancer sequence than the c-jun homodimer, whereas c-fos alone is not able to form homodimers and cannot bind to DNA on its own (Halazonetis 1988, Busch 1990). Many studies indicate that c-jun (but not c-fos) is the component of the AP-1 which induces cell differentiation (de Groot 1990a, Nishina 1993, Yamaguchi-iwai 1990, Yang-Yen 1990, Angel 1988). Differentiation is tightly linked to growth arrest particularly within the colonic crypt as cells move from base to surface (Colony 1989). Therefore, it is interesting that the differentiating agent and inhibitor of malignant and pre-malignant proliferation, butyrate predominantly induces c-jun whereas the tumor promoter deoxycholate predominantly induces c-fos. Further studies are required to determine if these differential effects on c-fos and c-jun expression in fact explain butyrate's and deoxycholate's potential protective and promotive effects on proliferation. The study of effects on proto-oncogene expression may increase our understanding of the *in vivo* mechanisms by which diet by-products affect proliferation.

3. BUTYRATE IN COLON CANCER TREATMENT: MECHANISTIC ROLE OF MEVALONATE METABOLISM

Carcinoma of the colon is the third most common cancer in the United States, with more than 150,000 new cases and 60,000 deaths per year (Boring 1994). Liver metastases complicate colorectal carcinoma in 20–30% of patients (Scheele 1990) and at least 30% of patients undergoing intended curative resections of hepatic tumors succumb to metastatic disease (Moertel 1995). As described earlier in this text, butyrate modulates proliferation and differentiation of normal and neoplastic colonocytes and regulates expression of various proto-oncogenes and adhesion molecules which are important in determining colonocyte biologic behavior and metastatic properties (Young 1994, Velázquez 1996 a,b,c). Butyrate inhibits growth and enhances differentiation in many neoplastic cell types (Kruh 1994), and these findings have led to treatment studies of butyrate in patients with leukemia (Newmark 1994). Whether these effects occur *in vivo* or have clinical relevance in the treatment of colorectal carcinoma in humans is unknown.

Mevalonate (a precursor in the synthesis of cholesterol and all isoprenoids) is another short-chain fatty acid which is functionally disparate but structurally similar and metabolically related to butyrate (Velázquez 1996b,c). Mevalonate or metabolic interme-

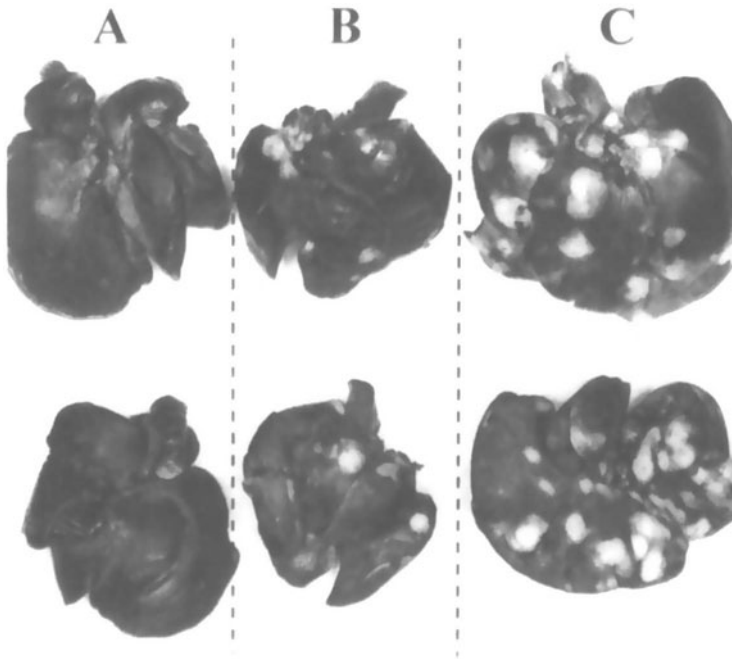

Figure 4. Photograph from livers of three representative study animals: A) no tumor B) tumor plus butyrate 2g/kg/d × 7 days, C) tumor plus PBS. B and C received 1×10^5 MC 26 cells implanted 24 hours after the start of treatment. Adapted and reproduced with permission (Velázquez 1996 b).

diates of mevalonate are essential for the activation of ras proteins that are important in signal transduction cascades leading to cell growth (Schafer 1989, Maltese 1990, Khosravi 1992). Within the colonocyte, the oxidative metabolism of butyrate (Roediger 1994) may yield metabolic intermediates for the synthesis of 3-hydroxy-3-methylglutaryl coenzyme A, the precursor for mevalonate synthesis. The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors such as mevastatin, inhibit mevalonate synthesis and may have significant *in vivo* anti-tumor activity (Yu 1995).

Despite extensive *in vitro* data documenting the growth inhibitory and differentiating effects of butyrate in neoplastic colonocytes (Chung 1985, Young 1994), few *in vivo* studies have investigated these responses (Otaka 1989, Perrin 1994). Moreover, the possible mechanisms of action for the selective antineoplastic effects of butyrate are poorly understood. We recently investigated butyrate's antineoplastic effects, in an *in vivo* model of colorectal carcinoma metastatic to the liver, and in subsequent *in vitro* studies, we examined the hypothesis that butyrate's anti-proliferative effects may be linked to the inhibition of mevalonate-mediated cell growth (Velázquez 1996b). Hepatic metastases were induced by injecting 1×10^5 MC-26 (N-methyl-N-nitrosourea-induced murine colorectal carcinoma) cells into the spleen of BALB/c mice. Mice were treated with a continuous intravenous infusion of butyrate (2 g/kg/d) for 7 days, starting 24 h prior to tumor cell injection. Study variables included liver weight and number of hepatic surface metastases. Butyrate treatment resulted in decreased seeding and growth of MC-26 cells within the liver (Figures 4–6). Proliferation studies on MC-26 cells were performed *in vitro* to examine the effects of butyrate alone, or combined with mevalonate or mevastatin (an inhibitor of mevalonate synthesis). Butyrate inhibited DNA synthesis in a concentration dependent manner with

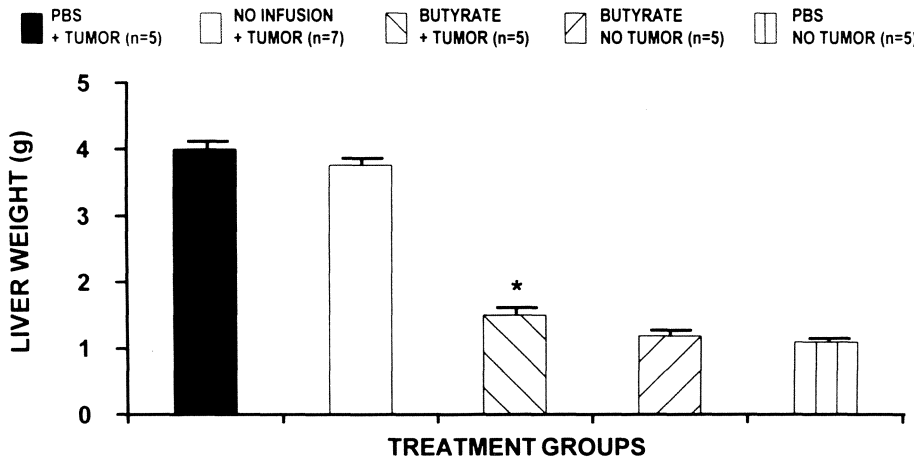

Figure 5. Liver weight of study animals. Data expressed as mean ± SEM; *p ≤ 0.001 (versus groups receiving tumor plus either PBS or no infusion) by ANOVA. Adapted and reproduced with permission (Velázquez 1996 b).

50% inhibition at concentrations of 0.5–2.0 mM and 100% inhibition at concentrations greater or equal to 8 mM (Figure 7). These effects were cytostatic and reversible upon withdrawal of butyrate from the media. Mevalonate alone did not significantly affect DNA synthesis or cell counts. Mevastatin alone showed only a 10–35% inhibition of DNA synthesis and decrease in total viable cell counts (Figure 8) at 64μM. Incubation of MC-26 cells with butyrate and mevalonate reduced butyrate's cytostatic effects (1.9×10^5 mean viable cells after 5 day co-incubation with 0.5mM butyrate plus 2.5mM mevalonate versus 8.4×10^4 cells with 0.5mM butyrate alone, experiment repeated 4 times, p=0.001). Co-incubation with butyrate plus mevastatin produced synergistic cytostatic effects (Figure 8), with over 90% reduction in total viable cell count after 5 day co-incubation with 0.5 mM

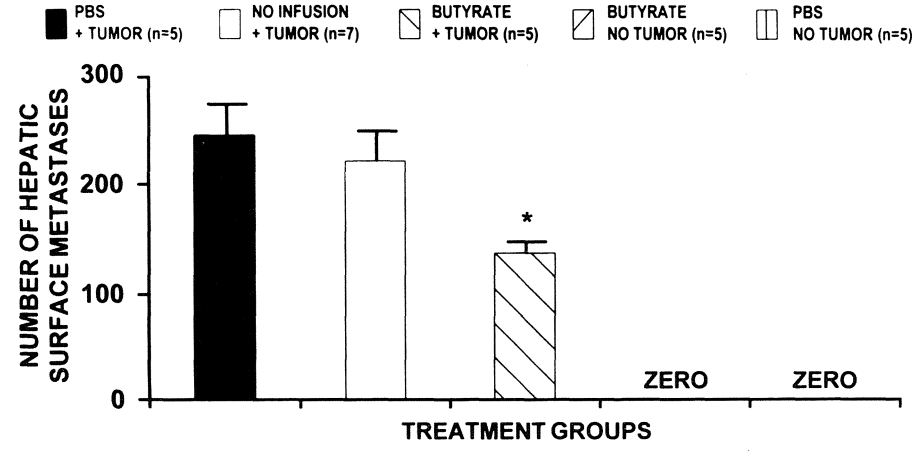

Figure 6. Number of hepatic surface metastases of study animals. Data expressed as mean ± SEM; *p = 0.027 (versus groups receiving tumor plus either PBS or no infusion) by ANOVA. Adapted and reproduced with permission (Velázquez 1996 b).

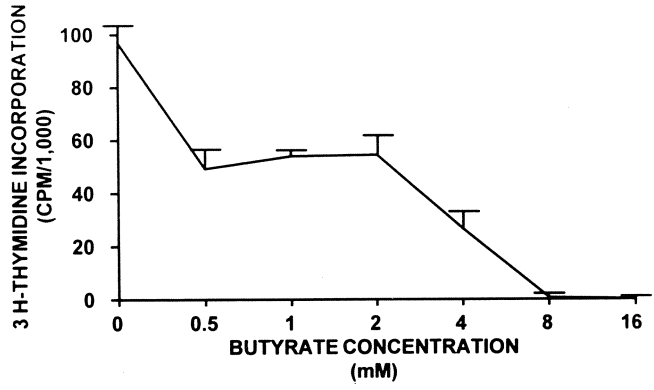

Figure 7. ^{3}H-Thymidine incorporation in MC26 cells after 24 hrs of incubation with butyrate at different concentrations (0–16 mM), 1 × 10^{6} cells plated. Data expressed as mean ± SEM. Experiment repeated 4 times in triplicate. Adapted and reproduced with permission (Velázquez 1996 b).

butyrate plus 64 μM mevastatin compared with complete media or either agent alone (Figure 8). These studies 1) demonstrate that butyrate inhibits seeding and growth of hepatic colorectal metastases *in vivo*, 2) associate butyrate's anti-proliferative effects with inhibition of mevalonate-mediated cell growth, and 3) indicate that HMGCoA reductase inhibitors may have synergistic anti-proliferative effects when combined with butyrate.

The experimental model in our study (Velázquez 1996b) is relevant to the human disease since the liver is the "preferred" organ for colon cancer metastases, and hepatic metastasis are particularly resistant to chemotherapy and contribute significantly to patient mortality (Gutman 1995). Moreover, a model that uses immunocompetent hosts with syngeneic metastases is important in the study of metastatic disease since host immune responses are integral factors which can modify the biologic response of metastatic disease and chemotherapeutic agents (Goldrosen 1980, Perrin 1994). A treatment regimen

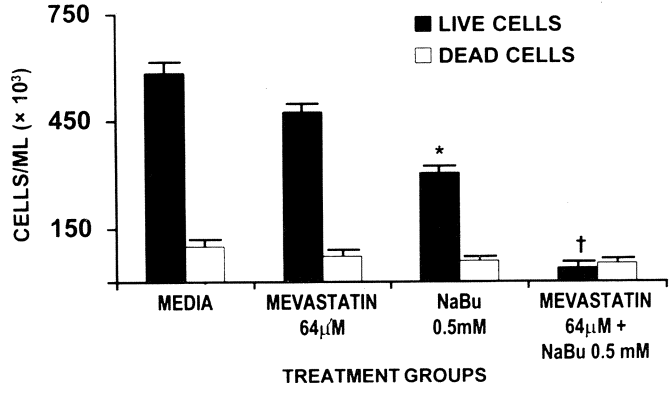

Figure 8. Viable and dead cell count after 5 day incubation with complete medium alone, or complete medium plus: butyrate alone, mevastatin alone, or butyrate plus mevastatin, at the specified concentrations. 5 × 10^{5} cells plated; experiment repeated 4 times in triplicate Data expressed as mean ± SEM; *$p \leq 0.05$ (versus media), $^{\dagger}p \leq 0.001$ (versus media, mevastatin, and NaBu (butyrate) alone) by ANOVA. Adapted and reproduced with permission (Velázquez 1996 b).

that starts 24 h prior to tumor cell implantation optimizes the potential effects on hepatic tumor cell seeding since therapeutic drug levels are present at the time of tumor cell inoculation. Effects on hepatic tumor growth are also optimized since the treatment is administered throughout the period of micrometastatic growth. Further studies are required to determine if the observed anti-tumor effects *in vivo* will prolong host survival in this experimental model. The analogous clinical counterparts of this experiment are: (1) the presumed entrance of tumor cells into the mesenteric circulation at the time of primary cancer resection, leading to metachronous micrometastases, and (2) the undetectable synchronous micrometastases to which current adjuvant chemotherapy is targeted.

Our recent findings (Velázquez 1996b) are consistent with published *in vitro* studies identifying butyrate as an inhibitor of malignant colonocyte growth in many different cell lines (Chung 1985, Young 1994). Our findings also agree with prior *in vivo* studies in different colorectal cancer models. These *in vivo* studies have shown antineoplastic effects of butyrate alone or combined with other agents in nude mice with subcutaneous tumors (Otaka 1989), and rats with syngeneic colon cancer intraperitoneal tumors (Perrin 1994). Otaka, *et al* selectively delivered butyrate to targeted tumor tissue by using a monoclonal antibody (Otaka 1989). Perrin, *et al* observed that butyrate plus interleukin-2 synergistically produced profound anti-tumor effects under conditions where neither agent alone was effective (Perrin 1994). In the aforementioned study, it is possible that butyrate alone (IP injections) did not affect tumor growth because of the dose and method of administration used. Sodium butyrate has a short metabolic half life of about six minutes which results in peak blood levels below 0.05 mmol/L when given in concentrations of 500 mg/kg/d continuous IV infusion (Miller 1987). The *in vitro* antineoplastic effects of butyrate occur at concentrations of 0.5 mM to 16 mM (Young 1994); therefore, effective systemic therapy probably requires high-dose continuous intravenous infusion.

The mechanism by which butyrate decreases *in vivo* malignant cell seeding to the liver is unknown. We speculate that this effect may be related in part to *in vitro* butyrate-induced regulation of important adhesion/migration molecules (Young 1994, Kruh 1994, Perrin 1994, Velázquez 1996b) which may influence the processes of: recognition by the immune system, adhesion to the endothelium within the liver capillaries, and extravasation into the hepatic parenchyma. Butyrate induces *in vitro* expression of ICAM-1 in a rat colon carcinoma cell line and this effect is positively correlated with *in vitro* increases in tumor cell immunogenicity and *in vivo* increases in antineoplastic activity (Perrin 1994). Butyrate decreases urokinase secretion, increases the expression of cell surface glycolipids, and decreases adhesion to laminin (Velázquez 1996c). Each of these effects of butyrate is associated with decreased metastatic potential of tumor cells in experimental systems.

The mechanism by which butyrate reduces growth of hepatic metastatic nodules *in vivo* is also unknown. Our *in vitro* studies in MC-26 cells suggest that one of the mechanisms by which butyrate inhibits neoplastic colonocyte growth may be through inhibition of mevalonate or a mevalonate intermediate. While mevalonate is similar in structure and related metabolically to butyrate, it has opposite effects on malignant cell proliferation. Whereas butyrate inhibits growth in many malignant cell types (Khruh 1994, Young 1994), mevalonate is essential for mediating cell growth in both normal and malignant cells (Gregg 1986, Schafer 1989, Maltese 1990, Khosravi 1992). We speculate that in the neoplastic colonocyte, mutated signal transduction proteins (e.g. ras proteins) may have altered binding affinity to butyrate, mevalonate, or mevalonate intermediates, leading to butyrate inhibition of mevalonate-mediated cell growth. Mevalonate is the precursor for the synthesis of all isoprenoids which are required for the post-translational modification of proteins including p-21 ras, prelamin A and lamin B, each of which plays an essential

role in signal transduction and proliferation (Gregg 1986, Maltese 1990). Our findings that butyrate's growth inhibitory effects on MC-26 cells are attenuated by mevalonate and potentiated by the HMGCoA reductase inhibitor mevastatin are consistent with our hypothesis linking butyrate's effects with inhibition of mevalonate-mediated cell growth. These are novel findings and further studies are required to confirm this metabolic link and to characterize the specific molecular sites of the interaction. If these effects are also present *in vivo*, they will establish the scientific basis for the study of a new chemotherapeutic approach in the treatment of advanced metastatic colon cancer. Moreover, since butyrate has *in vitro* anti-neoplastic effects in many different malignant cell types, these findings suggest the need for further studies to characterize butyrate's potential as a chemotherapeutic agent in other types of cancer.

4. CONCLUSIONS

The many effects of butyrate on the colonocyte probably reflect its ability to both specifically and nonspecifically affect genomic expression. The study of butyrate's varied effects may clarify the process of colorectal carcinogenesis at a molecular level. Future work is needed to evaluate the *in vivo* "balance" between colonic co-carcinogenic substances such as secondary bile acids and potentially protective agents such as butyrate, which can be modulated by dietary interventions. Additionally, further investigations are needed to identify the mechanism(s) of action of butyrate and to identify its role in the pathogenesis, prevention and treatment of colon cancer.

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19

EFFECT OF SHORT CHAIN FATTY ACIDS ON CALCIUM ABSORPTION IN HUMANS

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INTRODUCTION

Increased intake of dietary fiber is now being recommended for the prevention and management of chronic diseases. However, dietary fiber can bind with minerals and reduce mineral availability. We hypothesize that although some fibers may bind minerals in the small intestine, fermentation of the fiber in the colon releases the bound minerals which then are absorbed in the presence of the product of fermentation, short chain fatty acid (SCFA). Thus, fermentable fibers may not cause a reduction in mineral availability, but may shift a portion of the mineral absorption from the small intestine to the colon.

Calcium (Ca) has been shown to be transported and absorbed in the colon of animals (1–9) but little evidence is available in humans (10). SCFA, mainly acetic (Ac), propionic (Pr) and butyric (Bu) have been suggested to enhance the absorption of Ca and magnesium in the rat colon (1, 2, 11) but the mechanism by which SCFA increases the absorption of Ca is not clear and there is only indirect evidence that it occurs in man. This chapter describes some of our work which supports our hypothesis.

IN VITRO STUDY

Unabsorbable carbohydrates may differ in the degree to which they bind Ca in the small intestine and the degree to which they bind Ca in the colon. However, the site of Ca absorption cannot be easily determined in vivo, therefore, we used an in vitro method to estimate Ca availability in the small and large intestine by simulating conditions that prevail in the small intestine and colon (12, 13). We tested the effect of two fibers on the availability of Ca: (a) pectin which binds mineral in the small intestine but is fermentable in the colon; and (b) psyllium which can also potentially bind mineral in the small intestine but has limited fermentability in the colon. A basal meal consisting of milk, cheese,

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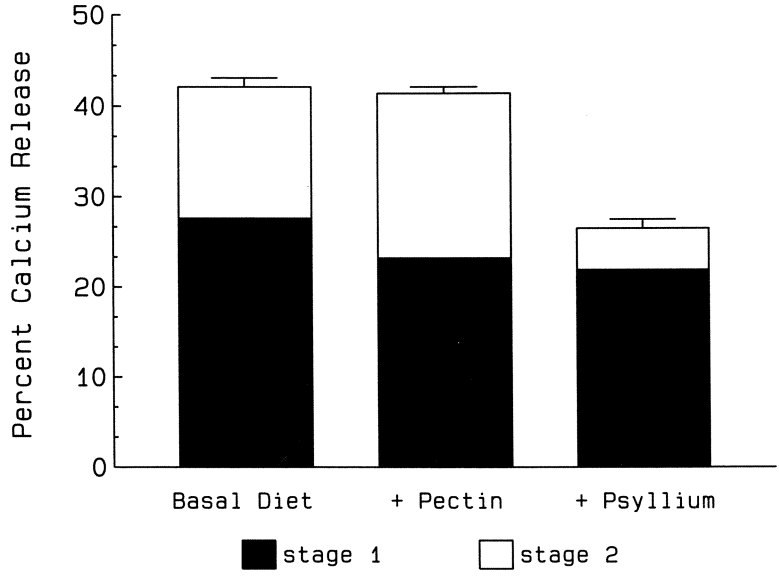

Figure 1. Calcium released after *in vitro* small intestinal digestion (stage 1) and colonic fermentation (stage 2).

apple juice and white bread with and without pectin or psyllium were digested with pepsin for 3 hours. Aliquots of the pepsin digest containing 5 mL of pancreatin-bile solution were dialyzed with 0.5 M sodium bicarbonate for 12 hours. The dialysate was collected every three hours and replaced with double distilled water. The dialysate was analyzed for Ca using the atomic absorption spectrometer to estimate the Ca released under small intestinal conditions. The retentate was freeze-dried and fermented using human faecal inoculum. The fermentation digest was dialyzed with double distilled water for 6 hours and the dialysate analyzed for Ca content to estimate the Ca released under colonic conditions.

Results showed that both pectin and psyllium caused inhibition in the release of Ca under small intestinal conditions but fermentation of pectin caused the release of Ca resulting in the total Ca released being equal to that of the control (Figure 1). On the other hand, the less fermentable psyllium continued to bind the Ca under colonic conditions and thus the overall Ca release was lower than that of the control. Thus we conclude that fermentable fibers such as pectin have no effect on total Ca availability. They tend to shift the Ca release potentially absorbable in the small intestine to the colon. The Ca released ranged from 21–28% under small intestinal conditions and 4–18% under colonic conditions. However, these in vitro results only indicate how much Ca might potentially be available for absorption; they do not necessarily mean that available Ca is actually absorbed. Therefore the next question was whether the Ca released in the colon is absorbed and whether Ca absorption is affected by the presence of SCFA.

IN VIVO STUDY

To study colonic Ca absorption in humans, we used a rectal infusion method which essentially measured the Ca absorption in the rectum and distal colon. Based on the average

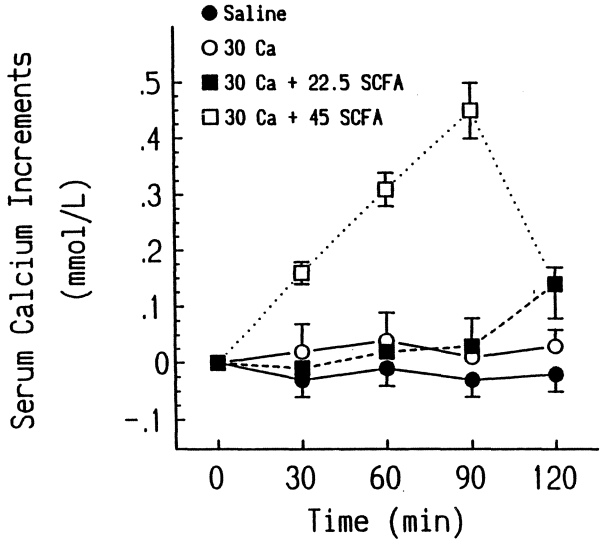

Figure 2. Mean±SEM serum calcium increments of six healthy subjects after rectal infusion of solution containing sodium chloride or 30 mmol calcium chloride with and without short chain fatty acids. This figure was adapted from reference #17.

total length of the colon (110 cm with the distal segment comprising 1/3 of the total length) (14, 15) and the diameter of the rectum and distal colon (4 cm) (16), the volumes of the rectum and distal colon are approximately 150 and 460 mL, respectively. The volume of the rectal infusion solution used in our studies ranged from 300–600 mL. Therefore, a significant amount of the infused solution would be expected to reach the distal colon. The rectal infusion apparatus consisted of a 1 m Tygon plastic tubing with one end (around 5 cm) inserted into the rectum and the other end connected to a 3-way valve to which are attached a 60 mL syringe (containing infusion solution) and a 10 mL syringe used to mixed the colonic contents (by withdrawing and infusing) before a sample is collected.

To determine the influence of SCFA on serum Ca response as an index of Ca absorption, we studied healthy subjects who were given in random order rectal infusions of isotonic solution of saline with or without Ca and graded doses of SCFA (17). The treatment solutions (600 mL) infused into the rectum at a rate of 30 mL/min were as follows: 45 mmol sodium chloride (NaCl); 30 mmol calcium + 45 mmol NaCl; 30 mmoles Ca + 22.5 mmol SCFA (15 mmol Na acetate + 7.5 mmol Na propionate); 30 mmol Ca + 45 mmol SCFA (30 mmol Na acetate + 15 mmol Na propionate); 10 mmol Ca + 45 mmol SCFA; and 45 mmol SCFA. Results showed that serum Ca did not change after 30 mmol Ca alone but significantly increased after addition of high dose of SCFA ($P<0.05$; Figure 2). Although the mean 30–120 min increment was not increased after adding 22.5 mmol SCFA to 30 mmol Ca, at 120 min a significant increase in serum Ca was observed. The serum Ca response for the treatment with 10 mmol Ca + 45 mmol SCFA did not differ significantly from that with 30 mmol Ca + 22.5 SCFA but significantly differed from the saline control solution. These results suggest that Ca is absorbed in the colon and this absorption is enhanced by SCFA.

Since the serum Ca response is an indirect measure of Ca absorption and also the SCFA used in this study is a mixture of Ac and Pr in a 3:1 molar ratio, we studied another group of healthy subjects to investigate the differential effect of Ac and Pr on Ca absorp-

Table 1. The effect of Ac and/or Pr on Ca absorption in the rectum and distal colon of man (Ca disappearance expressed as Ca/PEG ratios)

	Time (min)				
Treatment	0	10	20	30	Total
Study1					
Ca+NaCl	0^{ax}	-1.9 ± 0.1^{bx}	-2.3 ± 0.8^{cx}	-4.0 ± 1.0^{dx}	-5.5 ± 1.4^{cx}
Ca+3Ac	0^{ax}	-3.0 ± 1.0^{bx}	-8.6 ± 2.7^{cx}	-18.3 ± 5.5^{dy}	-22.6 ± 2.8^{dy}
Ca+1Pr	0^{ax}	-2.0 ± 0.7^{bx}	-5.8 ± 1.5^{cx}	-13.4 ± 3.1^{dy}	-23.2 ± 3.2^{cy}
Ca+3Ac+1Pr	0^{ax}	-6.0 ± 3.5^{bx}	-9.6 ± 3.5^{bx}	-10.2 ± 4.7^{by}	-19.7 ± 4.6^{cy}
Study 2					
NaCl	0^{ax}	$-.02 \pm .01^{bx}$	$-.06 \pm .02^{bcx}$	$-.05 \pm .02^{bcx}$	$-0.1 \pm .04^{cx}$
Ca+1Ac	0^{ax}	-2.4 ± 1.1^{bxyz}	-6.6 ± 1.7^{cy}	-11.1 ± 1.2^{dz}	-14.4 ± 0.4^{cyz}
Ca+1Pr	0^{ax}	-4.9 ± 1.1^{byz}	-7.2 ± 1.4^{by}	-10.8 ± 1.3^{cz}	-15.7 ± 1.4^{dyz}
Ca+3Ac	0^{ax}	-1.2 ± 0.3^{bxy}	-3.2 ± 0.6^{cxy}	-4.3 ± 0.6^{cxy}	-11.2 ± 3.6^{dy}
Ca+3Pr	0^{ax}	-6.5 ± 1.5^{bz}	-8.2 ± 1.5^{by}	-10.9 ± 1.8^{bz}	-20.3 ± 2.3^{cz}
Ca+1Ac+3Pr	0^{ax}	-2.1 ± 0.6^{bxyz}	-5.2 ± 1.4^{cxy}	-7.0 ± 2.0^{cyz}	-13.0 ± 1.1^{dyz}

abcde represents significant differences between time at different treatments within a study, ($P<0.05$).
xyz represents significant differences between time within a study, $P<0.05$).
Total represents absorption in the distal colon and rectum while 30 min represents absorption in the rectum alone. This table was adapted from reference #18.

tion in the distal colon of humans using actual disappearance of Ca as an index of absorption (18). Subjects were given a low fiber diet consisting of spaghetti and sauce the night before the study and were made to fast overnight. The following morning they were made to clean their colon by infusing an enema of 500 mL of double distilled water and immediately after they were given rectal infusions of 300 mL solution containing 50 mmol/L Ca with or without 56.3 mmol/L Ac or 18.7 mmol/L Pr or both plus polyethylene glycol (PEG) as an unabsorbable marker. Ca disappearance was used as an index of absorption and was expressed as the difference between the ratio of Ca/PEG in the baseline sample (0 min) and the total colonic sample collected at 30 min. Ca disappearance was significantly higher in treatments with Ac or Pr or both compared to the control (Table 1, study 1). The concentration of Ac was 3× that of Pr but both had the same effect on Ca disappearance suggesting that Pr may be more involved than Ac in enhancing Ca absorption in the distal colon of humans. When another group of healthy subjects were given similar rectal infusions as above or Ca with Pr concentration 3× that of Ac, results showed that at higher concentration, Pr was more effective than Ac in enhancing Ca absorption while at lower concentration both Ac and Pr were equally effective (Table 1, study 2) (18). This indicates that Ac and Pr may have different transport mechanisms in enhancing Ca absorption. Moreover, Ca absorption was significantly higher from the rectum and distal colon (estimated from total colonic sample collected immediately after the 30 min sample) than that from the rectum alone (estimated from samples collected at 30 min) suggesting that there were segmental differences in the absorption of Ca in the colon.

To characterize the transport mechanism involved in Ca absorption alone as well as in the presence of Ac or Pr, the relationship between Ca concentration and the rate of Ca absorption with and without Ac or Pr was investigated. Healthy human subjects were given rectal infusions of 300 mL of different Ca concentrations (4, 11, 24, 46 mmol/L). Each Ca concentration was tested with saline, 56.3 mmol/L Ac or 56.3 mmol/L Pr. Thus, there were a total of 12 treatments tested by each subject in random order. Samples were collected at 0 and 30 min.

Ca absorption in the absence or presence of Ac or Pr significantly increased at different concentrations of Ca ($P<0.05$). The treatment with either Ac or Pr was almost two times greater than that of the control for all concentrations ($P<0.05$). At 4.0–24 mmol/L Ca, Ac and Pr were equally effective in enhancing Ca absorption. However, at higher concentrations of Ca (46mmol/L), a higher absorption was seen with Pr than with Ac indicating that Pr was more effective than Ac. This result was similar to that seen in our previous study (18). For all treatments, the absorption of Ca was linearly related to Ca concentration indicating that across the concentrations of Ca studied, the absorption of Ca with and without Ac is by a non-saturable diffusion process. However, the rate of absorption as indicated by the slopes of their curve was higher in the presence of Ac (123 umoles/30 min/mM Ca)and even greater with Pr (179 umoles/30 min/mM Ca) than with Ca alone (67 umoles/30 min/mM Ca).

DISCUSSION

The above studies have shown that Ca bound by fermentable fiber can be released and absorbed in the rectum and distal colon of humans. This process is enhanced by SCFA such as Ac and Pr, Pr being more effective than Ac. It was also shown that across the concentrations of Ca studied, Ca with and without Ac or Pr is transported by a non-saturable diffusion process. We propose three possible mechanisms of absorption: (a) Ca can be absorbed by a Ca/H exchange similar to that proposed by Lutz and Scharer (1) in the distal colon of rats. The carbon dioxide that diffuses from the blood to the mucosal cells reacts with water to form carbonic acid which in the presence of carbonic anhydrase form bicarbonate and hydrogen ions. The intracellular hydrogen ion is then secreted outside the cell (which is in the lumen) and exchanged with Ca for absorption. However, the activity of the carbonic anhydrase declines towards the distal colon and rectum; (b) Ac and Pr are absorbed in the colon in the dissociated form (19–22). They are protonated by the hydrogen ion produced from the luminal hydration of carbon dioxide or secreted to the lumen. Because HAc and HPr have pKa values that are lower than that of the pH of the cell, they tend to ionize into hydrogen ion and Ac or Pr. The hydrogen ion produced is secreted outside of the cell in exchange for Ca. The presence of Ac or Pr may then enhance the production of hydrogen ion needed by Ca for absorption. The ability of Pr to be more effective than Ac may be related to its being more lipid soluble. The longer the SCFA, the more lipid soluble it is and the faster it diffuses into the colonocyte membrane. Therefore, Pr may supply more hydrogen ion needed by Ca for absorption; and (c) Ac can form a complex with bivalent cation such as Ca i.e. $[CaAc]^+$ (23). This complex is less charged than that of the Ca ion and since less charged ion has higher permeability to enter the cell, it is likely that the complex passes more readily through the cell than the Ca ion. However, the affinity of the $[CaAc]^+$ complex over that of HAc or HPr in passing through the colonocyte membrane is unknown.

The presence of Ca in the colon may have some important implications. When Ca is absorbed in the colon, it helps maintain the rate of cell turnover, thus maintain colonic health (24–26). However, an extensive change in intracellular Ca concentration can lead to changes in physiological behaviour of the cell (27). A rise in cytosolic Ca can cause cell aggregation, cell transformation, cell division and activation of intermediary metabolism which are related to cell proliferation (28). Extensive cell proliferation has been associated with increased cancer risk (23, 26, 29). Therefore Ca absorption in the colon may help maintain colonic health but extensive absorption my also be damaging to the colon. On the other hand, Ca present in the colon can bind with bile acids and fatty acids render-

ing them insoluble and less damaging to the colon (29, 30). Therefore, the Ca that reaches the colon may exert a protective effect on colon cancer.

In conclusion, the presence of fermentable fiber has no effect on total calcium availability; it tends to shift calcium release potentially absorbable in the small intestine to the colon. Ca is absorbed in the rectum and distal colon of humans and that both Ac and Pr enhance Ca absorption, with Pr being more effective than Ac. Ca absorption in the human distal colon is linearly related to Ca concentration either alone or in the presence of Ac or Pr indicating that it involves a non-saturable diffusion process.

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INFLUENCE OF SHORT CHAIN FATTY ACIDS ON INTESTINAL GROWTH AND FUNCTIONS

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1. INTRODUCTION

1.1. Significance of the Organ Size of the Large Intestine

1.1.1. Surface versus Volume Ratio of the Large Intestine. The enlargement of the large intestinal lumen expands the living space for bacteria. If the organ grows without modifying the shape by haustration or by other means, the surface/volume ratio should decrease by the growth of the organ. If we assume that microbial metabolism per unit volume of microbes is more-or-less homogenous, then the microbial metabolism per organ increases as a linear function of its volume. However, the absorptive surface, which increases only at 2/3 powers of the volume, cannot catch up with the increase in microbial metabolic rate per organ. Thus, there should be a theoretical limit where the rate of absorption for microbial products cannot meet with the rate of production. This should increase the lumen concentration of microbial products.

1.1.2. Lumen Diameter and Microbial Ecosystem. Widening of the diameter of an intestinal segment increases the mean distance of diffusion from the lumen contents to the mucosa to make the lumen environment more heterogeneous and to provide more widely varied habitat for bacteria. This may partly explain why the number of bacterial species in the large intestine increases during the postnatal growth (1).

1.1.3. Size of the Large Intestine and Transit Time in the Organ. Larger fermentation chamber extends the transit time of the contents unless the flow rate is changed. Elongated retention should facilitate more thorough microbial breakdown of digesta. Thus, the lumen volume of the proximal large intestine by itself should have a significant effect on bacterial composition and their overall metabolism.

Accordingly, the gross size of the large intestine should be one of the major determinants of the large intestinal function including microbial breakdown of dietary fibers.

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1.2. Postnatal Growth of the Large Intestine

1.2.1. Bi-Phasic Growth of the Large Intestine. The most striking postnatal change of the large intestine should be its rapid increase in tissue mass during the suckling period (Sakata & Setoyama, unpublished results).

The mass growth rate of large intestinal segments (cecum, and proximal, middle and distal colon) is 1.7 to 2.6 times faster than that of the whole body during the suckling period in rats. Then, the mass growth rate suddenly slows down to 0.5 to 0.7 times of body weight gain after weaning.

There is a tendency that the more proximal part of the large intestine grows more rapidly in suckling animals and more slowly after the weaning. The mass growth rate of the small intestine during the suckling period is slower than that of the large intestine (1.5 times as that of the whole body) and becomes indifferent from that of the large intestine after the weaning.

The above facts reflect that the mass proportion of the large intestinal tissue in the whole body dramatically increases during the suckling period and continuously decreases after the weaning.

1.2.2. Meaning of Weaning for the Large Intestine. Weaning is a transit of the food from highly digestible mother's milk to a variety of food components including materials resistant to human digestive enzymes. Thus, weaning should increase the entrance of undigested materials to the large intestine and thereby increase the load on that organ.

1.2.3. Possible Involvement of SCFA on Intestinal Growth. Mechanism that regulate such a drastic change in the intestinal growth is not yet clarified. Weaning accompanies the introduction of solid materials and various fermentation substrates to the large intestine. Accordingly, it may be possible that bacterial metabolism in the large intestine stimulates the postnatal growth of the large intestine. However, there is so far no available information on how SCFA concerns the rapid growth of the intestine during the suckling period.

2. BACKGROUND EVIDENCES THAT SUPPORT TROPHIC EFFECT OF SCFA

2.1. Intestine of Germfree and Conventional Animals

Germfree rats that lack gut microbes and their production of SCFA have enlarged, however, lighter colon (2, 3). It is interesting that the tissue mass of the proximal and distal colon is heavier in conventional than in germfree rats but not in the middle (Sakata & Setoyama, unpublished results).

Epithelial cell proliferation is less active in small and large intestine of germfree rats than in conventional animals (4, 5).

These results suggest the trophic effect of gut microbial metabolism and probably of the metabolic products of hindgut bacteria.

2.2. Epithelial Cell Proliferation Depressed by the Lack of Fermentation Substrate

The lack of fermentable fiber in the diet (6) or total parenteral nutrition (7, 8) depresses epithelial cell proliferation of rat colon suggesting the importance of fermentable fiber to maintain normal level of epithelial cell proliferation of the colon.

2.3. Epithelial Cell Proliferation of Rats after Hindgut Bypass Surgery

Crypt cell production rate is lowered in rats with its cecum and colon bypassed (9). The lack of substrates for bacterial fermentation depresses the epithelial cell proliferation both in the small and large intestine, although the intake level of the dietary fiber was indifferent from control animals. Further, the above results indicate that the effect of bacterial metabolism in the large intestine is not restricted to the large intestine but can be mediated to other organs.

2.4. Scope of This Chapter

As stated above, conditions that avoided the presence of hindgut bacteria (germfree animals), substrates for these bacteria or substrate entrance into the bacterial habitat all reduce the proliferative activity of intestinal epithelial cell, suggesting the role of bacterial metabolite, namely SCFA, in maintaining normal tissue mass and epithelial cell proliferation of the intestine. Accordingly, the present paper focuses on influences of SCFA on tissue mass growth of the intestine and on epithelial cell production rate of the intestinal tract in weaned mammals including humans.

3. INFLUENCE OF SCFA ON INTESTINAL TISSUE MASS

3.1. Influence of SCFA on Tissue Mass

Infusion of a mixture of SCFA (acetic 100, propionic 20 and butyric 60 (mM), 3 ml, twice a day) for 2 weeks increases the wet tissue mass relative to empty body weight of the cecum but not of the distal colon of rats fed an elemental diet (10). Further, intracecal infusion of a mixture of SCFA for 36 days increased the cecal tissue mass in the rat but pH or co-existing succinic acid had no effect on the tissue mass (11).

A recent study also demonstrated that the instillation of butyrate to defunctioned distal colon increased the mass of the segment (12). Above results suggest that the effect of lumenally infused SCFA to increase gut tissue mass is confined to the site of administration.

3.2. Influence of SCFA on Mucosal and Non-Mucosal Tissues

The intestinal wall consists of mucosal and non-mucosal tissues. The latter includes submucosa, muscular layers, serosa and accompanying vascular and neural tissues. They vary in developmental origin and responses to dietary stimuli. For instance, SCFA stimulates mucosal development and physical stimulation stimulates muscular development of the rumen, another major gut fermentation organ (13, 14).

3.2.1. Influence of SCFA on Mucosal Growth. It is sure that SCFA stimulate mucosal growth. Kripke et al. (15) showed that intracolonic infusion of butyric acid stimulated mucosal growth of atrophied colon. They also found that hindgut infusion of SCFA stimulated mucosal growth in the ileum and increased mucosal DNA in the jejunum and ileum. Thus, SCFA in the hindgut increase the mucosal tissue mass of the large intestine as well as the mucosal cell number of distant gut segments. It is very likely that the effect of SCFA on the distant mucosa is mediated by a systemic mediatory mechanism.

3.2.2. Trophic Effect of SCFA on Non-mucosal Tissue. Trophic effect of SCFA is not restricted to the mucosa. Intracolonic infusion of SCFA enhanced the healing of a colonic anastomosis and increased the bursting strength of the anastomosis, suggesting the effect of SCFA on submucosal tissue and collagen synthesis (16). Further, a recent study (12) demonstrated that daily instillation of a mixture of sodium acetate, sodium propionate and sodium n-butyrate into isolated and defunctioned rat distal colon for 14 days increased the tissue mass of not only the mucosa but also the submucosa and muscularis externa of the distal colon.

Unfortunately, the mechanism for such a trophic action of SCFA on non-mucosal tissue has not been studied.

4. INFLUENCE OF SCFA ON EPITHELIAL CELL PROLIFERATION

4.1. Crypt Fission and Epithelial Cell Division

Epithelial cell division is confined to the crypt in normal mammalian intestinal epithelia. In other words, intestinal crypt can be considered as a proliferative unit for the epithelium. Accordingly, there are two ways to regulate the rate of epithelial cell supply for an intestinal segment. One is to change the number of proliferative unit, the crypt. Another way is to change the cell production rate per crypt.

4.2. Influence of SCFA on Crypt Fission

Studies on the influence of SCFA on crypt fission is scarce. The percentage of dividing crypts reaches the peak (25% in the small intestine, and 52% in the colon) at 21 days after birth in rats (17). This mechanism can be responsible for the very rapid growth of the small and large intestine during the suckling period (see above).

As the crypt fission is a relatively slow process that takes 5–6 days in rats (18), it is not easy to detect changes in crypt fission rate in short-term studies.

We have not detect any changes in percentage of crypts in fission when we incubated isolated pig colonic mucosa for 24 hours (Sakata and Sato, unpublished results). Interestingly, crypt fission rate and crypt cell production rate (CCPR) negatively correlated in this study. This may indicate the existence of an overall regulatory mechanism to integrate the total epithelial cell production rate per segment both by crypt fission and cell production in the crypt.

4.3. Influence of SCFA on the Proliferation of Isolated Cells

4.3.1. Inhibitory Effect of SCFA on Cultured Cell Division. SCFA inhibit the proliferation of isolated cells including colorectal cancer cell lines in a dose-dependent manner (19, 20). This effect is reversible at lower doses and becomes irreversible at higher doses (20). The effect varies among acids: butyrate > propionate > acetate (20). Histone hyperacetylation seems to be responsible for this inhibitory effect (19).

However, recent studies suggest that SCFA may stimulate normal (not transformed) gut epithelial cells in vitro (21). On the other hand, SCFA inhibited cell proliferation of the primary culture of rumen epithelial cells (22). More studies are needed to integrate these apparently contradictory findings.

4.3.2. Stimulatory Effect of SCFA on Epithelial Cell Proliferation of Isolated Mucosa of Large Intestine. SCFA stimulate epithelial cell proliferation of human cecal or colonic biopsy specimens or of short-term culture of pig colonic mucosa within 3 hours (23, 24). Strength of the effect was in the order of butyrate > propionate > acetate (24). The effect of butyrate saturates at 10 mM. Neither low pH nor high sodium concentration stimulate colonic cell proliferation, suggesting that SCFA anions seem to be responsible for this trophic effect (24). It is not yet sure if this is a direct effect of SCFA on epithelial cells or an indirect effect mediated by a local mechanism involving paracrine or local neural reflex mechanism.

The time-course and dose-response relationship of the trophic effect of SCFA on epithelial cells of isolated hindgut tissue differ from those in vivo (see below). Thus, there is a reasonable possibility that trophic effect of SCFA on isolated hindgut mucosa is different from that on the hindgut mucosa in vivo.

4.3.3. Acute Stimulatory Effect of SCFA on Gut Epithelial Cell Proliferation in Vivo. Acute instillation of SCFA into a temporarily isolated segment of rat colon stimulates DNA synthesis and mitosis of the colon with or without direct contact with SCFA as well as of the esophagus, stomach, jejunum and ileum, but not of the ear epidermis within a few hours (25). This indicates that the effect of SCFA can be transmitted to distant sites and have tissue specificity. This acute effect requires both vagal and sympathetic nerve transmission.

The time-course of the above results suggests that this acute effect may be different from the chronic trophic effect of SCFA in vivo.

4.3.4. Stimulatory Effect of Daily SCFA Administration on Gut Epithelial Cell Proliferation in Vivo. Daily intermittent or continuous administration of SCFA into the cecum or colon stimulates epithelial cell proliferation of the small and large intestine (for review 26). Non-fermentable dietary bulk (kaolin) neither stimulate epithelial cell proliferation nor influence on the effect of SCFA (10).

The effect of SCFA is dose-dependent and vary among acids (acetic < propionic < n-butyric) (5). The effect appears within 1 to 2 days and lasts at least for 2 weeks (5, 26). It should be protonized SCFA or SCFA-anions that have the stimulatory effect, but not protons (5). However, there is a possibility that pH modulates the effect of SCFA.

This effect of daily SCFA administration is mediated by a systemic mediatory mechanism (26). The afferent transmission involves neural transmission, very likely cholinergic mechanism (27, 28). Efferent transmission to the gut does not require mesenteric neural transmission (29), suggesting either humoral or cellular transmission. Recent studies indicated that gastrin is a likely stimulator for the trophic effect of hindgut SCFA on jejunal epithelium (28, 30).

4.3.5. Does the Trophic Effect of SCFA Vary among Hindgut Segments? Some authors suggest that SCFA are trophic on cecal epithelium but not on the epithelium of the distal colon (31). The basis for this idea was the positive relationship between the lumen concentration of SCFA and proliferative indices in the cecum, but not in the distal colon, of rats fed various fibers. However, such an idea seems to be still arguable.

Firstly, lumen concentration is a poor predictor of the production rate of SCFA, especially when we consider the rapid absorption of SCFA from the large intestine. Further, the average lumen concentration can be rather different from that at the very surface of the mucosa. This is supported by the finding that SCFA concentration in the periphery of the digesta is lower than that in the center of the contents in the rat cecum and colon (32).

Secondly, SCFA given into the isolated rat cecum (25) into the colon of cecectomized rats (15) or isolated and defunctioned colon showed trophic effect at least on the site of administration (12). Therefore, all these segments have the potential to react to SCFA by increasing the proliferative activity of the epithelium.

4.4. Effect of SCFA on Cell Death Rate of Colonic Epithelium

Epithelial tissue mass is the result of a dynamic balance between the cell production and cell loss. There are two routes of cell loss for intestinal epithelial cells, desquamation and apotosis. Butyric acid stimulates apotosis of transformed cells (33, 34). However, effects of SCFA on intestinal epithelial cell loss rate in vivo, whichever route it is, is entirely unknown.

5. EFFECT OF SCFA ON MUCOSAL BLOOD FLOW

SCFA locally increase blood flow and oxygen supply of dog colon (35). SCFA can act directly on smooth muscle cells of the artery involving the fall in intracellular calcium (36). However, it is not yet sure if SCFA can reach the mucosal resistance artery at concentration exceeding the minimal effective concentration for the vasodilatation effect in vitro.

Increase in blood flow should support the increased epithelial cell mass by the tropic effect of SCFA by increasing the delivery of oxygen and nutrients.

6. EFFECTS OF SCFA ON WATER AND SOLUTE ABSORPTION FROM THE LARGE INTESTINE

6.1. Short-Term Effects of SCFA on Solute Absorption from the Colon

SCFA stimulate NaCl and water absorption, and bicarbonate secretion across the hindgut in vivo (37, 38).

SCFA also stimulates the absorption of ammonia from the colon (39). Both SCFA anion and ammonium ion may become unionized form by the transfer of proton from ammonium ion to SCFA-anion when they co-exist. This makes SCFA-anion and ammonium ion more lipid-soluble than their ionized forms and easier to cross mucosal barrier.

Recent studies showed that the colon is an important site for Ca and Mg absorption and the colonic absorption of Ca and Mg is enhanced by SCFA (40, 41).

It is not yet clear if the above mentioned vasodilator effect (see above) of SCFA facilitates the absorption of fluid or solutes.

6.2. Long-Term Effect of SCFA on Solute Absorption from the Colon

Absorption of sodium and glucose from the jejunum is increased when its epithelial cell proliferation is stimulated by hindgut SCFA (27, 37).

7. EFFECT OF SCFA ON MUCUS SECRETION FROM THE LARGE INTESTINE

Large intestinal mucosa is characterized by abundant mucus producing cells, goblet cells and vacuolated cells (42). Mucus from the cecum and the proximal colon may be

degraded by bacteria and very likely provide sulfur and other nutrients to the bacterial ecosystem. In the distal colon a continuous and tight mucus layer exists between the mucosal surface and feces very likely protecting and lubricating the mucosal surface (42).

7.1. Effect of SCFA on Mucus Biosynthesis in Colonic Epithelium

A recent study (43) demonstrated that sodium butyrate increases the mucin synthesis by normal colonic epithelium in vitro in a dose dependent manner, with maximum effect (476%) at a concentration of 0.1 mM. The increase in response to butyrate was not seen when specimens were incubated in the presence of the beta oxidation inhibitor sodium bromo-octanoate 0.05 M. The authors considered that this may be an important mechanism affecting the rate of mucin synthesis in vivo and may also explain the therapeutic effect of butyrate in colitis.

7.2. Effect of SCFA on Mucus Release from the Cecum and Colon

A mixture of SCFA instilled into the cecal or colonic lumen locally stimulates the mucus release in vivo (44).

8. MODE OF SCFA ACTION

As stated above, most in vivo effects of SCFA on the colon is the sum of direct, local indirect, and systemic effects. Accordingly, one should not directly extrapolate in vitro results to in vivo conditions.

Most effects of SCFA are dose-dependent and vary among acids. Thus, it is important to know which and how much acid do the large intestinal bacteria produce when we consider the influence of fermentable dietary fibers.

It is also to note that the receptive mechanism for SCFA in the large intestine is highly adaptable; the mechanism does not respond to the prolonged stimuli of SCFA unless the mechanism is washed free of SCFA (45). Thus, the effect of fermentable fibers may be affected by the feeding history of the host animal and may vary by the entry mode (i.e. continuous or intermittent) of such fibers into the fermentation chamber. In this regard, influence of histological structure and particle size of the diet that contains fermentable fiber should be more seriously discussed.

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RESISTANT STARCH — AN UPDATE ON ITS PHYSIOLOGICAL EFFECTS

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ABSTRACT

Resistant starch (RS) has emerged as one of the main substrates for colonic fermentation, together with other undigestible polysaccharides and oligosaccharides. There are indications that RS may be a good source of butyrate, and that the rate and site of fermentation can be varied and optimized. This makes RS potentially important for colonic health, and production of food products containing RS challenging. The present RS content in most Western diets is probably low, but can be increased by foods high in RS. The physiological effects of RS are reviewed, as well as the formation of RS in foods and its analysis.

1. INTRODUCTION

Resistant starch (RS) was first defined as starch remaining undigested together with the non-starch polysaccharides in dietary fiber analysis, in spite of an extensive amylase digestion *in vitro* aiming at a complete removal of the starch[1]. Previously, Anderson *et al.*[2] provided indirect evidence of incomplete starch digestion in bread from all purpose wheat flour in man, using the breath hydrogen test. Johansson *et al.*[3], and Siljeström and Asp[4] demonstrated that resistant starch, measured as starch remaining in a dietary fiber residue prepared by enzyme digestion for gravimetric determination, was formed during bread baking. The amount of RS in bread was generally around 1% of the dry matter. This RS was recovered in the feces of rats treated with antibiotics, demonstrating its resistance to digestion also *in vivo*[5].

Studies with ileostomists by Englyst and Cummings[6–8] provided direct evidence that starch in foods such as cereals, green banana and potato was incompletely absorbed in the

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human small intestine. The amounts of malabsorbed starch found *in vivo* were generally larger than the starch recovered in dietary fiber analysis. Thus, the starch determined as RS *in vitro* was only part of that escaping digestion and absorption in the human small intestine[9,10].

Based on these *in vivo* studies, Englyst and Kingman[10] extended the definition of RS to include all the starch that escapes digestion in the small intestine of man. A similar definition was adopted in a recent major European collaboration on the physiological effects of RS: "Resistant starch is the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals"[11]. This project, called EURESTA has recently been reviewed[12].

2. BASIS FOR RESISTANT STARCH FORMATION

Starch — the quantitatively most important source of dietary carbohydrate — is composed of two principal macromolecules, amylose and amylopectin. For a review on the structure of starch, see e.g. Gallant *et al.*[13] Amylose is an essentially linear polymer consisting of 1,4-α-D-anhydroglucose units arranged in a helix conformation with six glucose units per turn. The hydrophilic hydroxyl groups are on the external side of the helix, creating a hydrophobic interior cavity into which other molecules can be complexed, for instance the carbon chain of fatty acids (amylose-lipid complexes) and iodine (blue color).

Amylopectin has a branched structure with about 5% of the anhydroglucose units being 1,6-α-linked. The structure is a clustering of many short chains (12–70, mean 20 anhydroglucose units).

Native starch is stored in dense granules with variable size and shape characteristic of each starch synthesizing plant. Parts of the granules are crystalline and whereas other areas have an amorphous structure.

Three different forms of crystalline structure have been identified, A, B and C. In the A pattern, characteristic for cereal starches, water molecules are located between starch molecules forming double helixes. In the B structure, present in potato and other tuber starches, the double helixes are packed densely in a hexagonal pattern with water molecules only inside this structure. The C pattern is found in legume starches. It is not yet clear if this is a distinct crystallographic pattern or a mixture of the A and B patterns.

When starches are heated above the gelatinization temperature in excess water the granules undergo swelling and amylose is solubilized first. With lower water content, which is often the case in foods, the granules melt at temperatures above 100°C. When lowering the temperature, gelatinized starches tend to reassociate, especially at the semi-dry conditions that are often present in foods. Gelation is the first step in this process, followed by development of a B-type crystallinity referred to as retrogradation. Repeated heating and cooling cycles increase the amylose chain associations and thereby the retrogradation (for a review, see e.g. Colonna *et al.*[14]).

The salivary and pancreatic amylases hydrolyze the α-1,4-bonds with maltose, glucose and dextrins containing the branching points as end products. The hydrolases of the brush-border membrane complete the hydrolysis to free glucose.

Starch in freshly cooked starchy foods, amorphous or dispersed-solubilized, is readily hydrolyzed by salivary and pancreatic amylase. The digestion can be inhibited by certain glycoproteins, tannins, polyphenols and hydrolysis products[14].

Starch hydrolysis from solid substrates, such as starch granules or retrograded amylose, is more complicated. Colonna *et al.*[14] differentiated four different phases: 1) Diffusion of the

enzyme molecule towards its substrate, 2) The porosity of the substrate, 3) The adsorption of enzymes on the substrate, and 4) The catalytic event. All these steps are influenced by properties in the raw material and alterations during processing.

3. FORMS AND FORMATION OF RESISTANT STARCH

The originally identified form of RS in processed foods has been characterized as being mainly retrograded amylose[15,16]. Englyst and Cummings[8–10] differentiated three main forms of RS: 1) Starch that is physically inaccessible to digestive enzymes due to enclosure in structures such as intact cells in legumes or partly milled or whole cereal grains, 2) Resistant B-type starch granules occurring in uncooked potatoes and green banana, and 3) Retrograded amylose occurring in processed foods. These three forms of RS are generally the predominant ones in foods, although a number of other reasons for resistance should be kept in mind: Amylose-lipid complexation, retrogradation of amylopectin, chemical modifications, and dry heating creating new resistant glycosidic bonds[17]. Main forms of RS and their occurrance are listed in Table 1.

Formation of the retrograded amylose type of RS, RS_3, in bread baking and other processing of wheat has been studied by several groups[3,4,18–21]. Recently Eerlingen *et al.*[22–27] made a series of studies on RS formation, investigating the importance of both starch composition and processing conditions. RS formed from amylose fractions with different chain length differed in amount but not in quality: In all cases it had a B-crystalline type with similar chain length (DP_n between 19 and 26 glucose units, corresponding to DP_w about two times higher). Storage of gelatinized starch at 0–60°C for several days produced 4–6% RS with B-type crystalline structure. Storage at 100°C, on the other hand gave RS with an A-type pattern.

Table 1. Main types and sources of resistant starch

Type	Nomenclature according to Englyst	Nature of the starch	Occurrence in foods
Physically enclosed starch	RS_1	Amylose and amylopectin enclosed in grains, intact cells and other structures	Whole grain cereals, legumes, pasta (?)
Resistant starch granules	RS_2	Ungelatinized starch granules with B-type crystallinity from e.g. potato, banana, high amylose corn	Unprocessed or gently processed foods containing or enriched with resistant granules
Resistant starch in processed foods	RS_3	Retrograded starch, mainly amylose with B-type crystallinity	Bread, cornflakes, products heated with prolonged and/or repeated moist heat treatment
Amylose-lipid complexes		May be partially resistant	
Pyrodextrins		Incomplete hydrolysis due to new glycosidic links formed by dry heat treatment	"Fiber" drinks
Chemically modified food starches		Incomplete hydrolysis due to substituents	Foods in which modified starches are used

Amylopectin inhibits RS_3 formation due to interference with the ordering of amylose[21,25]. The outer short chains of amylopectin are able to reorganize at storage of gelatinized amylopectin, but these crystals melt at 46–65°C, compared with 125–150°C for amylose crystals. Staling of bread is due to retrogradation of amylopectin. This has been shown to inhibit the rate of hydrolysis with pancreatic α-amylase *in vitro*, but without affecting the final extent of hydrolysis[25,28]. It is unclear if amylopectin retrogradation would yield *in vivo* resistant starch — a mechanism suggested to explain the high amount of RS demonstrated after feeding cooked and cooled potatoes to ileostomists[8].

4. ANALYSIS METHODS

With the abovementioned physiological definition, methods for RS determination should simulate as far as possible the starch digestion in the upper gastrointestinal tract, aiming at measuring all types of starch that would escape absorption in the small intestine of healthy individuals. Obviously, this is a difficult task, taking into account that the amount of RS may vary between individuals due to differences in amylolytic activity, variation in transit time and other "extrinsic" factors[29]. The methods published so far, summarized in Table 2, can be divided into three different categories, each one described under a separate sub-heading.

4.1. Starch Remaining in Dietary Fiber Residues

As mentioned above, RS was originally defined analytically as the starch remaining undigested after extensive amylase treatment for determination of dietary fiber. Englyst *et al.*[1] calculated RS as the difference in glucan content of the dietary fiber before and after solubilization in 2 M KOH or dimethyl sulphoxide (DMSO). Johansson *et al.*[3], on the other hand, determined the starch remaining in a dietary fiber residue prepared for gravimetric determination[30]. The method based on determining starch in a dietary fiber residue has been simplified for the purpose of measuring resistant starch[31]. However, all these methods employ milling and gelatinization steps, releasing and solubilizing physically enclosed starch (RS_1) and ungelatinized starch granules (RS_2), respectively. Accordingly, the RS measured in the gravimetric dietary fiber residue has been characterized as retrograded amylose (RS_3)[15,16].

Table 2. In vitro methods for determination of resistant starch

Type	Features	RS fractions included	Reference
Starch remaining in dietary fiber determination	Measures starch resistant at conditions of assay, including milling and heat treatment	Mainly RS_3	1, 3, 31
Starch remaining after extensive amylolysis	Samples are milled and digested with α-amylase at 37°C	RS_2RS_3	18, 33, 34
Starch remaining after "physiological" disintegration and amylolysis-standarized milling	Ball milling during amylolysis to simulate physiological disintegration	$RS_1RS_2RS_3$	29
Chewing	Standardized chewing is used as an initial disintegration step, followed by pepsin and pancreatin digestion	$RS_1RS_2RS_3$	36, 38, 39

4.2. Methods Based on Extensive Amylolysis without Gelatinization or Thermostable Amylases

A method based on extensive α-amylolysis at 37°C was published by Berry in 1986[18]. This method was slightly modified by collaborators of a recent European project (EURESTA, for review see ref.[12,32] A first modified version was tested[33] and then improved further to recover very high RS contents accurately[34]. The method would be able to recover RS_2 and RS_3, but still not the physically enclosed RS_1 due to an initial milling step.

Englyst *et al.*[29] developed a method for separating different nutritionally important starch fractions. Various modifications of the method measures either total RS or the different main fractions — RS_1, RS_2 and RS_3, respectively. After initial gentle disintegration of the food when necessary, a standardized shaking with glass balls is used during the α-amylase digestion, in order to recover physically enclosed RS_1. This critical step is designed to mimic the disintegration obtained at chewing. The method has been documented versus *in vivo* RS determination using ileostomists with good agreement[29], but there is still a lack of published documentation regarding foods and whole diets with more complex structure. In such instances, the simulation of the disintegration of foods as eaten is more difficult than in test foods enriched with resistant starch. A disadvantage of the total RS method that has been used most extensively, is that RS is obtained as a difference between total and digestible starch, making the precision at high total starch and low RS content poor[35]. In these cases varieties based on direct measurement of RS are preferable.

A method using chewing to disintegrate the food samples before analysis of RS was published by Muir and O'Dea[36] and documented versus human ileostomy studies[37]. After some modifications of the initial method, the authors considered the agreement excellent[38]. Björck *et al.*[39] developed another method[40] for measurement of RS, using standardized chewing for a physiological disintegration of the material before pepsin and pancreatin treatment for removal of the digestible starch. Although chewing has certain disadvantages in practice as a first step in *in vitro* methods, it remains the most reliable way to disintegrate foods in a physiological way. For review, see also ref.[40]

5. PHYSIOLOGICAL EFFECTS

Physiological and nutritional effects of RS have been explored recently, not the least within the EURESTA project, and are summarized below.

5.1. Thermogenesis and Satiety

Resistant starch is by definition not absorbed in the small intestine of man. Therefore, it does not provide glucose to body cells. Accordingly, studies by Tagliabue *et al.*[41] and Heinen *et al.*[42] showed no thermogenic effect of RS. Raben *et al.*[43] demonstrated a reduction in satiating power of a meal containing 27 g RS compared with a control containing only digestible starch. This is consistent with the fact that RS does not provide glucose to body cells. However, 30 g RS_2 or RS_3/day for a week had little influence on appetite and food intake[44].

5.2. Glycemic Response

Effects of dietary fiber in the upper intestinal tract on glucose absorption and plasma lipids are related to physical properties of the fiber, notably the viscosity. RS_2 in the form of raw starch granules or RS_3 (retrograded amylose) do not possess such physical properties. On the other hand, structures such as intact cells in legumes are important in reducing the rate of absorption of digestible starch, as well as in enclosing RS_1. In starchy foods, a high amylose content is related to lower glycemic index, and also increases the formation of RS_3 at processing[40]. It is therefore not surprising that there is an inverse correlation between glycemic index and malabsorbed carbohydrate[45]. However, in some foods such as cornflakes, the bulk of the starch is easily digestible giving a high glycemic index in spite of a substantial RS content. Granfeldt *et al.*[46] studied the glycemic response to corn bread (Arepa) prepared from ordinary (25% amylose) and high-amylose (70% amylose) corn, containing 4 and 30% RS, respectively. The considerably reduced glycemia after the high amylose variety was not simply related to a decreased proportion of digestible starch, but rather to a reduced enzymic availability of the digestible starch in the presence of RS. Thus, RS may interfere with the hydrolysis of the digestible starch fraction.

5.3. Plasma Lipids

Several animal studies have shown that resistant starch given to rats is capable of reducing both plasma cholesterol and triacyl glycerol concentrations (for review, see ref. 12). Verbeek *et al.*[47] investigated mechanisms behind such effects, and found an increased total fecal bile acid excretion. This may be the mechanism behind the serum cholesterol reducing effect. In humans, however, Heinen *et al.*[48] did not find any lipid lowering effect when giving 30 g RS/day to young, normolipidemic subjects. It is still an open question if realistic amounts of RS could contribute to the normalization of elevated plasma lipids in man.

5.4. Mineral Balance

In studies with rats, Schultz *et al.*[49] found that RS_2, but not RS_3, increased the apparent absorption of calcium and magnesium, probably by decreasing pH through fermentation, resulting in increased solubility of the mineral in ileal and cecal digesta. However, raw or pregelatinized high amylose corn starch, providing 30 g RS/day to healthy male volunteers did not significantly affect calcium, magnesium or phosphorous apparent absorption[50]. The relative amount of RS given — 17 g/kg body weigth/day in rats versus 0.4 g in the human study — is one possible explanation for the species differences.

5.5. Fermentation and Cell Proliferation

Since RS is generally insoluble without hydrophilic and viscosity properties, physiological effects can be expected mainly from its presence and fermentation in the large bowel.

Cummings *et al.*[51] reviewed studies on large bowel effects of resistant starch. A number of sources of resistant starch have been demonstrated to be fermented in both rats[20,52,53] and man. *In vitro* studies with human fecal inoccula[54,55] indicated that RS would be an especially good substrate for butyrate production. This has been confirmed in human feces[56,57], but not in animal studies using rats[58].

Van Munster *et al.*[57] demonstrated increases in fecal output and relative amount of butyrate in feces when feeding healthy volunteers 45 g raw high amylose corn starch. Fecal water toxicity and deoxycholate concentration in fecal water decreased as did crypt cell production rates.

When measuring the breath hydrogen excretion after ingestion of raw potato starch (mainly RS_2), Olesen *et al.*[59] found a late response, indicating a prolonged fermentation. Slowly fermented substrates, that produce short chain fatty acids in the distal colon may be especially interesting in the prevention of colonic cancer.

5.6. Fecal Bulk

Some form of RS are incompletely fermented, at least in the rat, e.g. RS in high amylose corn bread[60] and in hydroxy-propylated potato starch[61]. Differences in fermentabilities of RS fractions have also been observed for legume products, although processed under similar conditions[62].

An increased fecal bulk due to starch was demonstrated by Shetty *et al.*[63] whereas others[64] found no significant bulking effect of breakfast cereals (cornflakes) containing 10g RS/day. However, laxative properties of RS have been reported for raw potato and banana starch, and retrograded wheat or maize starch[65]. The bulking effect was less than for wheat bran, but corresponding to that for dietary fiber from fruits and vegetables. A number of studies, reviewed by Cummings *et al.*[51], have shown no effect on transit time. More studies are need on the fecal bulking effect of various forms of RS in man.

6. RESISTANT STARCH INTAKE

The RS content in Western diets has been estimated to 3–20 g/day, based on determination with various methods[40]. This is an amount comparable to the non-starch polysaccharide intake. A diet with bean flakes, used in an ileostomy study, contained 8 g RS/day[66]. However, when estimating the RS intake in different European countries within the EURESTA project, the mean intake was 4.1 g/day only[67,12]. This figure was derived from calculations on food balance sheets using RS estimates with the Englyst or modified Berry methods. It should be regarded as indicative only due to incompleteness of analytical data on foods as eaten. In any case that intake figure obtained is low, and can easily be increased with foods containing elevated levels of RS.

7. PERSPECTIVES

Resistant starch has been established as one of the main sources of carbohydrate substrate for the large bowel microflora. Although RS may influence the rate of digestion of available starch, and thus be a factor influencing the glycemic index of foods, its main physiological effects seem to be related to fermentation. Short chain fatty acids are probably mediating also the effects on plasma lipid levels and mineral absorption observed in rats, findings that need further studies in man.

There is evidence, although not yet conclusive, that RS is a particularly good substrate for butyrate production in the human colon. The effects of different types of RS regarding rate and site of fermentation, fermentation products, nitrogen metabolism and cell proliferation on the colon need futher exploration. Such data would enable optimation of RS properties in foods in relation to colonic health.

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HEALTH BENEFITS OF NON-DIGESTIBLE OLIGOSACCHARIDES

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ABSTRACT

Non-digestible oligosaccharides are complex carbohydrates of the non-a-glucan type which, because of the configuration of their osidic bonds, resist hydrolysis by salivary and intestinal digestive enzymes. In the colon they are fermented by anaerobic bacteria. Among the non-digestible oligosaccharides, the chicory fructooligosaccharides occupy a key position and, in most european countries, they are recognised as natural food ingredients. The other major products are the short chain fructooligosaccharides and galactooligosaccharides obtained by enzymatic synthesis using sucrose and lactose as substrates respectively, the soybean oligosaccharides, the xylooligosaccharides produced by partial hydrolysis of xylans and polydextrose or pyrodextrins prepared by a chemical treament of carbohydrates. The most well known effect of most non-digestible oligosaccharides, and in particular of the fructooligosaccharides, is the selective stimulation of the growth of *Bifidobacteria* thus modifying significantly the composition of the colonic microbiota. Such a modification, which has clearly been demonstrated in human volunteers, is meant to be benificial in part because it is accompanied by a significant reduction in the number of bacteria reported to have pathogenic potential. Within the framework of research and development of "functional foods", such an effect justifies a "functional claim" for fructooligosaccharides namely "bifidogenesis". They are also typical "prebiotics". Besides their bifidogenic effect, the chicory fructooligosaccharides have additional nutritional properties on digestive physiological parameters like colonic pH and stool bulking which justify their classification as dietary fibers. Moreover, in experimental models, it has also been reported that they improve the bioavailability of essentiel minerals and that they reduce serum triglyceridemia by lowering hepatic lipogenesis. Such effects demonstrate interactions between the chicory fructooligosaccharides and key functions in the body but their significance for humans still need to be proven before being used to justify additional claims.

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1. INTRODUCTION

1.1. Functional Foods: Concept and Strategy[1]

A "functional food" is a "*food which contains (in adequate concentration) one or a combination of components which affects functions in the body so as to have positive cellular or physiological effects which may, in due course, justify functional or health claims*". Research and development of "functional food" is part of the science of nutrition and the benefit associated with its intake has to be evaluated in the context of a balanced diet. The strategy for research and development of a "functional food" or a "functional food ingredient" requires:

a. The identification of its interaction with a function in the body
b. The understanding of the mechanism of this interaction
c. The demonstration of the functional effect in relevant biological systems
d. The use of the scientific data to formulate sound hypotheses
e. Testing of these hypotheses in human nutrition studies, the functional food being ingested as part of a balanced diet.

1.2. The Non-Digestible Oligosaccharides: Definition

Among the components likely to be used in functional foods, the non-digestible oligosaccharides show interesting properties and some are already recognised and included in foods as ingredients[2]. By definition a non-digestible oligosaccharide (NDO) is:

> "an oligomeric carbohydrate the osidic bond of which is in a spatial configuration which makes it resistant to the hydrolytic activity of the intestinal digestive enzymes, but still sentitive to hydrolysis by the enzymes of the colonic bacteria which then ferment its monomers to produce short chain carboxylic acids and gases but also cellular energy for metabolic activities, growth and proliferation".

The NDOs belong to the category of non a-glucans and, if they are hydrolysed-fermented only by a specific group of colonic bacteria, they are classified as "prebiotics" a concept we have recently introduced[3]. According to this concept, a "prebiotic" is:

> "a non-digestible food ingredient which beneficially affects the host by **selectively** stimulating the growth and/or activating the metabolism of one or a limited number of health-promoting bacteria in the intestinal tract thus improving the host's intestinal physiology".

In addition to their prebiotic effect, some of the NDOs may also have interesting systemic effects acting, in particular, as modulators of the metabolism of lipids and carbohydrates and/or endocrine secretions. Up to now, effects of the NDOs can justify "functional claims", but not "health claims" which will require further and extensive research. As opposed to an "health claim" which directly concerns disease prevention, a "functional claim" is limited to a change in a particular function of the body not implying that this change has any proven relevance to the prevention of a disease[1].

The most important class of dietary NDOs is that composed of short (up to 8 monomers with a mean degree of polymerisation of about 5) and medium (up to 50–60 units with a mean degree of polymerisation of about 10 or 20) chain-length homopolymers (table 1). The chicory NDOs (inulin and its hydrolysate oligofructose) are fructooligo-

saccharides classified as natural food ingredients[4]. Other NDOs are synthetic products resulting from the enzymatic and/or chemical modifications of natural disaccharides like saccharose or lactose and polysaccharides like starch or xylans.

1.3. Aim of the Paper

The aim of the present paper is to review the nutritional and physiological properties of the chicory fructooligosaccharides which are, so far, the most studied NDOs. Even though more partly, similar evidences exist also for other NDOs, and they will be referenced. These properties will be discussed by reference to the strategy for research and development of a functional food described above (see 1.1) with the objective to identify which functional interactions have been identified, which mechanisms have been explored, which hypotheses are made, which human data are available and, eventually, which claims are justified.

2. BEHAVIOR AND EFFECTS OF CHICORY FRUCTOOLIGOSACCHARIDES IN THE GASTROINTESTINAL TRACT

2.1. Non-Digestibility

The evidence that chicory fructooligosaccharides resist hydrolysis by digestive enzymes and are not absorbed in the upper part of the intestinal tract comes from *in vivo* human studies performed in ileostomy subjects which demonstrate that 90–95% of the ingested oligosaccharides are recovered at the end of the small intestine, the small loss of material being due to microbial contamination of the ileon[5,6]. A study in rats fed ^{14}C-labelled Neosugar® provided similar evidence for the resistance to intestinal hydrolysis and absorption[7].

2.2. Fermentation by Bifidobacteria

That chicory fructooligosaccharides are quantitatively fermented by the colonic microbiota during their passage through the colon is supported by many experiments which showed that these carbohydrates could not be detected in the faeces of animals or of human volunteers who consumed them (sometimes more than 50g/day)[14–16]. We have confirmed these results in rats fed a diet containing 10% and even 20%, and in human volunteers consuming 10g or 30g of chicory fructooligosaccharides (unpublished data).

Using an *in vitro* model of mixed faecal culture, Wang and Gibson[11] have demonstrated that, as compared to other carbohydrates like polydextrose, pectins, fructose or glucose, the chicory fructooligosaccharides are selectively hydrolysed and fermented by the *Bifidobacteria*, one of the health promoting population in the colonic microbiota. Because of such a selectivity, the *Bifidobacteria* are stimulated to overgrow and they become predominant. Using pure cultures, the same authors have demonstrated that the specificity of *Bifidobacteria* is due to their rather unique capacity to secrete a β-fructosidase which hydrolyses the β 1-2 osidic bonds of the chicory fructooligosaccharides[9,10]. The same specificity of *Bifidobacteria* to selectively hydrolyse and ferment fructooligosaccharides has been reported for the synthetic product Neosugar®. That the same selective stimulation of colonic *Bifidobacteria* to overgrow also occurs *in vivo* has clearly been demonstrated

Table 1. Non-digestible oligosaccharides

Name (abbreviation)	Chemical structure	Osidic bond	Origin	Trade name
Fructooligosaccharides (FOS) Inulin	Glucosyl (fructosyl)n fructose(n=2→60)	β 1→2	Plant	Raftiline® Fibrulin®
Oligofructose	Glucosyl (fructosyl)n Fructose (fructosyl)m fructose(n=1→6, μ=2→7)	b 1→2	Plant and enzymatic hydrolysis of inulin	Raftilose®
Neosugar	Glucosyl (fructosyl)n fructose(n=1→3)	b 1→2	Enzymatic synthesis from sucrose	Neosugar® Actilight®
Galactooligosaccharides (GOS or TOS)	Glucosyl (galactosyl)n galactose(n=1→3)	b 1→6	Enzymatic synthesis from lactose	Oligomate®
Transgalacto oligosaccharides	(Galactosyl)n galactose (n=2)	β 1→6	Enzymatic synthesis from lactose	Cup-Oligo®
Isomalto oligosaccharides (IMO)	(Glucosyl)n glucose(n= 2→7)	a 1→6 α 1→4	Enzymatic rearrangement of maltose	Isomalto®
Polydextrose	Randomly branched + citric acid(n=2→100?)	/	glucose pyrolysis - citric acid	Polydextrose®
Pyrodextrins	Complex mixture	/	Pyrolysis of corn or potato starch	/
Soyoligosaccharides(SOS)	Raffinose + Stachyose(n=3–4)		Enzymatic synthesis + pyrolysis	Soya-Oligo®

by Gibson et al.[11] who reported that, after ingesting chicory fructooligosaccharides (3x5g/day) for two weeks, the composition of the faecal flora of human volunteers had been modified dramatically, the ratio *Bifidobacteria/Bacteroides* changing from 1/4 to 4/1. A more recent study has shown the same effect after the ingestion of 8g/day for 5 weeks (N.Guggenbuhl et al. Institut Paul Lambin Brussels, Belgium personal communication). Similar studies performed with the synthetic Neosugar[12,13] have led essentially to the same conclusions. Fructooligosaccharides in general and chicory fructooligosaccharides in particular are thus **bifidogenic** dietary factors.

2.3. Bulking Effect

Both in experimental animal (rat)[17] and in human volunteers[11], adding chicory fructooligosaccharides to the diet is accompanied by an increase in stool weight. In average, this corresponds to an excess of wet faecal mass of 1–1.5g per g of fructooligosaccharides eaten. Comparable values have been reported for wheat and oat bran or pectin added to rat's diet[18]. In human studies, fructooligosaccharides are less efficient than wheat bran[19–22] but as efficient as pectin or guar gum[23–25] (table 2).

2.4. Chicory Fructooligosaccharides as Prebiotics and Functional Food Ingredients

Based on the data available, it can thus be hypothesized that chicory as well as other similar fructooligosaccharides have a significant impact on both the biomass and the composition of the gut microflora by selectively stimulating the growth of the *Bifidobacteria* which are potentially health promoting and, consequently, by reducing the number of potentially harmful anaerobes. These food ingredients are thus likely to be beneficial and for these reasons, they have classified as **prebiotics.** But the exact role of the gut microflora in health as well as the definiton of an healthy colonic microbiota still remain open questions[26].

By reference to the strategy for research and development of a "functional food", it has been demonstrated that:

a. chicory and similar fructooligosaccharides interact specifically with a key component of the colonic physiological functions i.e. the biomass and the composition of the microbiota,

Table 2. Bulking effect of chicory fructooligosaccharides as compared to dietary fiber

Species	Carbohydrates	Bulking index*	Reference
Rats	Oligofructose (10%)	+1	17
	Inulin (10%)		
	Pectin (10%)	+1	18
	Oat bran (14%)	+0.7	18
	Wheat bran (13%)	+1.3–2.0	18
Humans	Oligofructose (15g)	+1.2	11
	Inulin (15g)	+2.1	
	Pectin	+1-2	23–25
	Guar gum	+1-2	23–25
	Wheat bran (15g)	+2.5-5	19–22

*Bulking index = g of increase in fresh faecal weight /g of carbohydrates eaten

() Indicates either % added to rat diet or g eaten per day.

b. both in experimentam systems and in human volunteers, such an interaction leads to a dramatic change in the composition of the colonic microflora in which the *Bifidobacteria* become the predominant population.

The obvious conclusion of these data is that chicory and similar fructooligosaccharides are **functional food ingredients** for which, at the present time **functional** but not **health claims** can be justified.

Moreover, the non-digestibility of these carbohydrates as well as their stool bulking effect justify their classification as dietary fibre or complex carbohydrates.

3. SYSTEMIC EFFECTS OF CHICORY FRUCTOOLIGOSACCHARIDES

3.1. Effects on Lipid Metabolism

The main systemic effect of chicory fructooligosaccharides so far reported is their effect on lipid metabolism. Such an effect had previously been suggested by Japanese studies which had shown that the administration of short chain synthetic fructooligosaccharides reduced serum lipids concentration. In various protocols differing in the composition of the basal diet, the dose of chicory fructooligosaccharides and the duration of the feeding treatment we have been able to demonstrate a significant decrease in serum lipidemia, thus identifying a possible interaction between these food ingredients and systemic lipid homeostasis. In a standard protocol in which male rats are fed a diet containing 10% chicory fructooligosaccharides, a significant hypotriglyceridemia (–30 to –40%) appears after 2–3 weeks but lowering cholesterolemia requires a longer treament (10 weeks or more). The early hypotriglyceridemic effect is exclusively due to a reduction in the VLDL fraction of the serum lipoproteins[27].

In order to proceed with the development of chicory fructooligosaccharides as hypolipidemic functional food ingredients, and by reference to the strategy described above (see 1.1), it was necessary to formulate sound hypotheses to be tested in human nutrition studies. For doing so more mechanistic informations were needed based on the hypothesis that hypotriglyceridemia induced by chicory fructooligosaccharides feeding resulted from a metabolic adaptation in the hepatic lipid metabolism.

Using an *ex vivo* protocol Kok et al.[28,29] demonstrated that, as compared to control liver cells, hepatocytes isolated from rats fed a diet supplemented with chicory fructooligosaccharides (10%) incorporated less (–40%) ^{14}C-labelled acetate in both their intracellular triglycerides and extracellular VLDL. Furthermore they reported that all the liver enzyme activities related to lipogenesis including glucose-6-phosphate dehydrogenase and malic enzyme which produce NADPH, fatty acyl synthase, ATP-citrate lyase and acetyl CoA carboxylase which all catalyse key steps in the synthesis of fatty acids were significantly reduced and to a similar extent (–40%) (table 3). Preliminary results seem also to indicate that these changes in hepatic lipogenic activity, possibly due to a change in gene expression, could, at least partly, be due to a decrease in postprandial insulinemia and possibly an increase in insulin sensitivity (Kok and Delzenne personal communication).

Being still at the level of experimental evidences, these systemic functional effects of chicory fructooligosaccharides cannot yet be used to support functional claims but they serve to formulate new hypotheses and to design protocols for further human nutritional studies. Functional interactions have been identified in experimental models which now need to be tested in humans.

Table 3. Relative values of lipid parameters in rats fed a chicory fructooligosaccharides containing (FOS) or a control diet (CTRL) (See ref 28,29 for experimental protocols)

	Lipid parameters (FOS / CTRL) × 100	
Parameters	Serum	Liver
Triacylglycerols	60	74
Insulin	77	—
Fatty acyl synthase	—	57
Malic enzyme	—	54
Glucose-6-phosphate dehydrogenase	—	56
AcetylCoA carboxylase	—	60

3.2. Bioavailability of Micronutrients

The possible interference of dietary fibers with the absorption of ions has regularly been questionned. With chicory fructooligosaccharides the effect could be quite the opposite. Indeed, in growing rats fed a diet supplemented with 10% of these[30,31] and similar fructooligosaccharides[32], the balances of Ca, Mg and Fe were improved significantly possibly because of an increased intestinal absorption of the minerals. Neither Cu or Zn bioavailability, nor lipid soluble vitamins (A and E) homeostasis were affected by the chicory fructooligosaccharides.

3.3. Caloric Value

Like with the other non digestible carbohydrates, the colonic fermentation of chicory fructooligosaccharides produces short chain carboxylic acids mainly acetate, propionate and butyrate. But the colonic fermentation of inulin has been reported to produce 4 times more butyrate than other dietary fibre like wheat and oat brans, pea and carrot fibers[33], an effect which, if confirmed could be of great interest since it is hypothesized that butyrate could play a role in preventing carcinogenesis. Moreover the fructoligosaccharides serves as an energy source for the bacteria to support their metabolic activity as well as their growth and proliferation. This complex process has to be taken into consideration when evaluating the caloric value of such non-digestible oligosaccharides in other words their contribution to energy metabolism of the host. The key question is to evaluate quantitatively which part of the initial energy content of the non-digested but fermented carbohydrate is available for the metabolism of the host, which part is used by the bacteria for their metabolic activity, which part serves their growth and proliferation and is, consequently lost in the faecal biomass. Based on experimental data as well as on basic biochemical knowledges, a theoretical approach has been used to compare the metabolic efficiency (in term of ATP production) and thus the effective caloric value of a fructosyl unit in chicory fructooligosaccharides and in sucrose (as a model of fully digested carbohydrate). As compared to sucrose, a fructosyl unit in fructooligosaccharides has a relative caloric value of 25 to 35%, it means 1 to 1.5 Kcal/g or 4 to 6 KJ/g[17]. They are thus in the range of the values generally accepted for non-digestible carbohydrates which vary from 0 to 2 Kcal/g or 0 to 8 KJ/g. This justifies their use as bulking agent, sugar substitute and, for inulin, as fat replacer in miscellaneous food products[34].

4. DISCUSSION AND CONCLUSION

Research and development of "functional foods" opens new perspectives in nutrition and food sciences[35]. The systematic investigation of the interactions between food components or food ingredients and genomic, biochemical, cellular or physiological functions completed by the understanding of their mechanisms, is a unique way to improve both our knowledge and the role of nutrition in maintaining good health and preventing diseases. But such a basic knowledge is insufficient to justify "claims" unless it is completed by relevant nutrition studies aimed at demonstrating the relevance of these interactions and their positive consequences in humans. In a first stage, this demonstration will, in most cases justify "functional claims with no direct reference to any health benefit. A "true" health claim wil require further studies involving large populations and long term trials.

But it is anticipated that the better we understand the mechanism of interactions between food components and biological functions, the better we will be in a position to formulate sound hypotheses to be tested in properly designed human nutrition trials based on which we will demonstrate significant functional effects. We will then accumulate convincing arguments in favor of health promotion and disease prevention.

Within the vast group of dietary carbohydrates, the non-digestible oligosaccharides including the chicory fructooligosaccharides belong to the group of non a-glucans. From a physiological point of view, the fructooligosaccharides resist digestion by the intestinal enzymes but they are quantitatively fermented in the colon most exclusively by the *Bifidobacteria* which become stimulated to grow. This specific effect has been well established in humans, and it justifies their classification as prebiotics and the functional claim of "bifidogenesis". Moreover they can be and are indeed, in most european countries, classified as dietary fibre. Via their systemic effects, the chicory fructooligosaccharides might also modulate hepatic lipid metabolism, but such an effect still needs to be demonstrated in humans.

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GASTROINTESTINAL EFFECTS OF FRUCTOOLIGOSACCHARIDES

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1. INTRODUCTION

1.1. Nature of Fructooligosaccharides

Fructooligosaccharides (FOS) consist of a glucose (G) unit joined by an (α1-β2) linkage to 2 or more fructose (F_n) units, where n is the number of fructose units. Fructose units are joined to one another by a β(2-1) linkage. Structures for three common FOS are shown in Fig. 1.

1.2. Reported Gastrointestinal Effects of FOS

FOS are resistant to salivary amylases[1], gastric hydrolysis[2], and small intestinal digestion[3]. Like dietary fiber, they appear to pass virtually intact into the large intestine. Extensive fermentation of FOS by the large intestinal microflora with production of short chain fatty acids (SCFAs) has been demonstrated in rats[4,5] and humans[1]. High levels of FOS (10–20%) have been shown to increase fecal wet weight in rats, and hasten the appearance of a fecal dye marker[5]. In humans the effects on gastrointestinal function are less clear; FOS have been reported to be of benefit to individuals with both diarrhea and

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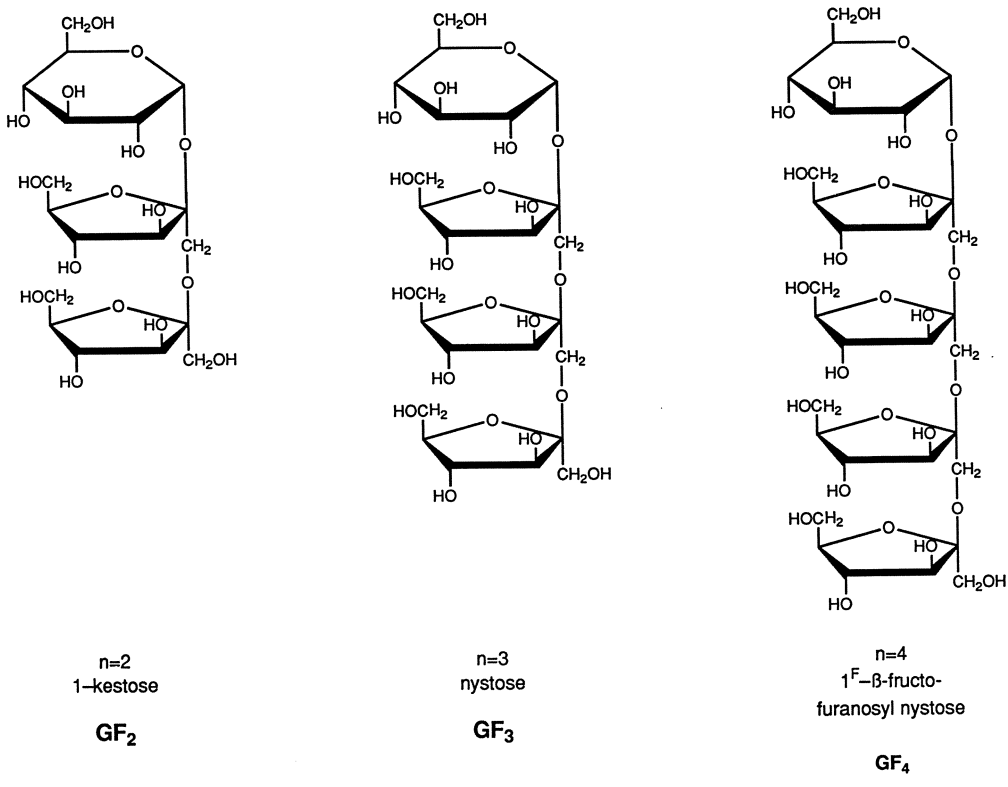


Figure 1. Chemical structures and nomenclature for three common fructooligosaccharides.

constipation[1]. Studies of cell proliferation in association with FOS feeding have shown either no effect[6], or enhancement of cell proliferation in the colon[7].

1.3. Hypothesis to Be Tested

In view of their apparent similarity to fermentable soluble dietary fibers, we hypothesized that the gastrointestinal effects of FOS would resemble those of soluble, fermentable dietary fibers. Given the limited and somewhat contradictory data available, we decided to test our hypothesis directly by inclusion of a guar gum group to facilitate direct comparison between this model soluble fiber and FOS. Two FOS preparations and inulin were studied. The control group consumed a fiber free diet used in previous long-term carcinogenesis studies[8]. A cellulose group was also included to provide a positive control as a model insoluble fiber.

2. MATERIALS AND METHODS

2.1. Animals

Sixty male F344 rats weighing initially 175–200 g were obtained from Harlan Sprague-Dawley, Indianapolis, In. Following one week on a non-purified commercial rat diet and

distilled water *ad libitum*, the rats were divided into six groups and placed on one of the six experimental diets described below.

2.2. Diets

Diets included a fiber free control diet, a 5% fructooligosaccharide mixture diet, a 5% nystose diet, a 5% inulin diet, a 5% guar gum diet or a 5% cellulose diet. The composition of the fiber free diet was (g/100 g): Dextrose, 34.9; Sucrose, 34.9; Casein, 19.8; DL-Methionine, 0.2; Corn Oil, 5.0; Modified AIN-76A Vitamin Mix[8], 1.0; Modified Bernhardt-Tomarelli Salt Mix[8], 4.0; Choline Bitartrate, 0.2. Dietary fibers or fructooligosaccharides were added at the expense of the dextrose/sucrose component of the diet. All diets were fed as a powder. The nystose (Meiji Seika Kaisha, Ltd., Tokyo, Japan) used was composed of 91% nystose (GF_3), 5% 1^F-fructosylnystose (GF_4), and 4% 1-kestose (GF_2). The fructooligosaccharide mixture used was a commercial product (Meioligo P, Meiji Seika Kaisha, Ltd., Tokyo, Japan) consisting of 42% 1-kestose (GF_2), 47% nystose (GF_3), 7% 1^F-fructosylnystose (GF_4), and 4% sucrose and glucose. Inulin isolated from chicory root (Sigma Chemical Company, St. Louis, Mo., catalog number I-2255), essentially free of polymers of length less than 10 by thin layer chromatography analysis, was used in the 5% inulin diet. The guar gum used was obtained from Meiji Seika Kaisha, Ltd. (Tokyo, Japan), and was 78% dietary fiber as measured by the AOAC method[9], without measurable mineral or protein content. Cellulose (Avicel FD-F20, Asahi Chemical Co, Tokyo, Japan) was a food grade material which was essentially 100% total dietary fiber. Animals had free access to diets and distilled water throughout the experiment for a six week period prior to necropsy.

2.3. Measurements during Feeding

Animal body weights and food intakes were determined weekly. After 4 weeks of diet consumption, total gastrointestinal transit time was determined by measurement of the fecal excretion of chromium (III) oxide following a 15 mg dose given by gavage[10]. The following week, feces were collected for 72 hours, lyophilized, and weighed to determine dry fecal weights.

2.4. Necropsy Procedures

Animals were necropsied after completing six weeks of feeding. Necropsies were performed between 9:00 AM and 11:30 AM. One hour prior to necropsy, animals were given an intraperitoneal injection of a 10:1 mixture of bromodeoxyuridine (BrdU)/fluorodeoxyuridine (60 mg/kg BrdU) to label cells in S phase. Following induction of anesthesia with sodium pentobarbital, the abdomen was opened by a midline incision. The internal pH of the cecum, proximal colon, and distal colon was measured after making small incisions in the intestinal wall and inserting the point of spear tip calomel pH electrode. The electronic pH meter was previously calibrated with standard buffers kept warm in a 37°C water bath. The colon and cecum were removed, and the colon divided into proximal and distal halves, and weighed. These segments of intestine were then placed on chilled glass plates. The cecum and colonic segments were slit open longitudinally, and the contents carefully transferred to vials which were then filled completely with chilled normal saline and frozen at −80°C for subsequent short chain fatty acid analysis. Intestinal tissues were rinsed, blotted, and reweighed following removal of the intestinal contents. The middle 0.5 cm of each colonic segment and a portion of the cecal wall were removed and placed in a vial of neutral buffered formalin to allow pres-

ervation for histology and cytokinetics. Following removal of tissues, the diaphragms were cut bilaterally to prevent the animal from recovering from anesthesia.

2.5. Analytical Procedures

2.5.1. BrdU Cytokinetics. Tissues were processed for embedding in paraffin within approximately 24 hours of necropsy to avoid degradation of antigenic sites. Following sectioning, the slides were incubated with a trypsin solution for 30 minutes. A 45 min incubation with 95% formamide in 0.15 M trisodium citrate at 70°C was used to partially denature double-stranded DNA and facilitate antibody binding. Slides were then treated with the primary antibody, a mouse monoclonal antibody to bromodeoxyuridine (Monoclonal Mouse Anti-Bromodeoxyuridine, DAKO A/S, Glostrup, Denmark), for 20 minutes at room temperature. After washing, they were incubated with a secondary antibody coupled to alkaline phosphatase which bound to the primary antibody. Color was developed by treatment with diaminobenzidine as a substrate for the linked alkaline phosphatase, resulting in dark brown staining of nuclei which had incorporated BrdU. A minimum of 20 well-oriented crypts was assessed for each anatomic site in each animal. The number of cells along one side of the crypt (crypt height), the percentage of labelled cells (labeling index), and the position (as a % of the crypt height) of the highest labelled cell (proliferative zone) was determined. All slides were encoded prior to interpretation, and were read by a single trained observer.

2.5.2. Short Chain Fatty Acid (SCFA) Analysis. After thawing, 5,000 ppm isocaproic acid was added to the intestinal content specimens as an internal standard. Short chain fatty acids within the specimens were converted to sodium salts by treatment with 5N NaOH. Specimens were dried in a vacuum, then redissolved in 1 ml of 0.3 N HCl, with 1 ml of 10 mM $HClO_4$ added. Short chain fatty acids were quantitated using high performance liquid chromatography. A Shodex KC811 column (Showa Denko, Tokyo, Japan) run at 60°C was used, as described[11]. Short chain fatty acids quantified included formate, acetate, propionate, butyrate, succinate, and lactate.

2.6. Statistical Analysis

Most data were analyzed initially by Analysis of Variance. If significant diet-related effects were found, pairwise comparisons were performed using the Least Significant Difference (LSD) test. Body weight data were analyzed by Analysis of Covariance with the body weight at week 0 as the covariate. Because of a limited number of samples and a large number of individual SCFA types to compare, a more conservative Tukey's Honestly Significant Difference test was used to compare SCFA differences among dietary groups. This test was performed using the SPSS for Windows computer software (SPSS, Inc., Chicago, IL). This more conservative test was used to avoid generation of spurious "chance" differences which might be interpreted as statistically significant if less conservative tests were used.

3. RESULTS

3.1. Body Weights and Food Consumptions

Initial mean body weights ranged from 240–241 g among groups. Final body weights (g, mean ± SEM) were as follows: control, 259 ± 3; FOS mixture, 268 ± 5;

Table 1. Fecal and cecal weights and gastrointestinal transit times for rats fed various fructooligosaccharides and dietary fibers

	Fiber free	FOS mixture	Nystos (GF_3)	Inulin	Guar gum	Cellulose
Fecal dry wt. (g/24h)	0.91 ±0.05	1.07 ±0.05	0.87 ±0.03	1.08 ±0.07	0.94 ±0.04	1.93 ±0.10*
Transit time (h)	43 ± 3	60 ± 7*	62 ± 7*	54 ± 5	64 ± 7*	30 ± 2
Cecal content, wet wt. (g)	1.8 ± 0.1	4.5 ± 0.4*	5.4 ± 0.7*	5.7 ± 0.4*	4.4 ± 0.6*	2.1 ± 0.1

*Differs significantly from fiber free control at $p < 0.05$.

nystose, 255 ± 5; inulin, 270 ± 5; guar gum, 262 ± 4; cellulose, 256 ± 6. These minor differences in body weights were not statistically significant. Mean food consumptions (g diet/day) varied from 14 g/d to 16 g/d. In all weeks except week 5, the cellulose group consumed a significantly greater mass of diet than control. In weeks 1 and 5, the nystose group consumed less diet than control. A few other statistically significant differences in diet consumption were detected, but seemed to follow no consistent pattern from week to week. Due to a technical error, food consumptions were not recorded for week 6.

3.2. Fecal Weights, Gastrointestinal Transit Times, and Weights of Cecal Contents

These data are shown in Table 1. Dry fecal weight differed significantly from control only in the cellulose group, where it was approximately 2-fold greater. Gastrointestinal transit time was prolonged in the FOS mixture group, the nystose group, the inulin group, and the guar gum groups. These differences were statistically significant for the FOS mixture, the nystose and the guar gum groups, which had transit times 40, 44 and 48% longer, respectively, than the fiber free control. Transit time was shortened (by about 30%) in the cellulose group compared to control. Weight of cecal contents was significantly greater in the FOS mixture, nystose, inulin, and guar gum groups as compared to the fiber free control.

3.3. Large Intestinal Cytokinetics

Significant differences in crypt height and labeling index were seen only in the cecum. These are illustrated in Figs. 2 and 3. Note that all FOS or fiber additions to the diet significantly increased crypt height compared to the fiber free control, while the labeling index was significantly increased by the FOS mixture, nystose, inulin, and guar gum, but not by cellulose. Values for crypt height and labeling index in the proximal and distal colon, which did not differ significantly from control values, are shown in Table 2.

There were no significant differences from control values for proliferative zone measurements in either the cecum, proximal colon, or distal colon (data not shown).

3.4. pH of Large Intestinal Contents

In general, the FOS mixture, nystose, inulin, and guar gum significantly reduced the pH within the large intestine. The resulting pH was significantly lower than that of the fiber free control group in the cecum, as well as the proximal and distal colon. Cellulose did not significantly alter pH compared to the fiber free control group. These results are shown in Fig. 4.

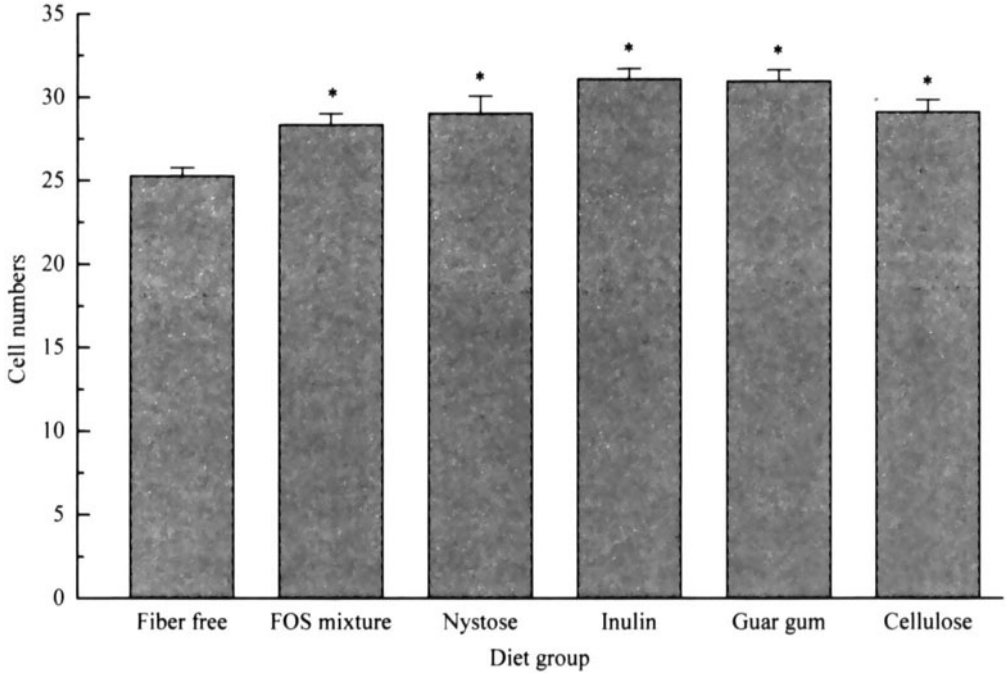


Figure 2. Cecal crypt heights (numbers of cells lining one side of a crypt) for rats fed various fructooligosaccharides and dietary fibers.

3.5. Short Chain Fatty Acid Analysis of Large Intestinal Contents

Total concentrations of SCFA in the three segments of large intestine examined are shown in Fig. 5. The only significant difference from control was in the cecum of animals fed guar gum, which had a significantly higher level of total SCFA compared to control. Proportions of SCFA types in the cecum, proximal colon, and distal colon are shown in Figs 6–8. In the cecum, the FOS mixture group had a significantly higher proportion of butyrate compared to control. The proportion of formate was significantly lower in the ceca of animals in the nystose, inulin, and guar gum groups. In the proximal and distal

Table 2. Crypt heights and labeling indices (LI) for proximal and distal colon of rats fed various fructooligosaccharides and dietary fibers

	Fiber free	FOS mixture	Nystose (GF_3)	Inulin	Guar gum	Cellulose
Prox. colon, crypt Ht.	28 ± 1	29 ± 1	29 ± 1	29 ± 1	27 ± 1	29 ± 1
Prox. colon, LI (%)	7.5 ±0.8	9.0 ±0.4	7.8 ±0.4	7.6 ±0.6	9.7 ±0.6	9.1 ±1.0
Distal colon, Crypt Ht.	31 ± 1	33 ± 2	31 ± 1	34 ± 1	29 ± 1	34 ± 1
Distal colon, LI (%)	7.4 ±1.5	6.5 ±0.8	7.0 ±1.3	8.6 ±1.5	7.3 ±0.7	7.7 ±1.4

None of the values shown above differed significantly from control (fiber free) values.

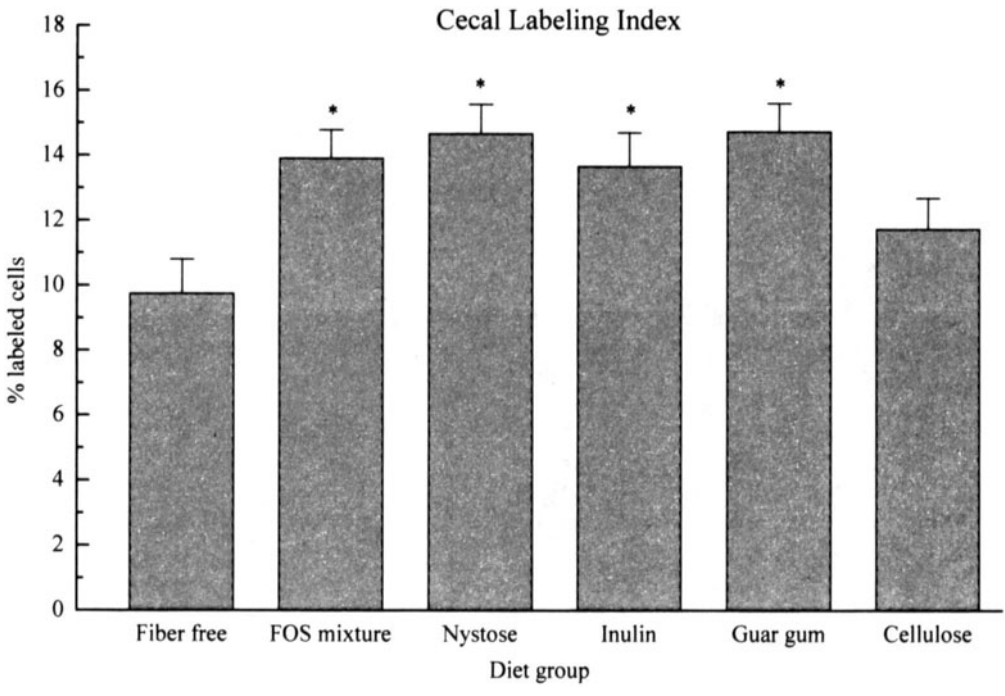
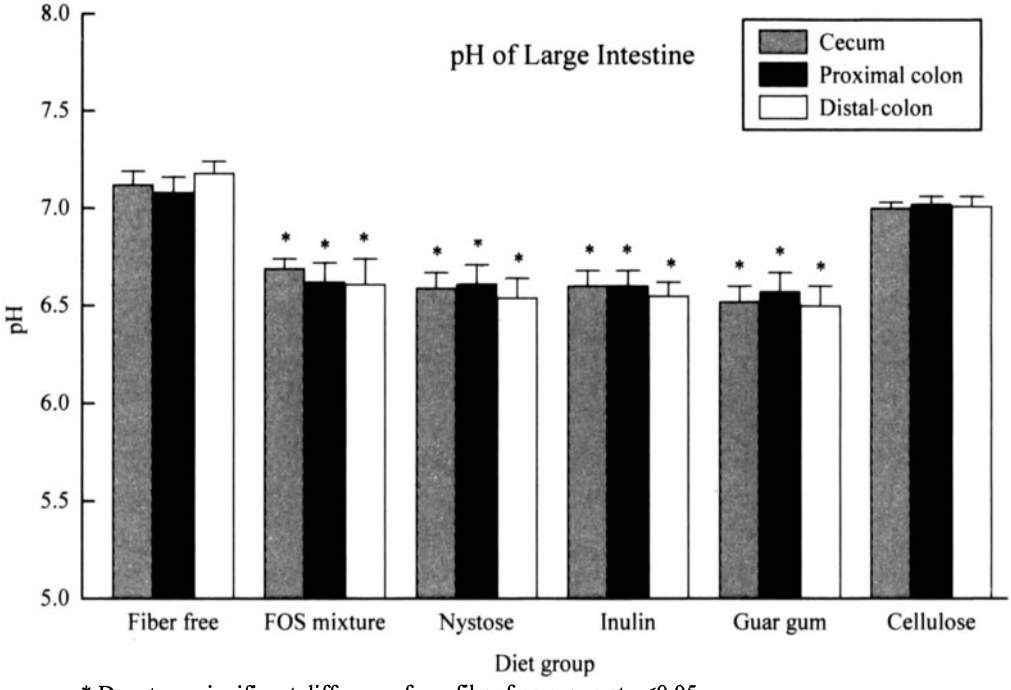


Figure 3. Cecal labeling indices [(number of BrdU-labeled cells/total cells) × 100] for rats fed various fructooligosaccharides and dietary fibers.

colon, none of the experimental groups had significantly different proportions of individual SCFAs compared to the control group.

4. DISCUSSION

Our original hypothesis was that fructooligosaccharides would have gastrointestinal effects similar to those reported for soluble, fermentable dietary fibers. In large measure, the hypothesis proved correct. Like soluble fiber, the FOS diets (FOS mixture and nystose) had a negligible effect on dry fecal weight. The FOS diets also increased the wet weight of the cecal contents, similar to guar gum. In our experiment, FOS diets also prolonged gastrointestinal transit time. Both FOS preparations stimulated cell proliferation in the cecum as assessed by crypt height and labeling index, as did inulin and guar gum. None of the experimental diets significantly altered cell proliferation in the proximal or distal colon. Like guar gum and inulin, both FOS diets resulted in a significantly lower pH within the cecum, proximal and distal colon. Only in their effects on total SCFA levels did there appear to be a measurable difference between the FOS diets and guar gum. Guar gum feeding significantly increased cecal total SCFA concentration compared to control, while neither of the FOS diets were associated with a significant alteration. No significant differences in total SCFA concentration were noted in the proximal



* Denotes a significant difference from fiber free group at $p<0.05$.
Each bar is a mean ± SEM.

Figure 4. Large intestinal pH in rats fed various fructooligosaccharides and dietary fibers.

or distal colon, however. Differences between the groups in the relative proportions of the various SCFAs measured tended to be minor and variable, precluding statistical significance except in the case of the higher proportion of cecal butyrate noted in the FOS mixture group. As in the guar gum group, acetate, propionate, butyrate, and succinate were the major SCFAs found in the cecal contents of animals fed both FOS preparations. Formate was noted in addition to these as major SCFA constituents in the proximal and distal colon.

In order to gain an overall perspective on the gastrointestinal effects of fructooligosaccharides, our results should be compared with those of other investigators. Our study differs from most other published studies in that a generally lower level of FOS was fed (5%) verses levels of 8–20% in some of the published studies, and therefore effects might be expected to be more moderate. Only one published study has examined the effects of FOS on fecal weight and transit time. Tokunaga[5] et al. noted, in contrast to our findings, that fecal weight was increased and transit time reduced by feeding FOS. However, their dietary levels of 10 and 20% were considerably higher than in our study, and transit time was judged only by the time of first appearance of a dye in the feces. Wet fecal weights were presented, unlike dry weights determined in our study. This factor, along with the higher feeding levels, likely accounts for these differences. Two published studies have examined the effects of FOS on large intestinal cell proliferation. Howard[7] et al. fed FOS at a 0.3% level to neonatal pigs as part of a liquid formula diet. They found that FOS feeding increased crypt height, proliferative zone, and labeling index in the proximal and distal colon but not in the cecum. We also found that FOS stimulated cell proliferation, but in

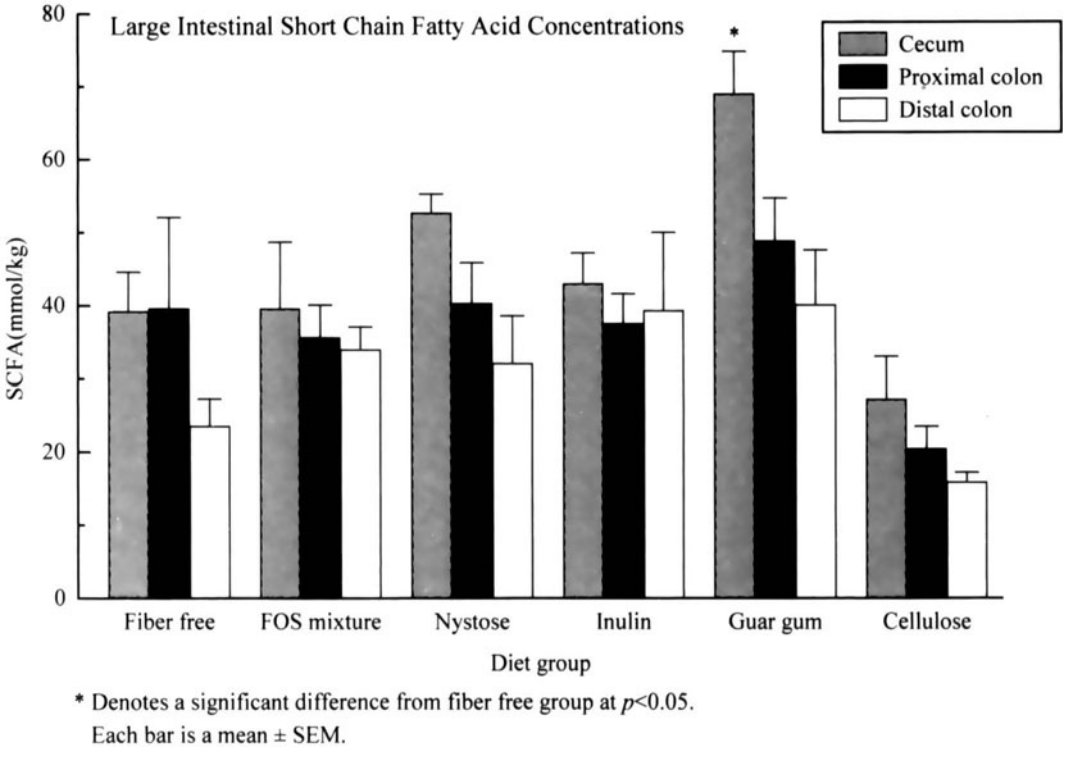
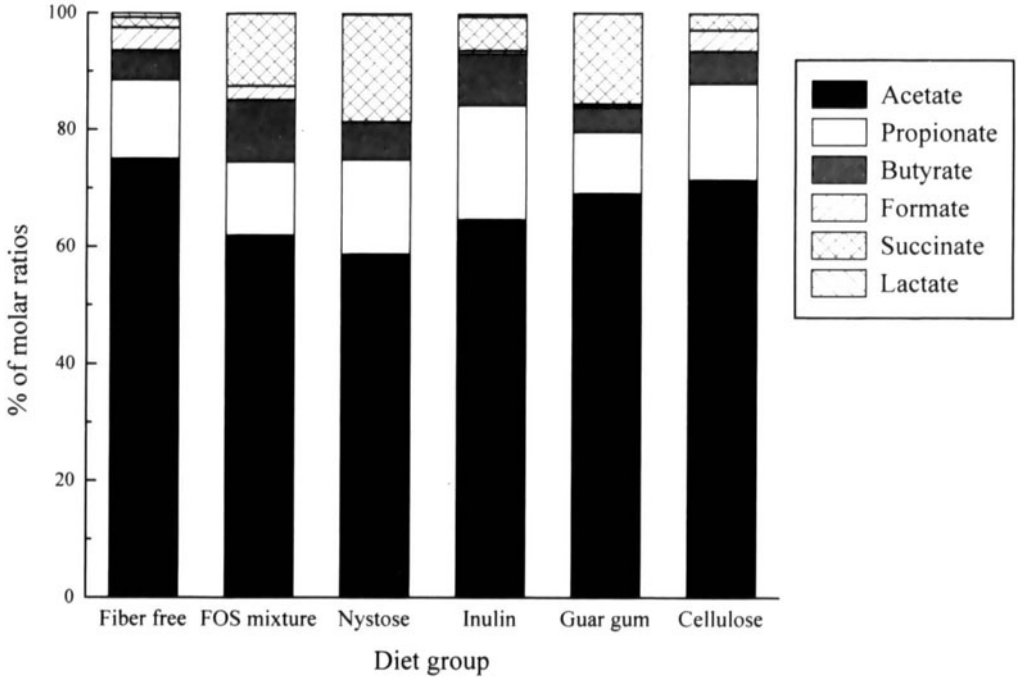


Figure 5. Large intestinal total short chain fatty acid concentrations for rats fed various fructooligosaccharides and dietary fibers.

our experiment enhanced cell proliferation was found in the cecum, not the colon. Differences in large intestinal anatomy, species, age, or level of intake of FOS may account for the difference observed with regard to the anatomic site of increased cell proliferation associated with FOS feeding. In contrast to this study and ours, a study in neonatal rats where FOS was provided in the drinking water (0.51 g FOS per day) showed no effect of FOS on indices of cell proliferation in the cecum or colon[6]. The overall high level of large intestinal cell proliferation these authors reported in their rapidly growing neonatal rats, possibly due to colonic fermentation of relatively high levels of dietary corn starch present in the diets, may have limited any observable enhancement of cell proliferation associated with FOS feeding. The pH of the intestinal contents has been infrequently determined in FOS feeding studies. Fermentation of FOS by rat microflora was reported to cause a decrease in fecal pH of approximately 0.3 and 0.7 units at the level of 2 and 10 % of FOS in the diet, respectively[12], consistent with our result of approximately a 0.6 unit decrease in the distal colon. In contrast to the consistently decreased large intestinal pH noted in our study and that of Hidaka[12] in association with FOS feeding, the only other published animal study in which pH was measured found no effect of FOS on the pH within the pig cecum, possibly due to a lower level of intake in this study[7] compared to ours. SCFA concentrations associated with FOS fermentation have been measured in prior studies using in vitro incubations of human[13,14] and rat[2] microflora, or following FOS feeding of rats[4,5] or pigs[7]. The in vitro studies have tended to produce disparate results regarding the relative



* Denotes a significant difference from fiber free group at $p<0.05$.

Figure 6. Proportion of various short chain fatty acids in the cecum of rats fed various fructooligosaccharides and dietary fibers. Besides the significantly higher proportion of butyric acid indicated by the *, the proportion of formate was significantly less than control in the nystose, inulin, and guar gum groups.

proportions of the various SCFAs. All three in vivo studies had results qualitatively similar to ours, with acetate, then propionate, then butyrate being the major SCFA in the cecum[4,7] or feces[5]. In addition to these SCFAs, we noted a substantial proportion of succinate in the cecum and proximal colon associated with FOS and guar gum feeding, and a reasonable proportion of formate in the distal colon associated with all of the diets. Data on formate or succinate were not presented in these previous studies. Over all, our results seem reasonably harmonious with published results, although differences in animal species and levels of consumption seriously limit comparability among published studies. The fact that we obtained the expected results from previously studied fiber sources (cellulose, guar gum) lends an additional level of confidence regarding the validity of the data in our study.

The reason for the prolonged gastrointestinal transit time observed in our study with FOS and guar gum feeding was not specifically investigated, but may relate to the observed cecal enlargement noted in the FOS mixture, nystose, inulin, and guar gum groups. Such an increase in cecal size has been previously observed when indigestible and soluble carbohydrates were administered to rodents[15]. In our study, when transit time and the weight of the wet cecal contents were statistically correlated, there was a significant correlation (r=0.651, n=38) between individual weights of cecal contents and transit times. Therefore, prolonged transit times associated with feeding FOS and soluble fibers may be considered to be in part due to dilution of the fecal marker in the enlarged cecum, resulting in slower marker excretion and a longer transit time.

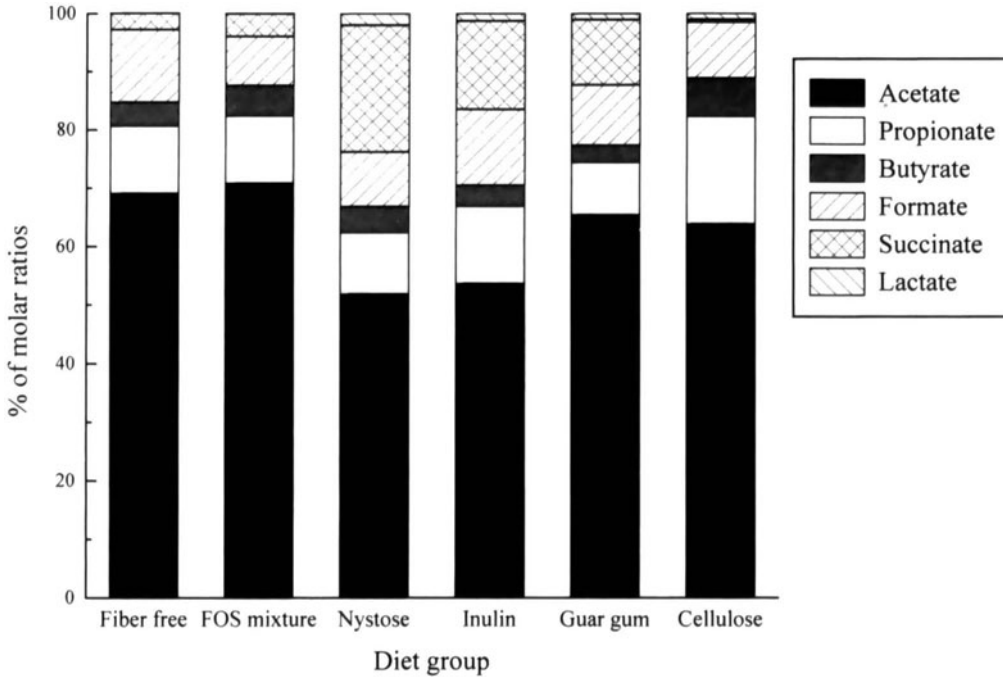


Figure 7. Proportion of various short chain fatty acids in the proximal colon of rats fed various fructooligosaccharides and dietary fibers. There were no significant differences between control and the treatment groups.

The mechanism by which various types of dietary fibers alter large intestinal cell proliferation is not known. Fiber-induced alterations in large intestinal pH, SCFA concentration, or butyrate concentration are currently popular hypotheses to explain the effects of fiber. In our study, addition of fermentable materials to the diet resulted in significant alterations in measures of cell proliferation only in the cecum. A casual observation of the data for cecal pH and crypt height or labeling index suggests that these variables may be correlated. Formal statistical correlation of individual cecal pH values with cecal crypt heights and labeling indices confirms that there are statistically significant correlations between these measures of cell proliferation and pH ($p = 0.007$ and 0.001 respectively). A similar type of correlation analysis revealed no significant correlations between total SCFA concentration or butyrate concentration and crypt height or labeling index in the cecum. Our data do not support a role for lowered pH as a mediator of altered cell proliferation in the colon. While pH was similar to cecal values in the proximal and distal colon, we observed no significant enhancement in cell proliferation in these areas of the intestine. Similarly, published data suggest that pH is much less well correlated with cell proliferation in the more distal parts of the large intestine[16,17], and it has been suggested that dietary fiber may exert its effects via a nonfermentative mechanism in the distal colon[16].

Published fiber feeding studies which have attempted to correlate pH, SCFA concentration, or butyrate concentration with intestinal cell proliferation often contradict one another. Fiber feeding regimens which increase butyrate concentrations have been reported to increase cell proliferation[16,17], decrease cell proliferation[18], or have no consistent effect[19–21]. The same investigators also examined pH[16,19–21] and total SCFA concentration[16–21] in the studies discussed above, with the same pattern of contradictory results.

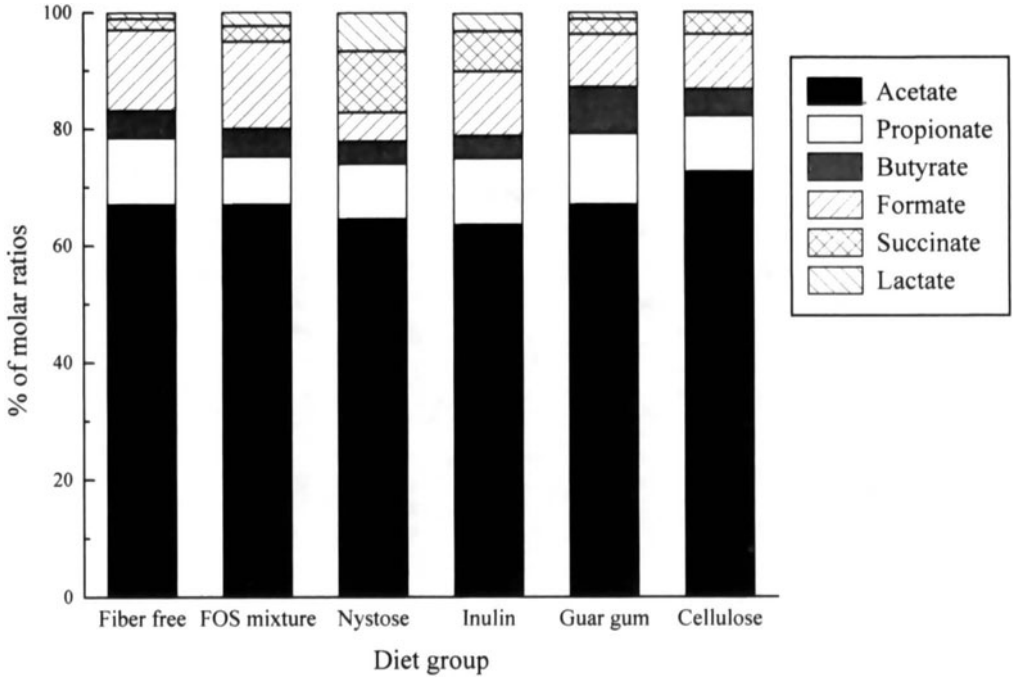


Figure 8. Proportion of various short chain fatty acids in the distal colon of rats fed various fructooligosaccharides and dietary fibers. There were no significant differences between control and the treatment groups.

Considering these published results with our own, several facts seem apparent. First, it is probably premature at this time to attribute to a specific SCFA (such as butyrate) a causal role in determining the large intestinal proliferative response to dietary fibers, given the contradictory in vivo data in the literature and lack of correlation of butyrate and cell proliferation in our study. Second, a reconsideration of other determinants of luminal pH besides SCFA might be warranted. Bile acids, urea, and bicarbonate, among other substances, might be expected to alter pH and might be responsible for alterations in cell proliferation associated with fiber feeding. Third, consideration should be given also to the importance of nonluminal factors, such as alterations in levels of enteroglucagon or mediation of effects by the autonomic nervous system (as suggested in the review by Roberfroid[22]). Fourth, it must be borne in mind that both fiber and the gut environment are extremely complex, and that many factors (often unknown) may act simultaneously to alter a parameter such as cell proliferation.

In summary, our examination of the gastrointestinal effects of FOS feeding largely confirmed our hypothesis that they would resemble the effects of consumption of soluble dietary fibers such as guar gum. Similar functional effects included lengthened transit time, negligible fecal bulking, increased cecal cell proliferation, and decreased large intestinal luminal pH. Guar gum produced a significantly higher concentration of cecal total SCFA, and there were other minor differences in SCFA types between groups. Our fecal weight and transit time results differ from published results, likely due to lower intake levels in our study. Cell proliferation and SCFA data from our experiment are fairly similar to that reported by others, but differences in intake levels and experimental animal models make a strict comparison impossible. Finally, we noted that pH in our study seemed the

best correlate of cecal cell proliferation, rather than butyrate or total SCFA concentration. Examination of other acidic substances in the lumen and consideration of nonluminal mechanisms for the effects of fiber on cell proliferation might be profitable in future investigations.

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24

PHYTOSTEROLS

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Sterols are important structural components of cell and organelle membranes of higher organisms. The primary sterol found in animal organisms is cholesterol. Analogs of cholesterol in the plant kingdom are, as a class, termed phytosterols. Plant sterols resemble cholesterol structurally in that they all have a steroid nucleus, a 3β hydroxyl group, and a double bond between carbon atoms 5 and 6. The major differences are in side chain substitution and/or unsaturation. Bean[1] has described 44 sterols that are present in plants. The principal plant sterols are β sitosterol (24α ethylcholesterol) which comprises 45–95% of the total sterol present in plant oils; campesterol (24α methylcholesterol) which may total 30% of the total sterols of seed oils; and stigmasterol (Δ^{22}, 24α ethylcholesterol) which may account for as much as 25% of the total sterol of seed oils. Brassicasterol ($\Delta^{7,22}$, 24α methylcholesterol) is also present in appreciable quantities in plant oils and a sterol found principally in yeast, ergosterol (Δ^{22}, 24α methylcholesterol) is present in significant amounts in corn, cottonseed, peanut, and linseed oils. Weihrauch and Gardner[2] have summarized the sterol content of a number of foods of plant origin. Tables 1 and 2 present data on the phytosterol content of selected vegetable oils and foods.

Shipley et al.[3] reported on long-term feeding studies of sitosterol in rats, rabbits, dogs, and man and found no gross or microscopic alterations of any tissues nor histologic evidence of plant sterol deposition. A more recent toxicity study in rats[4] showed β sitosterol to be well tolerated and found all parameters studied to be in the normal range except for serum cholesterol which was reduced in a dose-dependent manner. Ellis and Gardner[5] reported on the non-absorbability of phytosterols in 1912. About 20 years later Schoenheimer and his colleagues showed that sitosterol, stigmasterol, ergosterol, and brassicasterol were not absorbed by rabbits or rodents[6–9]. Schoenheimer reviewed the studies in 1931[10]. It is to be noted that the studies were carried out without the benefit of isotopic tracers and at a time when none of the sterol structures, including that of cholesterol, had been elucidated.

The advent of isotopically labeled sterols and development of more precise methodology improved the accuracy of sterol-feeding studies and it became evident that small amounts of plant sterols could be absorbed and metabolized. Gould and Cook[11] reviewed knowledge of absorption of sterols in 1958. Kritchevsky and Defendi[12] injected tritium labeled cholesterol, sitosterol, or lanosterol into yolk sacs of newly hatched chicks and

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Table 1. Major sterols of selected refined vegetable oils* (mg/100 g oil)

Oil	Total sterol	Beta sitosterol	Campesterol	Stigmasterol
Coconut	91	53	6	14
Corn	952	696	158	76
Cottonseed	327	303	20	tr
Linseed	338	180	100	29
Olive	176	153	6	2
Palm	49	30	10	6
Peanut	206	131	31	19
Rice bran	1055	571	215	82
Soybean	221	123	47	47
Wheat germ	553	370	122	tr

*After Weihrauch and Gardner[2].
tr = trace.

recovered small, but detectable amounts of isotope from the brain, liver, and spleen. Gould et al.[13] fed tritium labeled sitosterol to rats and recovered label in the sterol fraction of various tissues and estimated absorption to be about 30–40% of an equivalent dose of cholesterol. Studies on direct lymphatic absorption of β sitosterol in the rat indicate a finite level (3–6%) of absorption[14–16]. Vahouny et al.[17] compared lymphatic absorption of

Table 2. Major sterols of selected foods* (mg/100 g edible portion)

Food	Total sterol	Beta sitosterol	Campesterol	Stigmasterol
Vegetables				
Beet root	25	—	—	—
Brussels sprout	24	17	6	—
Cabbage	11	7	2	—
Carrot	12	7	1	3
Cauliflower	18	12	3	2
Onion	15	12	1	—
Yam	10	7	2	1
Fruits				
Apple	12	11	1	—
Banana	16	11	2	3
Cherry	12	12	tr	—
Orange	24	17	4	2
Peach	10	6	1	3
Pear	8	7	—	—
Nuts				
Almond	143	122	5	3
Cashew	158	130	13	tr
Pecan	108	88	4	2
Pistachio	108	90	6	2
Walnut	108	87	6	—
Legumes				
Broad bean	124	95	8	9
Kidney bean	127	91	3	31
Pea	135	106	10	10

*After Weihrauch and Gardner[2].
tr = trace.

Table 3. Absorption of plant sterols*

Species	No. studies	% Absorption
Rat	4	4
Rabbit	2	0
Dog	1	15
Fowl	1	2
Guinea pig	1	<5
Man	5	6

*Summarized from Pollak and Kritchevsky[22].

sitosterol, stigmasterol, and fucosterol (24-ethylidinecholesterol) in rats. Levels of absorption were 3.1, 4.3, and 3.7% for sitosterol, stigmasterol, and fucosterol, respectively.

In contrast to findings with cholesterol, a significant amount of fed sitosterol remains within the intestinal wall rather than being transported into lymph[15]. This finding may explain higher levels of plant sterol absorption reported by investigators using the balance method[18]. Ergosterol is absorbed to an extent of 2–5%[19] and there are suggestions of differential rates of absorption of different plant sterols. Thus, the level of campesterol absorption in dogs is intermediate between absorption of sitosterol and stigmasterol[20]. Heinemann et al.[21] compared absorption of cholesterol, campesterol, campestanol, stigmasterol, and sitosterol in 10 healthy subjects using an intestinal perfusion technique. Mean absorption (%) was: cholesterol, 31.2 ± 9.1; campesterol, 9.6 ± 13.8; campestanol, 12.5 ± 4.8; stigmasterol, 4.8 ± 6.5; and sitosterol, 4.2 ± 4.2. Pollak and Kritchevsky[22] have summarized data on phytosterols absorption by different species (Table 3).

Salen et al.[23] carried out a thorough study of sitosterol absorption and metabolism in man (Table 4). The level of absorption is about 1.5 to 5% when "normal" levels (240–320 mg) are fed and acidic products are recovered. Similarly, acidic steroids have been recovered from the feces of vervet monkeys fed sitosterol[24]. It has also been shown that ergosterol can be oxidized to acidic products by mouse or rat liver mitochondria[25]. Thus, phytosterols are indeed absorbed and metabolized but to a very small extent.

In 1951 Petersen[26] showed that addition of soy sterols to a cholesterol containing diet normalized plasma and liver cholesterol levels in chickens. In a typical experiment male chicks fed a diet containing 1% cholesterol demonstrated a significant increase in plasma cholesterol levels compared to controls (553 ± 95 mg/dl vs. 85 ± 8 mg/dl). Addition of 1% soy sterols to the same diet reduced plasma cholesterol to 109 ± 19 mg/dl. In later studies Peterson et al.[27,28] fed chicks for 4 weeks on a diet containing 1% cholesterol and 4% cottonseed oil and plasma cholesterol levels rose from 73 mg/dl to 892 mg/dl. Addition of 1% soy sterols, β sitosterol, ergosterol or stigmasterol to the diet resulted in cholesterol levels of 142, 105, 143, and 191 mg/dl, respectively. Soy sterol caprates were somewhat hypocholesterolemic (37% lowering) in chicks fed cholesterol. Ergosterol (2.4%) reduced plasma cholesterol levels in chicks by 84% but ergosteryl palmitate had

Table 4. Metabolism of β sitosterol in man* (n = 12)

1) Plasma concentration 0.30–1.20 mg/dl
2) % Plasma sitosterol ester = % plasma cholesterol ester
3) β sitosterol absorption < 5% (cholesterol absorption; 45–54%)
4) 20% of absorbed sitosterol converted to cholic and chenodeoxycholic acids

*After Salen et al.[23]

virtually no effect (7% reduction). In rats, β sitosteryl or stigmasteryl acetate reduced cholesterol absorption to the same extent (about 25%) as the free sterols[29].

Peterson et al.[30] rendered chickens atherosclerotic by feeding them a diet containing 1% cholesterol for 28 weeks. Additional of 1.3% mixed soy sterols to the diet inhibited cholesterol-induced atherogenesis. After 28 weeks, two of 13 chickens fed a control diet exhibited atheromatous lesions. The average grade of severity (arch + thoracic ÷ 2) was 0.15. Eight of 10 birds fed cholesterol were atherosclerotic (avg. grade, 2.2) and two of 9 birds fed cholesterol plus soy sterols had lesions (avg. grade, 0.17). Pollak[31] fed rabbits 1 gm/day of cholesterol or cholesterol-sitosterol mixture in ratios of 1:1, 3:1, 5:1, 6:1, or 7:1. At a 1:1 ratio serum cholesterol levels were 72% lower than the cholesterol-fed control. At a cholesterol-sitosterol ratio of 5:1 the plant sterol did not exert any cholesterol-lowering effect. Pollak and Kritchevsky[22] have summarized the animal data obtained between 1950 and 1980 (Table 5).

Pollak[32] was the first investigator to demonstrate that plant sterols could prevent cholesterol absorption and reduce plasma cholesterol levels significantly in man. He used the healthy subjects whose average starting cholesterol level was 267 ± 14 mg/dl. They were fed daily doses of 5.7 to 10 g plant sterols (avg. dose 8.1 g) for an average of 13 ± 1 days. Cholesterol levels were reduced by as much as 28%. Best et al.[33] fed 5–6 gm of β sitosterol immediately before meals to 9 men and saw an average fall in cholesterol by almost 13% after 12 weeks. Farquhar et al.[34] fed 15 subjects with previous myocardial infarction 12–18 g/day of β sitosterol for 16 weeks and reported a total serum cholesterol fall of about 5% ($p < 0.001$). Lees et al.[35] compared the effects of soy or tall oil sterols given as an emulsion or in the powdered form. Plasma cholesterol reduction was 12% on either soy sterol preparation and somewhat less on tall oil sterols. Pelletier et al.[36] added soybean sterols to the diets of 12 normolipidemic subjects. The ratio of phytosterols/cholesterol was 1.88. After 4 weeks, total and LDL-cholesterol levels had fallen by 11 and 15%, respectively and the LDL-cholesterol/HDL cholesterol ratio had fallen by 19%. Table 6 summarizes the results of 52 studies relating to plant sterol effects on cholesterolemia. The average reduction in serum or plasma cholesterol was 20%.

Sugano et al.[37] found in rats that sitostanol was a more efficient hypocholesterolemic agent than sitosterol. On a cholesterol free diet fecal excretion of sitosterol was 85% and that of sitostanol was virtually 100%. Heinemann et al.[38] fed 1.5 g sitostanol daily to six hypercholesterolemic subjects and found that total serum cholesterol levels had fallen significantly by the third week. Becker et al.[39] observed that diet alone did not influence lipemia in 9 hypercholesterolemic children. Their cholesterol levels fell when 6 gm/day of sitosterol was added to their diet and fell even further when treated with 1.5 g sitostanol

Table 5. Phytosterol effects on serum cholesterol levels and atherosclerosis in various species*

	Species		
	Rat	Chicken	Rabbit
No. studies	13	13	20
Avg. phytosterol dose (%)	5.4 ± 1.9	2.3 ± 0.5	2.8 ± 0.5
Avg. cholesterol dose (%)	1.6 ± 0.4	1.2 ± 0.4	0.9 ± 0.13
Cholesterolemia	10/13↓	12/13↓	↓17/19
Atherosclerosis	—	6/13↓ (7/13 ND)	↓11/16 ↑3/16

*Summarized from Pollak and Kritchevsky[22].
ND = no data.

Table 6. Influence of phytosterols in human cholesterolemia* (52 studies)

Avg. number of subjects	17 ± 3
Avg. phytosterol dose	1.3 ± 1.1 g/d
Avg. duration of study	27 ± 4 weeks
Avg. cholesterol reduction	20 ± 1.5%

*Summarized from Pollak and Kritchevsky[22].

daily. Denke[40] placed 33 men on an AHA Step 1 (cholesterol-lowering) diet. Addition of sitostanol to the dietary regimen did not affect total cholesterol levels but the LDL-cholesterol/HDL-cholesterol ratio fell by 6.5%.

Miettinen and his co-workers have interesterified sitostanol into rapeseed oil to provide a fat containing an appreciable amount of sitostanol esters. This fat can be used to make margarine or mayonnaise. Using the sitostanol ester-rich margarine they investigated cholesterol and lipoprotein metabolism in a group of eleven hypercholesterolemic non-insulin dependent diabetic men[41]. The men received 3 g/day of sitostanol and the study was carried out in a double-blind crossover design. Serum total-, VLDL-, and LDL-cholesterol and apoprotein B fell by 6, 12, 9, and 6%, respectively (all significant) and HDL-cholesterol rose by 11 ± 4% ($p < 0.05$). They concluded that the mode of action of sitostanol ester involved decreased cholesterol absorption and decreased transport rate of LDL-apoprotein B (Tables 7 and 8). A second study[42] involved use of a rapeseed oil mayonnaise containing sitostanol ester. Fifteen mildly hypercholesterolemic subjects were placed on diets in which 50 g of their usual dietary fat was replaced by rapeseed oil mayonnaise (no sitostanol) for 6 weeks. Eight subjects were continued on this regimen for 15 weeks and seven were given the mayonnaise plus a low dose of sitostanol ester (800 mg/d) for 9 weeks followed by 6 weeks on the mayonnaise plus a high (2000 mg/d) of sitostanol ester. Serum cholesterol levels fell by 14% (249 ± 9 to 214 ± 11) during the rapeseed oil period, fell 3% further on 800 mg/d of sitostanol ester and fell another 5% on 2000 mg/d of ester. LDL-cholesterol fell by 19, 6, and 12%, on the margarine, low or high sitostanol periods, respectively. HDL-cholesterol ended up increased by 8% and the LDL-cholesterol/HDL-cholesterol ratio which was 2.52 at the beginning of the study had fallen to 1.56 after the high sitostanol period. Triglyceride levels were also reduced. Overall cholesterol absorption was reduced significantly and fecal neutral steroid excretion was increased by 18.7% ($p < 0.05$). In a 1 year study, 102 mildly hypercholesterolemic subjects received 1.8–2.6 g/day of margarine containing sitostanol esters and the control

Table 7. Influence of sitostanol ester margarine on lipid metabolism in diabetic subjects* (mean ± SEM)

Variable (mg/dl ± SEM)	Control	Test
Total serum cholesterol (C)	231 ± 9	217 ± 8[a]
Serum triglycerides	189 ± 15	184 ± 19
VLDL-C	28 ± 3	24 ± 3[a]
IDL-C	12 ± 1	11 ± 0.8
LDL-C	148 ± 6	134 ± 6[a]
HDL-C	44 ± 1	48 ± 2[a]

*After Gylling and Miettinen[41].
Eleven subjects on 3 g sitostanol/day.
[a]$p < 0.05$.

Table 8. Cholesterol metabolism in diabetic men receiving sitostanol ester* (mean ± SEM)

Variable	Control	Test
Cholesterol absorption (%)	25.3 ± 2.1	8.6 ± 1.8[a]
Dietary cholesterol absorbed (mg/kg/d)	0.96 ± 0.12	0.42 ± 0.12[a]
Total cholesterol absorbed (mg/kg/d)	4.5 ± 0.4	1.8 ± 0.4[a]
Fecal steroids of cholesterol origin		
Neutral sterols	13.5 ± 1.1	18.7 ± 0.8[a]
Bile acids	8.6 ± 1.1	8.4 ± 1.2
Cholesterol synthesis(mg/kg/d)	18.2 ± 1.6	22.7 ± 1.3[a]
Intestinal cholesterol flux(mg/kg/day)	18.0 ± 1.1	20.6 ± 1.0[a]

*After Gylling and Miettinen[41].
Eleven subjects fed 3 g sitostanol/day.
[a]$p < 0.05$.

group of 51 subjects were given sitostanol-free margarine[43]. At the completion of the study there was a 10.2% mean reduction of total cholesterol in the test group ($p < 0.001$) and LDL-cholesterol was reduced by 14.1% ($p < 0.001$). Neither HDL-cholesterol levels nor triglycerides were altered (Table 9).

There has been little interest in sitosterol in the last 20 years and hence little work on its mode of action. The modes of action recently attributed to sitostanol[41] may also hold for sitosterol. Among the reasons given for the discontinued use of sitosterol were its chalky taste and inconvenience of dosage regimen (before every meal) but these have not discouraged use of bile acid sequestrants. Levere et al.[44] tested a sitosterol preparation in 25 ambulatory subjects over 9–23 months and reported a variation in their cholesterol levels ranging from +39 to −33%. The swings in cholesterol level were extreme but they were seeing the variations in cholesterol levels that have been seen by many other authors[45–49]. Very likely they would have seen similar fluctuations in control subjects. It is possible that Levere's paper[44] and an earlier one which reported no effect of sitosterol[50] on serum cholesterol levels may also have discouraged further investigation. The phytosterols merit renewed testing under the rigorous conditions of clinical trial currently in vogue and using the large new array of analytical methods. They may still have much to offer the clinician.

At least eight different mechanisms have been proposed to explain the hypocholesterolemic effect of sitosterol (Table 10).

Table 9. Serum lipid changes in 48 subjects fed sitostanol for one year*

	Sitostanol		
Variable	None	2.6 g/d**	p <
Total cholesterol (C)(mg/dl)	−1	−25	0.001
LDL-C (mg/dl)	−3	−24	0.001
HDL-C (mg/dl)	0	+0.4	NS
HDL-C/LDL-C	+0.008	+0.059	0.002
Triglycerides (mg/dl)	+9	−4	NS

*After Miettinen et al.[43]
Sitostanol fed as margarine containing sitostanol esters.
NS = not significant.

Table 10. Suggested mechanisms of hypocholesterolemic properties of sitosterol*

1)	Mixed crystal formation
2)	Adsorption aggregation
3)	Decreased solubility of cholesterol micelles
4)	Enhanced cholesterol excretion
5)	Interference with cholesterol synthesis
6)	Competitive esterification
7)	Competition for "acceptor" sites
8)	Reduced membrane permeability

*Summarized from Pollak and Kritchevsky[22].

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ADDING CERTAIN FIBER-RELATED NUTRIENTS TO FOOD PRODUCTS

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1. INTRODUCTION

In the session "Nutrients Contributing to the Fiber Effect", four components — resistant starch, oligosaccharides, phytosterols, and lignans — were discussed. Where commercially available, these components are usually marketed under a trade name.

While some information is becoming available on the use of resistant starch (RS) and oligosaccharides (OSs) in commercially produced food products, virtually no information is available on the other two components. All four components are briefly discussed here, with food-use information, however, limited to RS and OSs. When added to foods, finished products containing the four discussed components may be viewed as "Functional Foods", a term synonymous with terms such as "Designer Foods", "Medical Foods", "Nutraceuticals", "Pharma Foods", and the likes. The food industry in North America is interested in the concept of "Functional Foods", but regulatory environment, unlike the situation in Europe and Japan, is prohibitive for this category of food products to be promoted and marketed.

2. ADDING RESISTANT STARCH

The term RS was first coined by Englyst and Cummings[1]; they also classified RS into different types. The type currently commercially available in North America results from subjecting high-amylose cornstarch to repeated heating and cooling cycles. This RS is being added to a variety of foods, especially grain-based, extruded products.

The two sources marketed in North America are: Novelose™ and CrystaLean™. Both contain about 30% RS, which measures as insoluble fiber when determined by the enzymatic-gravimetric fiber method[2]. Their energy value is listed as 2.8 kilocalories per g. This is based on the assumption that RS is non-caloric; a recent study[3] indicates such may be the case.

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These sources are promoted as bland white powders, with no color or taste and as not altering flavor and textural properties of foods to which they may be added. More importantly, they do not retain much water and, thus, may be suitable for use in low-moisture products such as cookies, crackers, and some snack (extruded) food items. A high level of RS, unlike most other fiber sources, may be used in extruded products since these products treat RS as normal (digestible) starch.

Although isolated high-amylose cornstarch is the normal source for commercially prepared RS, studies have shown that white flour itself can be treated in a similar manner to increase RS levels[4]. This increase, expressed as total dietary fiber (Table 1), is usually not as high as achievable when high-amylose cornstarch is used as the starting material. Treated white flour, however, also adds protein and micronutrients when used in a product, although the flour does lose its gluten functionality and may be suitable for use in some products only.

3. ADDING OLIGOSACCHARIDES

In Japan and some European countries, OSs are widely used in food products. In 1991, Japan marketed 450 food products containing OSs. OSs function as bulking agents and as reduced-calorie sweeteners. Some commercial sources are: Soy Oligo™, Raftiline™, and Raftilose™; several other sources are also commercially available.

The syrup form of Soy Oligo™ contains 24% OSs (stachyose and raffinose), 52% other sugars, and 24% water. Stachyose and raffinose are marketed as "Functional" food ingredients in Japan, primarily because they appear to promote beneficial intestinal bacteria (bifidobacteria). Soy Oligo™ syrup is 70% as sweet as sucrose and contains 50% fewer calories. Its potential applications include use in beverages, baked goods, ice cream, yogurt, puddings, infant foods, health foods, and various other products.

In Japan, beverages are the main product where Soy Oligo™ syrup finds its wide use. It can replace high fructose corn syrup. A cultured milk soft drink (Calpis™), formulated with Soy Oligo™ syrup, is one of the best selling products in Japan[5]. It is approved by the Ministry of Welfare as a food for health, although overdose may cause diarrhea.

Raftilose™ and Raftiline™ (inulins), prepared from chicory root, are also used in many foods—confectioneries, baked goods, meat products, ice cream and sorbet, chocolate, yogurt and fresh cheeses, milk desserts and fruit preparations.[6] Their use levels may be as high as 25% (confectioneries). Studies have shown that Raftiline™ can replace sucrose in white cake, with a 50% replacement not requiring an adjustment for sweetness but a 100% replacement needing acesulfame K, an intense sweetener, to maintain desired sweetness.

Table 1. Resistant starch in white wheat flour

	Total dietary fiber, %	
	Untreated flour	Treated flour
Five bread flours	2.7 ± 0.1	11.2 ± 0.6
Five pastry flours	2.4 ± 0.1	12.4 ± 1.3

Reference 4.

Raftiline™ and Raftilose™ are marketed as natural ingredient (i.e., not considered as "additives"), a fiber (soluble) source, a fat substitute, a bifidus promoter, and a low-calorie sweetener. Table 2 shows the caloric value of these OSs; for Raftiline, we recently reported a caloric value of 1.5 kilocalories per g[7].

Maga and Fapojuwo[8] analyzed 39 drinks from 12 different countries to assess the level of OSs in these drinks. Virtually all drinks were found to contain raffinose, with only a few containing stachyose and verbascose. The drinks sampled in the United States contained more total oligos (1.72 g/100 ml), but only a few were sampled and, thus, the values obtained may not be representative. Drinks sampled in Spain, United Kingdom, and Hong Kong contained less than 0.1 g oligos per 100 ml.

4. ADDING PHYTOSTEROLS

The main sources of phytosterols — fruits, vegetables and leguminous seeds — are linked to a reduction in chronic diseases. Although, these sources (dried, pureed, powder, and paste forms) are added to many grain-based and other foods, no information appears available where isolated phytosterols are being added to foods. In part, this could be because the safety and toxicity issues have not been resolved. Where safety is not a concern, some phytosterols like beta carotenes are added to foods such as isotonic sport drinks, margarines, baked goods, and nutritional bars.

5. ADDING LIGNANS

Lignans, which are reported to possess antioxidant and antiestrogenic properties, are currently not available in commercial quantities for addition to food products. Concentrated source of lignans such as flaxseed (linseed) and sesame seed are, however, widely used in many grain-based and other foods. They may be added as seeds, as pastes, or as powders.

Analyzing defatted flaxseed meal, Muir and Westcott[9] recently reported a lignan content of 9–30 mg/g. In white and whole wheat breads commercially produced in Canada, Muir and Westcott[9] were unable to detect lignans. However in flax-containing baked products (maximum permissible level in baked goods in Canada, 7%), lignan levels ranged between 0.24–0.90 mg per g (flax breads) and 0.60–1.20 mg per g (muffins/bagels/cookies). Based on these values, a 50-gram (two slices) serving of flax-containing bread would provide 12–45 mg lignans. Studies carried out at the American Institute of Baking show that a good quality variety bread can be produced containing 22.2% (based on flour equal to 100%) pre-soaked flaxseeds in the formula.

Table 2. Caloric value of raflilose™ and raftiline™

Component	Kilocalories/g
Raftilose 60 (syrup)	2.5
Raftilose 85 (syrup)	1.9
Raftilose 95 (syrup or powder)	1.6
Raftiline (powder)	1.0

Reference 6.

6. CONCLUSIONS

Although the evidence for health benefits is better for whole foods and food groups than for individual food components, the evidence for protective effects against chronic diseases is also persuasive for isolated components discussed in this section. These components can be added to foods for distinct health benefits. To bring such foods to the market place, however, food industry, academia and regulatory agencies need to be fully involved.

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FIBER AND CANCER PROTECTION — MECHANISMS

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Since we do not currently understand all the changes that accompany the various stages of transition from normal colonic epithelium to colon cancer, it is impossible to talk with great certainty about how dietary fiber protects against colon cancer. Nevertheless, enough is currently known in this complicated process to discuss potential mechanisms by which dietary fiber may operate. This chapter will first examine the effects of dietary fiber within the lumen of the bowel and then concentrate on the tissue effects that might occur as a result of exposure to high fiber diets or specific metabolites of dietary fiber.

1. BULKING EFFECTS

While the association of reduced colon cancer incidence has been linked with the consumption of high fiber foods for three decades, the physiological effects that lead to this relationship are not well established. Although fiber is one component of foods that may be protective against cancer the epidemiology, by its very nature, precludes definitive conclusions about what constituent(s) of foods may protect against development of colon cancer. There are a number of potential mechanisms by which a protective effect of dietary fiber may be operative. The earliest proposed mechanism for the protective action of dietary fiber against colon cancer was that of intestinal bulking espoused by Denis Burkitt (1,2). To paraphrase the words of Burkitt, small stools in a society necessitated large disease palaces but large stools required only small clinics. This hypothesis has the attraction of being very simple; however, simple answers to complex problems like colon cancer are likely to be too simplistic at best and incorrect at worst. However, the value of this hypothesis was to focus both scientific and lay public attention on the importance of dietary fiber and its potential for reducing the incidence of a number of chronic diseases. In its original iteration, the fiber hypothesis suggested that bulking of colonic contents would dilute fecal carcinogens or promoters and decrease transit time. While dietary fiber does

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hold several times it weight in water, the majority of the bulking effect is probably due to increased bacterial mass. An other effect that dietary fiber may have on intestinal mass is to bind carcinogens or promoters, making them less available for contact with the colonic epithelium. However, fermentation of fiber in the colon by the microflora will release those compounds into the intestinal contents to freely contact the mucosa. Another simple relationship that has been suggested previously by the author (3) is that consumption of adequate dietary fiber will result in softer stools. The reduced amount of abrasion in the rectosigmoid portion of the colon will lead to lower cell turnover and less tumor promotion. It is well established that irritation acts as a strong tumor promoter in the colon and other tissues. For example, there is significant increase in tumor formation at suture lines in the colon (4) or at the edges of burns or scars of the skin (5). Most of the excess tumors found in affluent countries are in the left colon and specifically in the rectosigmoid area of the colon where the lesser water content makes the stool firmer, and presumably more abrasive.

2. BILE ACIDS

A related, subsidiary mechanism to the original stool bulking hypothesis is that of bile acids as tumor promoters. While there are good statistical associations of higher fecal bile acid concentrations in a population with incidence of colon cancer, the studies that have examined fecal bile acids in individuals with adenomas or carcinomas have not consistently found many differences (3). The bile acids are clearly promoters at pharmacologic levels but it is not clear that these compounds alone are promoters at physiologic levels (6,7). While bile diversion studies suggest that bile is a promoter, this fluid is a mixture of several components that may play a role in increasing tumorigenesis; in addition many xenobiotics undergo enterohepatic circulation via the bile. Studies of patients or animals with cholecystectomies have been inconclusive on whether the constant versus pulsatile release of bile results in greater risk of colon cancer. In studies of bile, as opposed to bile acids or salts, one cannot discount the effects of other constituents that might play a role in the tumorigenic process such as phospholipids, bile pigments or cholesterol. Secondary bile acids are more active promoters than primary bile acids and this may be related to the greater detergent effects of the secondary structures on cell membranes at the surface of the colonic crypts (8,9). The principal physiologic effect of high concentrations of bile acids in the colon appears to be increased cell turnover. This will be discussed below in greater detail. An as yet unanswered question is whether the concentration of water soluble bile acids is more important than the concentration of total bile acids in the intestine.

3. FERMENTATION

Our understanding that fiber is not inert and the majority of it is fermented, even on typical western diets, has altered thinking in this area. Fermentation products that could play a role in development of colon cancer include the short-chain fatty acids (SCFA), ammonia, and urobilinogens such as stercobilin and biliverdin (10–12). Since fiber may change the amount and type of bacterial cells in the hindgut, there are a large number of compounds and activities produced by the biomass in the colon that could impinge upon the tumorigenic process in the colon. Examples include diacylglycerols which have been shown to be mitogens for colonic epithelium (13,14); these compounds are produced by

bacteria, presumably from fats that escape absorption in the small intestine. Glucuronidation by the bacteria of various toxic molecules should make them water soluble and more readily excreted (15). The use of probiotics, or combinations of specific microorganisms to colonize the intestine, can alter the colonic environment which could affect tumorigenesis. One of the difficulties in dealing with this area of bacterial metabolites is that the processes and products will be multi-substrate dependent. That is, the absolute and relative amounts of many nutrients reaching the colon will have an effect on the bacterial environment.

An hypothesis that seems to have supplanted the bile acid mechanism as the most likely explanation for the protective effects of dietary fiber against colon cancer is that of fermentation of fiber to SCFA. Although there are three principal SCFA produced in relatively specific ratios (dependent on diet) by fermentation in the colon of monogastric animals — acetate, propionate, and butyrate — most of the experimental work has been done with individual SCFA. Butyrate has been, by far, the most intensively studied of the SCFA partly because it has been shown to be the preferred substrate for colonocytes (16). In addition, butyrate is a differentiating agent in vitro and induces apoptosis, or programmed cell death (17). However, there are a number of unanswered questions in this area that must be scrutinized before one can make conclusive statements concerning the preventive role of butyrate in colonic carcinogenesis. All SCFA are volatile and are supposed to be rapidly absorbed from the lumen (18). This suggests that most of the SCFA produced in the proximal colon will not be present by the time the intestinal contents get to the distal colon. Slowly fermented fiber sources such as wheat bran and psyllium may increase butyrate contents of the distal colon but rapidly fermented forms of fiber may be completely gone by the time they leave the proximal colon. It is unknown to what extent fecal SCFA measurements reflect intracolonic levels. In addition to these methodologic issues of SCFA measurement, a critical question in this scenario is if there are differential responses to butyrate by normal and transformed cells (19) and whether the ratios of other SCFA or conditions such as pH modulate the physiologic effects of butyrate.

Acidification of the colonic contents has been proposed to protect against the development of colon cancer (20). Although it is widely accepted that SCFA are the major constituents that acidify the colon, the author knows of no objective data on this point. These organic acids are weak anions but make up 75% of total positive charges in the intestine (21). The buffering capacity of the colonic contents must be quite potent and it is unclear what percentage of acidification is due to SCFA. Changes in the pH will affect solubility of many metabolites as well as metabolic activity of the numerous bacterial species in the colon. In particular, exoenzymes released by some bacteria might be particularly susceptible to alterations in the acidity of the intestinal environment.

4. LUMENAL ANTIOXIDANTS

Another group of compounds that might modulate the carcinogenic process in the colon is lumenal antioxidants which are found in fiber-rich foods. These include: phytic acid, which has been shown to inhibit chemically-induced colon and breast cancer in rats (22,23); lignans, which are similar in structure to estrogen and inhibit some types of experimental tumors (24,25); bioflavonoids, which are abundant in plant foods and modulate the immune system, cell cytokinetics, and other parameters that can affect tumorigenesis (26–28); carotenoids have been shown to reduce incidence of a variety of cancers (29–31); and glutathione is supposed to be bioavailable from fresh vegetables. People con-

suming a typical western diet are estimated to consume up to one gram per day of bioflavonoids; there are estimates that people consume up to 2,400 individual bioflavonoids (32). These compounds have numerous biological effects, ranging from inhibition of platelet aggregation to altering protein kinase C levels (33,34). The beneficial effect of high carotenoid levels in the plasma for reducing incidence of a variety of cancers, including lung cancer, is well established (35,36) but the clinical intervention trials in which β-carotene supplements were administered have been dismal failures. These studies have shown either no benefit or a significant increase in lung cancer in individuals taking the supplements (37–39). These results indicate that the protective factor is not β-carotene or that its administration in high doses reduces absorption of other bioprotective carotenoids or that high doses of this antioxidant results in prooxidant activity.

5. CYTOKINETICS

In addition to the effects in the intestinal lumen of dietary fiber and other compounds found associated with it, there are a number of demonstrated actions on the intestinal tissue. The most widely studied has been that of cytokinetics. Increased proliferation is clearly a risk factor for enhanced risk of developing colon cancer (40). This is particularly true when proliferation expands out of the lowest third of the crypt. However, one must also distinguish proliferation rates of normal and transformed cells as these two types of cells may not respond in the same manner to similar stimuli. The converse of proliferation is the phenomenon of apoptosis and this has been studied only minimally with respect to dietary fiber and colonic carcinogenesis. A relevant question in this regard is why are small bowel tumors so rare when the proliferation rate is higher there than in the colon? One must assume there is increased desquamation of surface epithelium as a result of apoptosis in the colon as compared to the small intestine. The conditions in both the upper and lower intestines are quite harsh with respect to the other environments in which cells are normally found. Therefore, the surface cells of the intestine must have a well developed system for shedding cells that are altered genetically or otherwise by exposure to lumenal factors.

6. EICOSANOIDS

Another area with seemingly great potential for contributing to our understanding of the carcinogenic process in the colon is the observation that cyclooxygenase inhibitors such as aspirin, sulindac, and piroxicam significantly inhibit the formation of polyps and carcinomas in humans (41,42) and these compounds have a similar protective effect on chemically-induced colon cancer in rats (43). The tissue response to these eicosanoids may be influenced by interactions with SCFA or result from altered membrane fatty acids or phospholipid classes (44–46). The data on this aspect of colon carcinogenesis are probably the strongest at the present time for identifying a protective factor. Since consistent, chronic use of these nonsteroidal antiinflammatory drugs is associated with a 50% reduction in tumor formation this is potentially one of the most promising areas for inhibiting colon tumors.

7. TISSUE METABOLISM

There are many other tissue alterations that have been documented in response to feeding of dietary fiber. Decreased ornithine decarboxylase (ODC) has been shown with

high levels of some fiber types (47,48); however, there may be specific isoforms of ODC, rather than total enzyme activity, that are more important in the development of colon cancer (49). Recently, there has been emphasis on the potential role of protein kinase C and its translocation between the cytoplasm and cell membrane in response to dietary fiber (50,51). Consumption of dietary fiber probably plays a role in altering xenobiotic metabolizing enzymes of the colonic tissue which would modulate a cell's resistance to transformation. A concept that relates back to the original bulking hypothesis is whether stretch receptors in the colon modify adhesion molecules and, subsequently, alter cell-cell communication in the colonic epithelium.

8. MUCIN

The synthesis and release of mucins and mucous by the intestinal epithelium can be influenced by dietary fiber intake (52). Since mucin release washes lumenal contents away from dividing cells, it is reasonable to assume that changes in both the quantity and quality of mucin and mucous my alter susceptibility of colonic cells to initiators and promoters in the intestinal lumen. Theoretically, a decrease in thickness or viscosity of mucin layers on the crypt surface should increase access of lumenal compounds to the epithelial cells. Intestinal mucin has been demonstrated to bind carcinogens (53) and this may inhibit interaction of these compounds with target cells. Since fiber also has the ability to bind carcinogens, competitive binding between fiber and mucin may determine whether a particular compound reaches the epithelium. Finally, the solubility of mucin may be altered by the presence of SCFA; mucin is insoluble in acetic acid so small concentrations of this acid may alter the partitioning of water into the mucin layer or its permeability.

9. GENETIC CHANGES

There has been an explosion of research into the sequential genetic changes that are involved in the stepwise process of transformation from normal epithelium to cancer. In this book, Michael Hill (54) describes the transition from normal mucosa to small adenoma to large adenoma to severe dysplasia to cancer. He points out that small adenomas are distributed throughout the colon, suggesting that systemic rather than local factors are important in the genesis of these lesions. In addition, this change is rather widespread with 50% of males and 30% of females showing these lesions at age 70. In contrast, the distribution of large adenomas is the same as that for colon cancer, there is a equal gender distribution, and their incidence is much less. These facts suggest that progression of small adenomas to large adenomas is the rate limiting step in progression to colon cancer. Vogelstein and colleagues (55) have described a sequence of genetic changes that is involved in this process. Genes included in this process are p53, DCC (deleted in colon cancer), K-ras, C-myc, CEA (carcinoembryonic antigen), and DRA (down regulated in adenoma) (56–61). This last gene was recently identified and codes for a membrane glycoprotein that functions as a sulfate transporter. One way in which dietary fiber may affect gene expression is via formation of SCFA, particularly butyrate (62). Since there appears to be a cascade of genetic changes that occurs during the carcinogenic process, it is theoretically possible to intervene at several steps. However, this will only be possible if each step is both necessary and sufficient for progression to the next stage. It has already been shown that some tumors may not show all the genetic changes that are seen in the typical carcinogenic cascade.

10. PEPTIDE GROWTH FACTORS

An area that has received relatively little attention is the response of colonic tissue to a variety of peptide hormones. There is little reason to suspect that peripheral blood levels of these growth factors play a role but local tissue concentrations and/or receptor expression for these hormones may be key. A considerable amount of data exist for trophic effects of gastrin and peptide YY, including their influence on colonic carcinogenesis in animals (63,64) although some data oppose this linkage (65). Epidermal growth factor levels in rat colon are reduced by feeding rats a wheat bran diet compared with those fed a fiber-free ration (66). Insulin and insulin-like growth factor I (IGF-I) are well established mitogens for both normal and transformed cells and respond primarily to protein and energy intake. Several studies have linked these peptides and the IGF binding proteins to colonic carcinogenesis (67–69). Finally, vitamin D has a variety of hormonal activities, particularly in the gastrointestinal tract, and may be responsible for the epidemiologic observations that dairy product consumption is protective against colon cancer, although endogenous synthesis of vitamin D may may also be related to lower incidence of colon cancer (70–72).

11. SUMMARY

There is no reason to believe that a single lumenal or tissue factor will hold the key to understanding the of dietary fiber's effect on reducing the risk of colon cancer. In fact, the data suggest that multiple, interacting factors will be revealed. After years of research, it appears that the bile acid hypothesis is not nearly as strong as first envisaged. Additionally, the theory that SCFA protect against colon cancer has little clinical or experimental support. There is no doubt that identification of genetic alterations, and their controlling factors, will play a major role in our understanding of this issue. The appeal of the original fiber hypothesis has not diminished but simply requires updating based on discoveries made since it was first proposed. It is this author's opinion that dietary fiber will likely be found to modulate human colon cancer and the mechanisms of its beneficial effect will probably be through multiple actions within the lumen and at the level of the target tissue. Based on our current knowledge of the pathogenesis of colon cancer we cannot make definitive statements about what percentage of colon cancer might be prevented by a specific type or amount of dietary fiber but it is reasonable to conclude that consumption of fiber-rich diets is associated with reduced risk of colon cancer. It is quite plausible that the combination of dietary fiber, or its metabolites, in conjunction with other phytochemicals may be necessary to realize inhibition of the tumorigenic process.

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DIETARY FIBER AND BILE ACID METABOLISM — AN UPDATE

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Metabolism of bile acids plays a key role in the etiology of two of the most common causes of death, coronary heart disease and colon cancer. Bile acids are the major route of excretion of steroid from the body and thus a determinant of changes in sterol balance, a risk factor for coronary heart disease. Risk for colon cancer has been shown to increase with increased concentrations of bile acids in the colon, both in epidemiological studies in humans and in experimental animal studies. Dietary fiber plays a major role in regulating the metabolism of bile acids and altering risk for these diseases. However we know little of the specific mechanisms involved in the role played by dietary fiber in bile acid metabolism.

Dietary fiber sources fed to humans with predominantly water soluble components have proved to be more effective in reducing serum cholesterol than insoluble types of fiber[1–5]. Among these effective sources of dietary fiber many have also been shown to prevent cholesterol accumulation in cholesterol-fed experimental animals. Soluble fibers, such as pectin, guar gum and psyllium hydrocolloid, significantly reduce serum cholesterol concentrations in rats fed normal and cholesterol supplemented diets[6–8]. Oat bran, a fiber source with both soluble and insoluble components, reduces cholesterol accumulation in rats[8–11].

One of the first suggested mechanisms for the effects of dietary fiber on sterol balance was that these dietary fiber sources adsorbed bile acids, reducing their reabsorption and increasing their excretion in feces[12]. In humans this hypothesis is supported by most studies[1,3–5] but the magnitude of increase in fecal bile acids is often not in proportion to the reduction in plasma cholesterol levels. In experimental animals the results are less consistent.

The ability of dietary fiber sources to prevent cholesterol accumulation in experimental animals has been examined extensively. The following briefly reviews a portion of those studies which are germane to our discussion. In a direct comparison of ten sources of dietary fiber in rats fed a diet containing cholesterol and cholic acid, Anderson et al.[13] found several sources of dietary fiber significantly reduce the accumulation of serum cholesterol caused by the diet. Psyllium, oat gum, guar gum and pectin all caused significant

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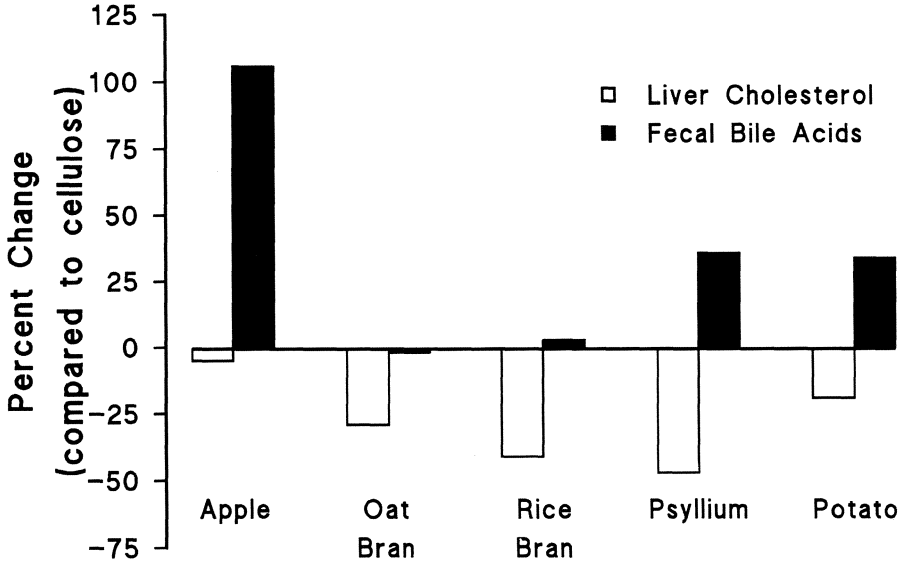

Figure 1. Percent change in liver cholesterol accumulation and fecal bile acid excretion in rats fed various sources of dietary fiber.

reductions while soy fiber, oat bran and corn bran resulted in reductions that were not significant and wheat bran and rice bran caused small increases in serum cholesterol. Shinnick et al.[11] found that oat bran altered serum cholesterol in rats fed this cholesterol/cholic acid diet in a does-response fashion.

In similar studies in rats employing a semipurified diet containing cholesterol (but not cholic acid) which causes an increase in liver but not serum cholesterol, we have found that oat bran, psyllium, pectin and rice bran all resulted in significantly reductions liver cholesterol accumulation[8]. Arjmandi et al.[9] made similar observations for psyllium, oat bran and pectin.

Concomitant examination of bile acid excretion in rats has yielded interesting results that indicate the inadequacy of our initial hypothesis. As can be seen in Figure 1, accumulation of liver cholesterol was reduced in comparison to cellulose by all of these sources of dietary fiber from 5–47%. However the change in bile acid excretion on a daily basis ranges from an increase of more than 100% to only minor changes and the magnitude of change in bile acid excretion does not correlate well with the ability of the dietary fiber source to alter cholesterol accumulation. Most interesting may be oat bran which results in a very consistent lowering of cholesterol levels[11,13] but has not been shown to increase bile acid excretion in rats[9]. In humans oat bran has consistently resulted in an increase in bile acid excretion[1,5]. This would suggest that several mechanisms might be involved in the effects of on cholesterol balance, i.e., that bile acid excretion might be a part of the effect but some other mechanism is at work in some or all of the dietary fiber sources which have an effect on cholesterol levels.

In an effort to better understand these alternate mechanisms we examined the effects of psyllium and pectin on bile acid pool size in rats in comparison to cellulose[14]. We employed the washout technique that involves insertion of a bile duct canula and collection of bile as the pool "washes" out to a point where synthesis = secretion. As can be seen

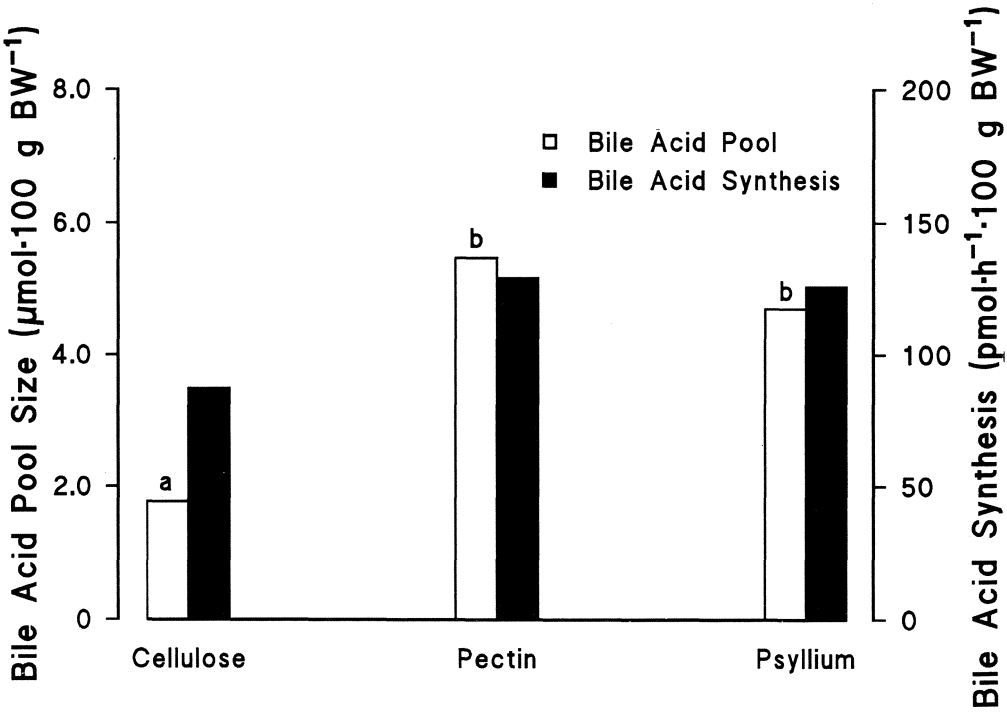

Figure 2. Bile acid pool size and synthesis rates in rats fed cellulose, pectin or psyllium. Bars with different letters are significantly different ($p<0.05$).

in Figure 2, both pool size and synthesis increased in response to pectin and psyllium but only the increase in the size of the bile acid pool was statistically significant. Turley et al.[15] have reported similar results in hamsters, i.e., increased bile acid pool size in response to psyllium.

To determine if the change in apparent synthesis measured by the above methods was substantiated by increased activity in the rate limiting enzyme in bile acid synthesis, we measured the activity of cholesterol 7α-hydroxylase in groups of rats fed several sources of dietary fiber[8]. As can be seen in Figure 3, activity of cholesterol 7α-hydroxylase was increased in animals fed pectin, psyllium and cholestyramine (the positive control) but was not significantly different among those fed cellulose, oat bran or rice bran.

Studies in guinea pigs have supported our observation that cholesterol 7α-hydroxylase is stimulated by pectin and psyllium. Fernandez[16] reported an increase in cholesterol 7α-hydroxylase activity of 39% in response to pectin feeding and 59% when psyllium was fed, both differences being significantly different. Guar gum, on the other hand, actually resulted in a nonsignificant reduction in activity (10%). Guar gum did however result in lower plasma cholesterol levels, the change seemingly as a result of a greater reduction in cholesterol absorption. Horton et al.[17] have also reported increased activity of cholesterol 7α-hydroxylase in hamsters fed psyllium (compared to Avicel) as well as an increase in hepatic cholesterol 7α-hydroxylase mRNA levels.

The availability of stable isotope techniques have allowed similar studies on bile acid pool size and synthesis rates to be carried out in humans. Everson et al.[3] fed psyllium (15 g/d) to hypercholesterolemic men for 40 days. The observed 6% reduction in

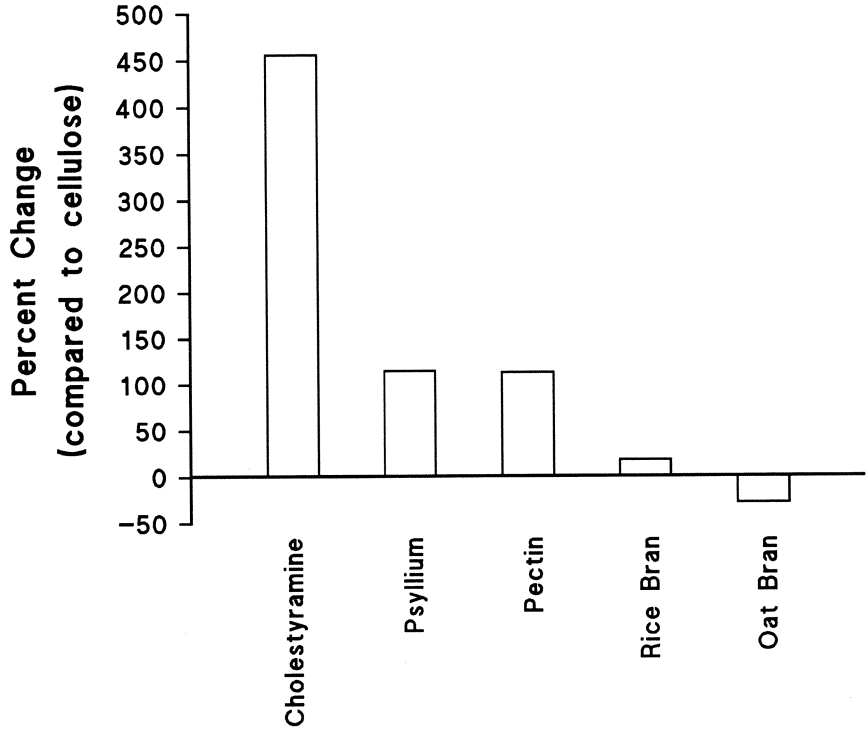

Figure 3. Change in hepatic microsomal cholesterol 7α-hydroxylase activity in rats fed various sources of dietary fiber in comparison to cellulose.

plasma cholesterol level was accompanied by an increase in bile acid pool size which was not statistically significant and a significant increase in bile acid synthesis (Figure 4). Marlett et al.[5] fed oat bran (100 g/d) to normocholesterolemic men for 4 weeks and compared the results to a similar period when they were fed a low level of dietary fiber (Figure 5). Serum cholesterol levels were reduced significantly (9%) and, as in the earlier study, bile acid pool size was increased 11% (N.S.) and bile acid synthesis was significantly increased (58%). This response to oat bran is notably different than that reported above for rats[8,14].

What effector is causing the increase in bile acid synthesis observed in response to these sources of dietary fiber? The nature of bile acids, as natural detergents that solubilize lipids in the intestine and in bile, is a hydrophobic/hydrophilic balance. The number of hydroxyl groups, presence of side chain esters (taurine or glycine conjugation), and other factors determine this balance for each bile acid. Heuman[18] has described a method for estimation of the hydrophobicity of bile based on the hydrophobic/hydrophilic balance of mixtures of bile acids and salts, i.e., in bile. He suggested that the hydrophobic index (HI) can be calculated using the formula:

$$HI = \sum_{x=1}^{n} HI_x F_x$$

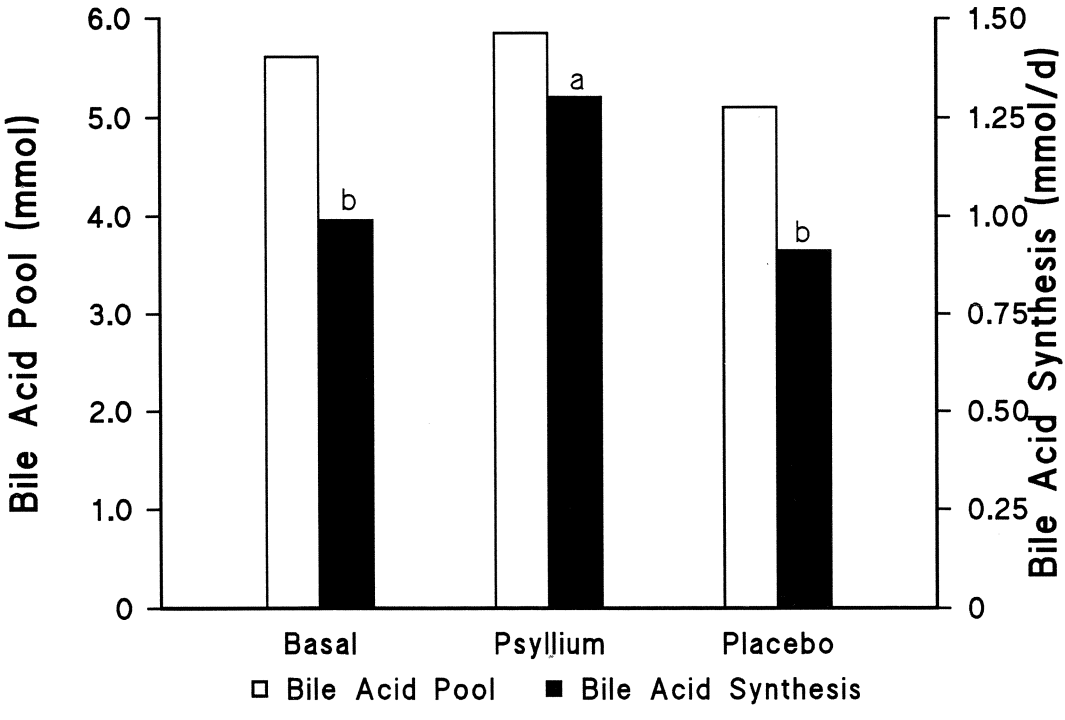

Figure 4. Bile acid pool size and synthesis rates in hypercholesterolemic men given psyllium or cellulose (placebo). Bars with different letters are significantly different ($p<0.05$).

Where HI_x is the hydrophobicity index for each bile salt and F_x is the mole fraction of that bile acid in bile. He found that various species produce bile of widely varying hydrophobicity depending on the composition of their bile, the rat having very hydrophilic bile (HI = −0.31), as a result of the presence of the muricholic acids, and humans having relatively hydrophobic bile (HI = +0.32). He suggested that, in addition to the role relative hydrophobicity would play in lipid solubility in bile (e.g., gallstone formation), HI would also be an important factor in regulation of cholesterol metabolism.

Subsequently Heuman et al.[19] examined the effects of HI on activity of cholesterol 7α-hydroxylase, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG CoA) reductase, and acyl CoA:cholesterol acyl-transferase (ACAT) in rats by feeding various bile acids to alter the relative sizes of the bile acid pools and thus alter HI. They found that increased HI was directly correlated with reduced activity of both cholesterol 7α-hydroxylase and HMG CoA reductase and, to a lesser degree, ACAT activity. This was accomplished by addition of many different bile acids and thus is not unique to a single bile acid or combination of bile acids but appears to be related only to the resultant HI of the bile.

In our work with various sources of dietary fiber[20] we observed changes not only in the level of excretion of bile acids but also in the relative amounts of bile acids excreted. Our studies which examined bile acid pool size[14] were designed to further clarify the nature of the change in the relative size of the individual bile acid pools. Data on the HI derived from this study and the activity of cholesterol 7α-hydroxylase which was reported for these same animals in a later paper[8] have been combined in Table 1. Both sources of dietary fiber resulted in less hydrophobic bile and increased activity of the cholesterol

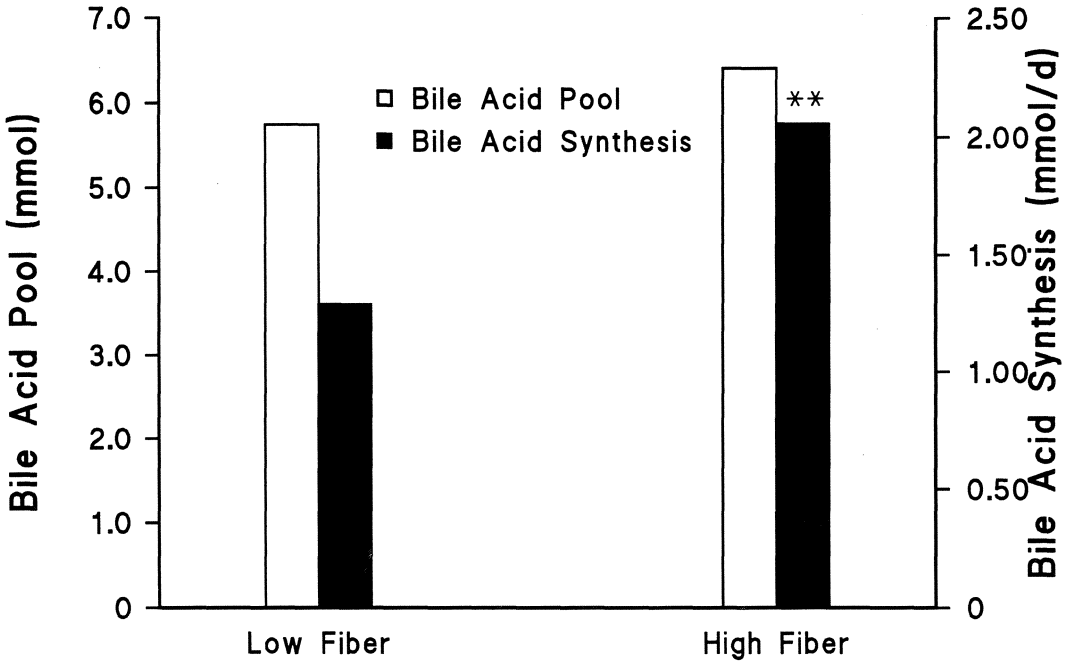

Figure 5. Bile acid pool size and synthesis rates in young males with normal cholesterol levels when fed a diet containing oat bran and compared to a low fiber diet. Asterisks indicate statistical significance ($p<0.05$).

7α-hydroxylase enzyme. In addition it should be noted that the HI in this experiment, for rats fed a semipurified diet, is substantially more hydrophobic than that reported by Heuman[18] for rats fed Rat Chow®, correlating well with the general observation that semipurified diets result in lower activity of cholesterol 7α-hydroxylase that those fed a commercial ration. Trautwein et al.[21] recently reported a decrease in the hydrophobicity of bile in response to psyllium but not pectin or guar gum in hamsters.

A primary site for possible changes in HI is the 12α-hydroxylation of bile acids during their synthesis. Of the two primary bile acids in humans, chenodeoxycholic acid is more hydrophobic than cholic acid which contains a 12α-hydroxyl group. Thus a reduction in hydrophobicity could result from increased 12α-hydroxylation in response to dietary fiber. In rats this is also an important control point but the rat can also add a 6β-hydroxyl group, forming the muricholic acids which are very hydrophilic and thus have a significant impact. We have measured the activity of these two enzymes in rats fed either cellulose, pectin or psyllium (Figure 6). Activity of the 12α-hydroxylase was increased by both sources of

Table 1. Relationship between hydrophobicity index (HI) and cholesterol 7α-hydroxylase activity in response to dietary fiber

Dietary fiber	HI	Cholesterol 7α-hydroxylase $pmol \cdot min^{-1} \cdot mg^{-1}$
Cellulose	+0.073	16.9
Psyllium	+0.015	36.3
Pectin	–0.074	36.0

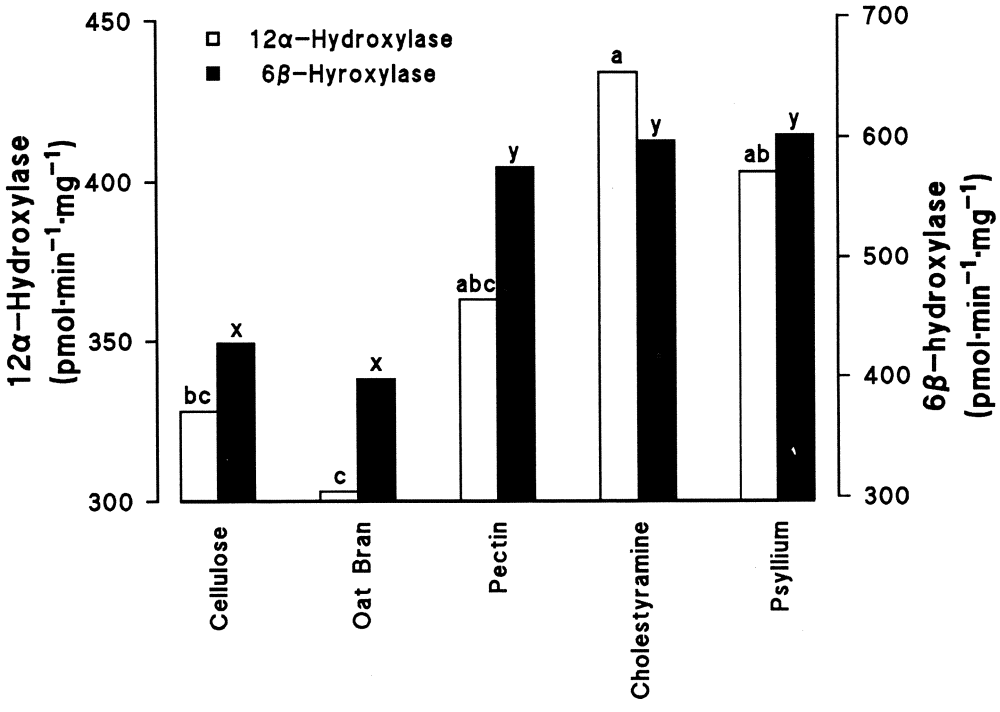

Figure 6. Changes in activities of 12α-hydroxylation of 7α-hydroxycholest-4-en-3-one and 6β-hydroxylation of lithocholic acid in rats in response to various sources of dietary fiber. Bars with different letters are significantly different ($p<0.05$).

dietary fiber but neither difference was statistically significant. However both sources caused a significant increase in activity of the 6β-hydroxylase. Increased synthesis of the muricholic acids would cause a greater reduction in hydrophobicity of bile than would a shift from synthesis of cholic to chenodeoxycholic acid.

Available evidence concerning the mechanisms by which some sources of dietary fiber alter cholesterol balance in humans and experimental animals would suggest the involvement of several mechanisms. In many cases bile acid excretion is increased in response to the dietary fiber source thus increasing the loss of sterol from the body. As a result of this loss of bile acids or through other mechanisms, the rate of synthesis of bile acids increases as does the size of the bile acid pool. Changes in the relative sizes of the individual bile acid pools occurs in response to these sources of dietary fiber. Changes in the hydrophobicity of bile as a result of these changes in bile acid spectrum alter regulation sterol balance at the molecular level and may be an important part of the effect of dietary fiber. Gaining an understanding of the mechanisms by which dietary fiber regulates sterol balance is an important step in being able to make better use of dietary fiber in treating abnormalities in sterol balance and in making recommendations for prevention of chronic disease.

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HARD WHEAT BRAN AND HARD WHEAT BRAN FIBER ENERGY VALUES MEASURED IN RATS AFTER 6 AND 16 WEEKS

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1. INTRODUCTION

The difficulties in determining the energy values of dietary fiber (DF) sources in human studies include the following: 1) The control over food intake, 2) the long-term maintenance of dietary changes (Stevens *et al.*, 1987), 3) the cost and the large number of dietary combinations to be assessed. Models for predicting the energy value of DFs in humans have improved over the last decade (Livesey, 1995) but energy-balance experiments in animals are important due to the limited number of human studies (Livesey, 1990a). The rat model is particularly useful for long-term experiments and it has been recommended for predicting the digestibility of dietary components and energy (Bach Knudsen et al., 1994; Wisker *et al.*, 1996).

It is important to verify if short-term rat assays can accurately predict long-term energy absorption. Changes in the colonic microflora and in the gastrointestinal (GI) tract over time may influence energy metabolism and body weight. The GI tract is the first group of organs to adapt to high DF intakes and an increase in the weight (muscles and mucosa) of the stomach, cecum and large intestine has been reported to occur with high fiber intake (Goodlad *et al.*, 1995; Jacobs and Lupton, 1984; Mongeau *et al.*, 1991; Slavin and Marlett, 1980). The question has been raised if the increase in the weight of the GI tract and its contents might limit or offset the potential "slimming" action of DF in wheat bran (Livesey, 1990a).

The aim of this work was to examine the short-term (6–8 weeks) and long-term (16–18 weeks) effect of hard wheat bran (HWB) on energy absorption, GI tract weight, body weight and composition, and metabolic parameters in rats. To this end, animals were fed fiber-free diets or diets containing 2% or 10% DF from HWB. Apparent and partial

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digestible energy values, the net energy value for maintenance and the net energy value relative to carbohydrate were determined for HWB and HWB fiber.

2. MATERIALS AND METHODS

2.1. Animals and Experimental Protocols

Weanling male Sprague-Dawley rats (47.5 ± 3.3 g for Experiment 1, 48.0 ± 3.5 g for Experiment 2, see below) were obtained from Charles River Co., Québec. Animals were randomly assigned to individual wire-bottom stainless steel cages and maintained on a 12 h light/dark cycle. The room temperature was 22 ± 1°C and the humidity was held between 45–55%. Rats were given free access to water and food throughout the experiment. Animal protocols were approved by the Animal Care Committee of Health Canada. Two different protocols were used. In Experiment 1 (performed from 15 February to 21 June, 1995), rats were fed Basal, 2%DF1 (made with HWB-1, see below) or 10%DF1 for 12 weeks at which time the rats fed diet 10%DF1 were switched to 10%DF2 (made with HWB-2, see below). Animals were maintained for the remaining 6 weeks on appropriate diets. In Experiment 2 (performed from 09 August to 12 December, 1995), rats were fed either 10%DF2 or 10%CS (corn starch) for 18 weeks (no switch in diet). Diets designed 10%DF1 and 10%DF2 were formulated using two different batches of HWB. The 10%DF1 diet was made using a batch of HWB that contained 39.6% fiber by weight (designated HWB-1). The 10%DF2 diet was made using a different batch (HB-195) of HWB that contained 49.4% fiber by weight (designated HWB-2). Hard wheat bran was obtained from the American Association of Cereal Chemists (St-Paul, MN). Detailed dietary compositions are shown in Table 1.

Table 1. Diet compositions

	per 100 g diet				
Ingredient[1]	Basal	2% DF1	10% DF1	10% DF2	CS
Basic diet mix[2] (g)	92.8	87.8	67.8	72.8	82.8
Vitamin/mineral mix[3] (g)	7.2	7.2	7.2	7.2	7.2
HWB (g)[4]	—	5.0	25.0	20.0	—
Cornstarch (g)	—	—	—	—	10.0
DF (g)[5]					
soluble	0.2 ± 0.1	0.7 ± 0.1	1.5 ± 0.2	1.4 ± 0.1	0.6 ± 0.2
insoluble	0.02 ± 0.02	1.8 ± 0.1	8.4 ± 0.8	8.9 ± 0.4	0.0 ± 0.0
total	0.2 ± 0.1	2.5 ± 0.1	10.0 ± 0.7	10.3 ± 0.4	0.6 ± 0.2
Energy (kcal)[6]	448 ± 2	445 ± 1	434 ± 1	438 ± 2	431 ± 1

[1]The diets were formulated to have identical vitamin and mineral densities.

[2]The Basic Diet Mix contained (per 92.8 g): 20 g ANRC casein, 32.8 g cornstarch, 31 g sucrose, 6.75 g soybean oil and 2.25 g lard.

[3]The Vitamin/Mineral Mix contained: 5.0 g AIN76 mineral mix, 1.5 g AIN76A vitamin mix, 0.4 g dl-methionine and 0.3 g choline bitartrate. The AIN76 mineral mix and AIN76A vitamin mix were obtained from Harlan Teklad (Madison, WI). Choline bitrartrate and dl-methionine were from Sigma Chemical Co. (St. Louis, MO).

[4]AACC certified hard wheat bran (HWB) was obtained from the American Association of Cereal Chemists. The percentage of total fiber in the HWB used to make the 2%DF1 and 10%DF1 diets was 39.6%. The percentage of total fiber in the HWB used for diet 10%DF2 was 49.4%.

[5]Dietary fiber (DF) was measured directly in samples of diet (N = 3). Soluble and insoluble fiber components are reported.

[6]Dietary energies were measured directly by bomb calorimetry (N = 2).

The experimental protocol for metabolic measurements was as follows: after 6 or 16 weeks in the wire bottomed cages, 8 animals from each dietary group were transferred to Nalgene metabolic cages. Animals were allowed a period of 2 days to adapt to the new environment. At this time 8 animals from each dietary group (not placed in cages) were sacrificed for body composition measurement. Over the next two weeks, food consumption (corrected for spillage) was measured and urine and feces were collected daily and frozen at –20°C. Urine and feces were freeze–dried prior to measurement of energy by bomb calorimetry (Parr Model 1261, Isoperibol bomb calorimeter). Two weeks after transfer to metabolic cages the animals were sacrificed. All animals were sacrificed by exsanguination while under Aerane anesthesia. Animals were not fasted prior to sacrifice. Plasma was frozen at –80°C until assayed. Liver samples were collected with freeze–clamp tongs and frozen at –80°C until use. The gastrointestinal (GI) tract was also dissected out and the carcasses (less liver and GI tract) were frozen at –20°C. The GI tract was kept on ice and the stomach, small intestine, cecum and large intestine were separated and weighed full. The stomach and the cecum were opened with scissors, rinsed with cold Ringer's solution, patted dry and weighed. The small and large intestines were rinsed with cold Ringer's solution (using a large syringe and flexible tubing) until all apparent food material or feces were removed. They were then patted dry and weighed. The tissues were kept frozen until further analysis.

2.2. Measurement of Body Composition

Carcasses (less liver and GI) were processed by homogenizing 1:1 (w:w) in water after autoclaving (Hartsook and Hershberger, 1963). Liver and digestive tract were homogenized separately 1:1 in water before processing for composition and energy analysis. Total fat was measured gravimetrically after extraction as described by Bligh and Dyer (1959). Protein concentrations were determined according to Brooks *et al.* (1995). Whole body values are reported for carcass + liver + digestive tract. Liver and whole body glucose and glycogen concentrations were measured using a kit designed for plasma glucose measurements (Abbott Laboratories, Mississauga, Ont., see below). Glycogen was hydrolyzed before analysis following the procedure of Huijing (1970). Moisture was determined by drying the homogenized carcasses to constant weight in a 60°C oven. Whole body energy was measured in 48 animals (12 animals from each of the first three dietary groups) by bomb calorimetry of the freeze-dried carcasses. Multiple regression of lean body mass (LBM = total mass – fat content – water content), body fat and total body energy gave factors for the carcass energy values of fat and protein (Figure 1). These were determined as 10.26 ± 0.23 kcal/g for fat and 3.78 ± 0.24 kcal/g for LBM. The value for fat is slightly higher than previously reported for other methods (Davies *et al.*, 1991; Pullar and Webster, 1977) which may be due to differences in fat extraction methods. A difference of 8% in fat extraction would account for the difference seen and would increase the LBM value to 4.2 kcal/g. This latter value is what would be expected based on our protein measurements assuming a gross energy value of 5.25 kcal/g protein.

2.3. Other Analyses

Plasma concentrations of glucose, triglycerides, and HDL-cholesterol were measured using kits (Abbott Laboratories, Mississauga, Ont.). These assays were performed with an Abbott VP discrete analyzer using a 340/380 nm filter. All materials for assay of enzyme rates were obtained from Sigma Chemical Co. (St. Louis, MO) or from Boehringer

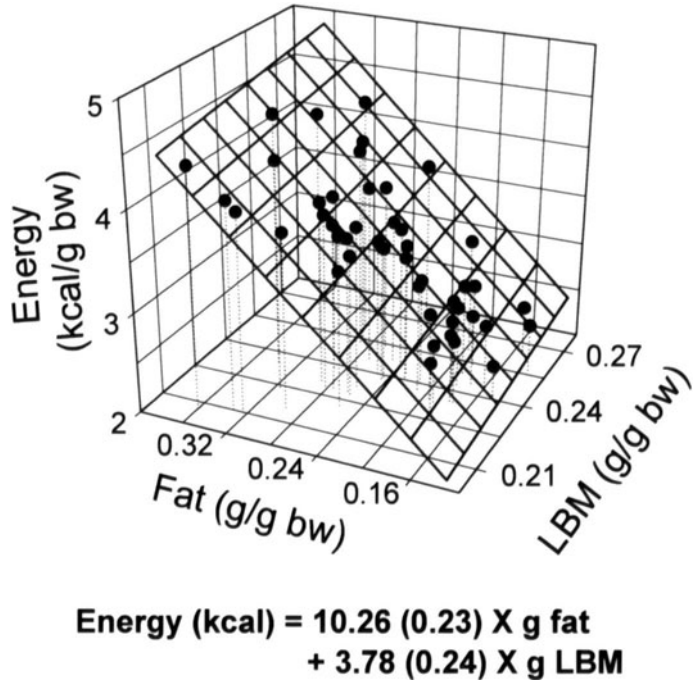

Figure 1. Carcass energy density as a function of carcass fat composition and carcass lean body mass composition (LBM). Carcass energy, fat composition and moisture were measured as described in the Materials and Methods section. The LBM was calculated as total mass – moisture – fat. Multiple regression gave 10.26 ± 0.23 kcal/g fat and 3.78 ± 0.24 kcal/g LBM.

Mannheim (Montreal, PQ). Enzyme assays were performed according to Brooks and Lampi (1995, 1996). Acetyl–CoA carboxylase was assayed essentially according to Tanabe *et al.* (1981). Citrate was added at concentrations of 0.5 mM and 3 mM as suggested by Thanpy and Wakil (1985). The apparent fermentability of DF was estimated by its disappearance upon passage through the GI tract. Dietary fiber was measured in the diet by the sequential analysis of soluble and insoluble fractions using AOAC method #992.16 (Mongeau and Brassard, 1993). The insoluble fiber was measured in the residue left following soluble fiber extraction. Dietary fiber was measured in fecal material collected on four non-consecutive days using the insoluble fiber procedure of the method mentioned above, assuming that negligible amounts of soluble fiber were present in the feces of animals fed bran diets. This assumption was supported by equivalent values obtained using the Englyst method and adding $KMnO_4$ lignin, for a wide range of results (7–45 g fiber/100 g dry feces, $r^2 = +0.97$, N = 6): Fecal fiber values for animals fed HWB were 46.3% and 46.7%, respectively.

2.4. Statistics

All values are reported as means ± one standard deviation. Significance was assessed by ANOVA analysis followed by Student Neuman-Keuls analysis. Where appropriate, food intake or food energy was used as a covariate in the ANOVA analysis. In this case, mean values adjusted for food intake or energy intake were analyzed by a Student Neuman-Keuls test. Values are reported as significantly different if $P < 0.05$.

2.5. Calculations

The apparent digestibility of a substance refers to the amount of the substance that is not recovered in the feces. If IM_s is the ingested mass of a substance and FM_s is the mass of the substance recovered in the feces, then the apparent digestibility is calculated as (Merrill and Watt, 1973):

$$\text{Apparent digestibility} = (IM_s - FM_s)/IM_s$$

The apparent digestible energy value (DEV_{app}) is then:

$$DEV_{app} = \text{apparent digestibility} \times dH_{c,s}$$

where $dH_{c,s}$ is the heat of combustion of the supplement.

Partial digestible energy values (DEV_{part}) describe the influence of a dietary substance on the energy digestibility of the whole diet (Livesey, 1990b). The energy digestibility (D) is defined by:

$$D = (IE_T - FE_T)/IE_T$$

where IE_T is the total ingested energy and FE_T is the total fecal energy. A plot of dietary energy digestibility as a function of the fraction of ingested energy due to supplement (IE_s/IE_T) yields a slope of $dD/d(IE_s/IE_T)$. The partial indigestibility of the substance (**S**) is then given by (Livesey, 1990b):

$$\mathbf{S} = dD/d(IE_s/IE_T) + (1 - D_0)$$

where D_0 is the energy digestibility of the basal (unsupplemented) diet. The DEV_{part} is then given by:

$$DEV_{part} = dH_{c,s} \times (1 - \mathbf{S})$$

The graphical procedure was used when more than one different level of bran substitution was present. However, since we changed the source of HWB during Experiment 1, the 10%DF2 diet of week 6–8 and the 2%DF1 diet of week 16–18 contained only one level of supplementation. In this case the DEV_{part} was calculated using an equation that minimized the errors of calculation (Livesey, 1989):

$$DEV_{part} = dH_{c,s} - [FE_t/(M_t - IM_s) - (FE_0/IM_0)]/[IM_s/(IM_t - IM_s)]$$

where FE_0, and FE_t are the energies of the feces produced by basal diet (subscript 0, unsupplemented) and the test diet (subscript t, supplemented). IM_0, IM_t and IM_s represent the ingested mass of the basal diet, the test diet and the supplement, respectively.

Net energy values (NEV) take into account all the additional digestion-associated losses. For dietary substances that are fermented, these losses can include: loss of energy to urine, loss to production of fermentation gasses, loss to the heat of fermentation, loss to increased bacterial mass in the feces and loss due to inefficient use of the fermentation products by the host. It is difficult to measure these values directly so other means have been employed. In the present paper we estimated the net energy value for maintenance

(XE) by taking the difference between the total ingested metabolic energy of the basal portion of the diet and the energy value of the accumulated body mass using the equation described by Livesey (1993):

$$XE = (IE_T - IE_s) \times Q_{part} + IE_s + E_f - 1.36 \times EF - 2.25 \times ELBM$$

Q_{part} is the partial metabolizability of the basal portion of the diet, including energy losses to urine and feces, and was 0.943 for our diet. E_f is the loss of body fat during the experimental period. Since our rats gained body fat throughout the experimental period, this value was 0.0 for our animals. EF and ELBM represent the accumulated energy of fat and lean body mass (LBM) during the experimental collection periods determined for our extraction methods (Figure 1). The values 1.36 and 2.25 represent the energy of accumulated body fat or body protein, respectively (including the cost of energy depostion; Pullar and Webster, 1977). The XE is normalized to the metabolic weight of the animal (average LBM over the course of the experiment) to give XE/ALBM. This value is plotted as a function of the mass of ingested supplement normalized to average LBM (IM_s/ALBM) and the net energy value NEV is estimated from the slope, defined as dXE/dIM_s (Livesey, 1990c):

$$NEV = dH_{c,s} - dXE/dIM_s$$

The net energy value (NEV) for HWB fiber was also estimated by a factorial model to give the NEV relative to carbohydrate (REV). Our factorial model was adapted from the original model proposed by the Dutch Nutrition Council for the estimation of the net energy values of sugar alcohols (Bär, 1990). In our model, the net energy value of the supplement is given by:

$$REV = [(A \times B) + (1 - A - D) \times C] \times dH_{c,s}$$

where A is the fraction of supplement digested and absorbed from the small intestine and B is the fraction of absorbed substance that is metabolically available. For fiber we assumed that both values are equal to 0.0. The variable D represents the fraction of energy lost directly to feces in the form of fiber. The value $(1 - A - D)$ is, therefore, the fraction that is fermented and C is the efficiency with which the host can utilize the energy resulting from fermentation. The value of C can be broken down into components so that (Livesey, 1992):

$$C = (1 - \boldsymbol{a} - \boldsymbol{b} - \boldsymbol{c}) \times \boldsymbol{d}$$

The variable ***a*** represents the energy lost to the heat of fermentation (0.07 kcal/kcal fermented; Livesey, 1992) and ***b*** represents the energy lost to the formation of fermentation gasses such as H_2 and CH_4 (0.02 kcal/kcal fermented; Livesey, 1992). The ***c*** variable is the energy lost to feces (usually associated with increased fecal bacteria). The value of ***c*** can be obtained from a plot of the fecal energy derived from fermentation versus the total fermented fiber energy (Figure 2). The slope of this line gives the proportion of fermented fiber energy lost to the feces (presumably through increased bacterial excretion). This value was 0.674 in our experiments. The ***d*** variable is the efficiency with which the host utilizes the products of fermentation (short chain fatty acids) and is approximately 0.825 (Livesey, 1992).

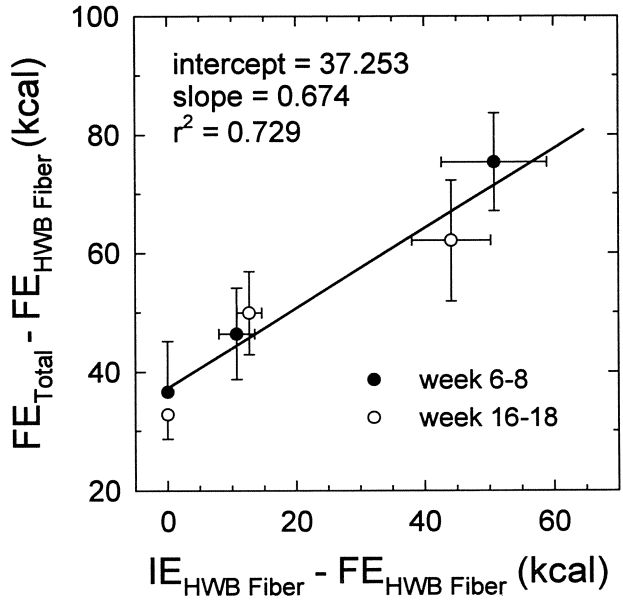

Figure 2. Loss of bacterial energy to feces: kcal energy lost per kcal DF fermented. The fecal energy derived from the fermentation processes (total fecal energy – fecal DF energy) was plotted as a function of the total fiber energy available for fermentation (ingested DF energy – fecal DF energy) in kcal. The slope gives the fractional energy lost to feces (kcal/kcal fermented). The closed circles represent weeks 6–8 and the open circles represent weeks 16–18. Symbols represent the mean ± one standard deviation for N = 8 to 16 animals.

3. RESULTS

3.1. Intake, Excretion and GI Tract Weight in Experiment #1

Food consumption was 19.1 ± 3.77, 20.2 ± 1.79 and 21.1 ± 1.51 g/rat/day for animals fed basal, 2%DF1 or 10%DF1 diets, respectively, during the 6–8 week collection period. Similar intakes (19.9–20.9 g/rat/day) were observed during week 16–18.

Fecal wet weight was 1.57 ± 0.46[a] (basal), 2.45 ± 0.35[b] (2%DF1), and 5.40 ± 0.89[c] (10%DF1) g/rat/day during week 6–8 and similar results with the same differences were observed during week 16–18 ($P<0.05$). The fecal moisture did not vary significantly during week 6–8 (47.1–53.6%) but it was higher than basal (51.6 ± 4.0%) in group 2%DF1 (55.4 ± 3.0%) during week 16–18 ($P<0.05$). Group 10%DF1 had the highest fecal output but not the highest percentage of moisture (52.8 ± 3.5%). The total fat excreted was 2 times that of basal in group 10%DF1 (0.11 ± 0.03 vs 0.22 ± 0.04 g/rat/day, $P<0.05$).

The wet weight of the washed cecum and large intestine generally increased with increasing levels of dietary HWB ($P<0.05$; Tables 2 and 3). The stomach weight was higher than basal in animals fed 10%DF1 in week 16 (g/kg live body weight). The total weight of the washed GI tract was affected differently in week 6 than in week 16 due to the weight of the small intestine. The latter was 32% lower than basal in group 10%DF1 at week 6 ($P<0.05$) but there was no difference between these groups at week 16. This effect was not due to a change in length of the small intestine, since for all groups it was within 122 ± 10 to 127 ± 6 cm in week 6, and 117 ± 3 to 120 ± 6 cm in week 16. The absolute weight of the small intestine was significantly lower than basal in animals fed 2%DF1 at

Table 2. Weight of washed gastrointestinal tract and luminal content at week 6 of experiment #1 (mean ± one standard deviation for N = 6 to 8 animals)

Diet	Wet weight (g)	Wet weight (g/kg live BW)	Moisture (%)	Dry matter (g)	Wet luminal content (g)
Stomach					
Basal	1.58 ± 0.13[a]	3.95 ± 0.39[a]	66.5 ± 4.1[a]	0.53 ± 0.09[b]	2.82 ± 2.39[a]
2%DF1	1.49 ± 0.19[a]	3.96 ± 0.38[a]	72.4 ± 1.4[b]	0.41 ± 0.06[a]	1.68 ± 0.75[a]
10%DF1	1.51 ± 0.15[a]	4.15 ± 0.27[a]	73.2 ± 1.2[b]	0.41 ± 0.04[a]	2.89 ± 1.74[a]
Small intestine					
Basal	8.04 ± 1.23[b]	19.9 ± 1.6[b]	76.4 ± 3.2[a]	1.90 ± 0.42[b]	1.87 ± 0.78[a]
2%DF1	6.31 ± 1.00[a]	16.7 ± 2.1[a]	76.8 ± 3.5[a]	1.47 ± 0.34[a]	2.48 ± 0.89[a]
10%DF1	5.50 ± 0.68[a]	15.1 ± 1.4[a]	75.9 ± 2.4[a]	1.34 ± 0.28[a]	3.72 ± 0.84[b]
Cecum					
Basal	0.68 ± 0.09[b]	1.70 ± 0.26[a]	61.4 ± 4.7[a]	0.27 ± 0.06[a]	2.49 ± 0.40[a]
2%DF1	0.64 ± 0.09[a]	1.70 ± 0.30[a]	62.5 ± 7.2[a]	0.24 ± 0.07[a]	2.94 ± 0.40[b]
10%DF1	0.75 ± 0.11[b]	2.04 ± 0.23[b]	68.4 ± 2.4[b]	0.24 ± 0.04[a]	3.57 ± 0.68[c]
Large intestine					
Basal	0.84 ± 0.11[a]	2.22 ± 0.34[a]	68.4 ± 3.9[a]	0.27 ± 0.05[a]	0.41 ± 0.27[a]
2%DF1	1.03 ± 0.13[b]	2.75 ± 0.33[b]	72.9 ± 1.5[b]	0.28 ± 0.04[ab]	0.56 ± 0.32[a]
10%DF1	1.37 ± 0.17[c]	3.74 ± 0.29[c]	75.7 ± 2.3[c]	0.33 ± 0.06[b]	1.49 ± 0.16[b]
Total gastrointestinal tract[d]					
Basal	10.50 ± 0.56[a]	27.9 ± 1.9[a]	73.8 ± 2.7[a]	2.75 ± 0.31[a]	7.59 ± 3.02[a]
2%DF1	9.46 ± 1.22[ab]	25.1 ± 2.5[b]	74.7 ± 2.8[a]	2.40 ± 0.44[ab]	7.66 ± 1.22[a]
10%DF1	9.13 ± 0.84[b]	25.0 ± 1.2[b]	74.8 ± 1.5[a]	2.31 ± 0.32[b]	11.67 ± 2.99[b]

[a–c]For each organ, mean values in the same column and not sharing the same superscript are significantly different ($P<0.05$).
[d]Total value may not equal the sum of organs because only complete GI tracts were counted.

Table 3. Weight of washed gastrointestinal tract and luminal content at week 16 of experiment #1 (mean ± one standard deviation for N = 8 animals)

Diet	Wet weight (g)	Wet weight (g/kg live BW)	Moisture (%)	Dry matter (g)	Wet luminal content (g)
Stomach					
Basal	2.17 ± 0.23[ab]	3.18 ± 0.20[a]	66.1 ± 2.6[a]	0.73 ± 0.06[a]	2.81 ± 0.79[a]
2%DF1	2.11 ± 0.18[a]	3.32 ± 0.34[ab]	67.9 ± 1.3[a]	0.68 ± 0.04[a]	2.30 ± 0.80[a]
10%DF1	2.37 ± 0.27[b]	3.63 ± 0.39[b]	68.7 ± 2.3[a]	0.74 ± 0.05[a]	3.15 ± 1.34[a]
Small intestine					
Basal	8.18 ± 0.86[b]	12.1 ± 2.0[a]	79.4 ± 3.7[a]	1.70 ± 0.43[ab]	0.39 ± 0.67[a]
2%DF1	7.05 ± 0.86[a]	11.0 ± 1.2[a]	80.4 ± 2.3[a]	1.38 ± 0.23[a]	0.77 ± 0.40[a]
10%DF1	8.04 ± 0.65[b]	12.4 ± 2.1[a]	78.6 ± 2.4[a]	1.73 ± 0.29[b]	1.83 ± 0.91[b]
Cecum					
Basal	1.01 ± 0.17[a]	1.50 ± 0.31[a]	57.4 ± 4.1[a]	0.43 ± 0.08[a]	1.60 ± 0.38[a]
2%DF1	1.00 ± 0.16[a]	1.56 ± 0.23[a]	58.7 ± 4.5[ab]	0.41 ± 0.10[a]	2.27 ± 0.29[b]
10%DF1	1.21 ± 0.10[b]	1.85 ± 0.25[b]	61.7 ± 3.0[b]	0.46 ± 0.05[a]	3.31 ± 0.67[c]
Large intestine					
Basal	1.07 ± 0.12[a]	1.57 ± 0.24[a]	65.7 ± 7.7[a]	0.37 ± 0.10[ab]	0.49 ± 0.48[a]
2%DF1	0.99 ± 0.12[a]	1.56 ± 0.21[a]	66.4 ± 5.2[a]	0.33 ± 0.07[a]	0.63 ± 0.18[b]
10%DF1	1.65 ± 0.23[b]	2.51 ± 0.26[b]	73.2 ± 3.5[b]	0.44 ± 0.08[b]	1.74 ± 0.84[c]
Total gastrointestinal tract					
Basal	12.43 ± 0.98[b]	18.3 ± 2.5[ab]	74.0 ± 2.6[a]	3.24 ± 0.45[b]	5.29 ± 1.09[a]
2%DF1	11.15 ± 1.13[a]	17.5 ± 1.6[a]	74.9 ± 1.9[a]	2.80 ± 0.34[a]	5.97 ± 1.08[b]
10%DF1	13.27 ± 0.88[b]	20.4 ± 2.7[b]	74.6 ± 1.1[a]	3.37 ± 0.27[b]	10.02 ± 2.59[c]

[a–c]For each organ, mean values in the same column and not sharing the same superscript are significantly different ($P<0.05$).

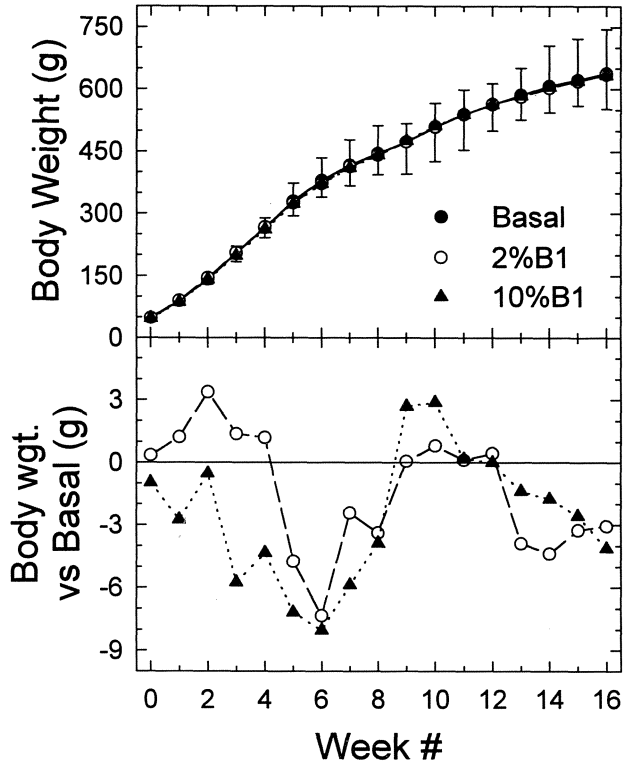

Figure 3. Live body weights and differences in live body weight as a function of diet for the three diets of experiment #1. The top panel shows live weight as a function of time and the bottom panel shows the difference in the body weights of animals fed 2%DF1 (open circles) and 10%DF1 (closed triangles) diets as compared to rats fed basal diet (set to 0). Each point represents the mean ± one standard deviation for N = 16 to 32 animals.

weeks 6 and 16 ($P<0.05$). The weight of GI tract organs slightly increased between weeks 6 and 16. The luminal content of the GI tract increased with increasing levels of dietary HWB, except in the stomach where results were variable (Tables 2 and 3). The difference in the total weight of GI tract (washed weight plus luminal content) between basal and 10%DF1 groups was <1% of total body weight and thus had no influence on measurement of body weight gain.

3.2. Dietary Intake and Whole Body Results in Experiments #1 and #2

A previous experiment with bran supplementation showed decreased body weight gain in rats fed 26% HWB (13% DF) as compared to controls fed a low bran diet (3% DF) (Mongeau *et al.*, 1991). This effect persisted until approximately 6 weeks feeding (~350 g body weight) and then reversed so that rats fed the high-bran diet had the same body weight as their low bran–fed counterparts after 29 weeks. This effect was also noted in the present experiments but the differences in body weights were not statistically significant (Figure 3, bottom). Thus, live weights were similar for all groups throughout the time course of the experiments (Figure 3, top). Slight differences in total dietary mass and total energy consumption were noted for the different diet groups. This effect was confined to

Table 4. Effects of dietary hard wheat bran and corn starch on the intake and fecal loss of dry matter and energy over 14 d in rats (mean values for N=8 animals)

Week	Diet	Total dry mass ingested (g)	Total energy ingested (kcal)	Total ingested DF (g)	Fecal dry weight (g)	Fecal energy (kcal)	Total fecal DF (g)	Digestibility of added dietary fiber (g/g)	Apparent digestibility of dietary energy (kcal/kcal)
6–8	Basal	277^{a}	1241^{a}	0.7^{a}	10.7^{a}	36.6^{a}	0.50^{a}	—	0.971^{a}
	2%DF1	289^{a}	1287^{a}	7.2^{b}	17.2^{b}	64.1^{b}	4.12^{b}	0.472^{a}	0.950^{b}
	10%DF1	309^{a}	1343^{ab}	30.6^{c}	36.5^{c}	148.7^{c}	11.51^{c}	0.408^{b}	0.889^{c}
	10%DF2	369^{b}	1613^{c}	37.1^{d}	44.3^{d}	179.3^{d}	—	—	0.889^{c}
	CS	345^{b}	1487^{b}	1.9^{e}	13.7^{e}	46.9^{a}	—	—	0.968^{a}
16–18	Basal	283^{v}	1269^{v}	0.7^{v}	11.0^{v}	32.8^{v}	0.48^{v}	—	0.974^{v}
	2%DF1	298^{v}	1324^{v}	7.4^{w}	17.7^{w}	63.3^{w}	3.29^{w}	0.390^{v}	0.952^{w}
	10%DF1	287^{v}	1260^{v}	28.9^{x}	35.1^{x}	134.8^{x}	10.71^{x}	0.378^{w}	0.893^{x}
	10%DF2	318^{v}	1389^{v}	32.7^{y}	38.6^{y}	149.5^{y}	—	—	0.892^{x}
	CS	322^{v}	1388^{v}	1.8^{z}	12.0^{z}	40.1^{v}	—	—	0.971^{v}

Diets as defined in Table 1. Mean values in a column with different letters within a single time period were significantly different ($P < 0.05$). Statistical tests on "Total Dry Mass Ingested" and "Total Energy Ingested" were performed using ANOVA followed by Student Neuman-Keuls analysis. Other values were compared with "Total Dry Mass Ingested" as a covariate. Adjusted means were then tested by ANOVA (one way) followed by Student Neuman-Keuls test when differences were indicated. Differences were noted for values reported during the same 2 week period only. Significant differences between 6-8 and 16-18 week periods were also noted for "Total Dry Mass Ingested", "Total Energy Ingested", "Total Ingested DF" and "Fecal Energy". See Table 2 for index definitions.

Table 5. Body composition: individual week summaries of pooled diet groups

	Week 6	Week 8	Week 16	Week 18
Live weight[1] (g)	371 ± 39[a]	438 ± 48[b]	577 ± 74[c]	603 ± 75[c]
Protein (%)	18.3 ± 1.4[a]	18.4 ± 1.2[a]	16.7 ± 1.2[b]	17.9 ± 1.5[a]
Fat (%)	17.0 ± 2.9[a]	16.7 ± 2.7[a]	26.6 ± 4.0[b]	24.6 ± 3.3[c]
LBM (%)	23.8 ± 0.9[a]	24.0 ± 1.7[a]	23.2 ± 1.7[b]	23.1 ± 1.0[b]
Moisture (%)	59.3 ± 2.8[a]	59.4 ± 3.4[a]	50.9 ± 3.0[b]	52.3 ± 2.7[c]
CHO (%)	1.07 ± 0.06[a]	1.03 ± 0.06[a]	0.63 ± 0.18[b]	0.61 ± 0.18[b]

Values are the mean ± one standard deviation for N=8 rats.

[1]Weights reported at 6 and 16 weeks are those of the animals sacrificed at 8 and 18 weeks. The body composition data is from animals sacrificed at the indicated dates. Values with different superscripts are significantly different as determined by the Student Neuman-Keuls test at the $P < 0.05$ level. Percentages were transformed by $\sqrt{\arcsin(y)}$ prior to statistical analysis. LBM; lean body mass, CHO; carbohydrate. See Table 2 for index definitions.

week 6–8 where diet consumption by rats from experiment #1 was significantly lower than that by rats from experiment #2 (Table 4). This effect disappeared by week 16–18 but necessitated the inclusion of dietary intake as a covariate in subsequent statistical analyses. No differences in body composition were noted between the different dietary groups (Table 5) although older animals had higher body fat content and lower LBM, moisture, carbohydrate and protein content when expressed as percentage of total body weight.

3.3. Apparent and Partial Digestibility

The apparent fermentability of DF was estimated as the disappearance of DF after passage through the GI tract. Fermentability values were 72.0 ± 13.0% and 65.9 ± 11.3% (basal), 57.3 ± 6.6% and 44.3 ± 10.1% (2%DF1), and 37.6 ± 5.7% and 37.1 ± 7.1% (10%DF1) during weeks 6–8 and 16–18, respectively. Correcting for the small amount of DF present in the basal diet gave the DF digestibility values listed in Table 4. Using the fermentability to calculate the apparent DEV of the HWB fiber showed that HWB fiber was a poor source of energy. It apparently provided only 1.85 kcal/g when measured 6–8 weeks after introduction of the diet. Contrary to expectations, the apparent digestibility of the fiber decreased with age to 1.59 kcal/g when measured 16–18 weeks after introduction of the diet (Table 6).

Table 6. Digestible and metabolizable energy values determined over two week periods

Week	Diet	DEV_{app} (kcal/g)	DEV_{part} (kcal/g)	NEV (kcal/g)	REV (kcal/g)
6–8	HWB-1	—	2.55 ± 0.05	2.80 ± 0.58	—
	HWB-2	—	2.16 ± 0.06	−1.22 ± 0.77	—
	HWB Fiber	1.82 ± 0.17	0.45 ± 0.10	−5.24 ± 1.30	0.35
	Corn Starch	—	3.84 ± 0.09	−3.83 ± 1.79	—
16–18	HWB-1	—	2.02 ± 0.19[a]	3.22 ± 3.98[a]	—
	HWB-2	—	2.18 ± 0.06	0.97 ± 0.93[a]	—
	HWB Fiber	1.59 ± 0.28[a]	0.51 ± 0.11	−2.12 ± 1.52[a]	0.31
	Corn Starch	3.83 ± 0.05	−2.61 ± 1.62[a]	—	—

The method for calculating the digestible energy value (DEV_{app}), the DEV_{part} and the NEV are outlined in the Materials and Methods section. The net energy value relative to glucose (REV) was calculated by a factorial method described in the legend to Table 8 and in the Materials and Methods section.

[a]Statistically different from the corresponding value for week 6-8 as determined by the two-tailed Student's *t* test. The values are the mean ± one standard deviation for N=8 rats except for the 6-8 HWB-1 and 16-18 HWB-2 values which were determined graphically (see text and Figures 4 and 5). See Table 2 for index definitions.

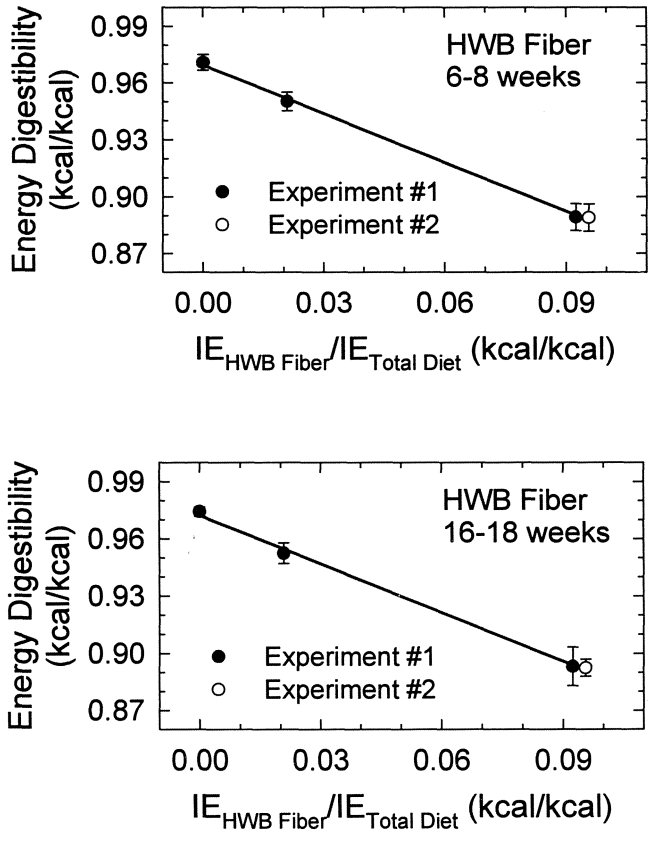

Figure 4. Calculation of DEV_{part} for hard wheat bran fiber. The energy digestibility of the total diet was plotted as a function of the fraction of dietary energy derived from HWB fiber. The points represent the mean ± one standard deviation for N = 8 animals. The closed circles represent data from experiment #1, the open circles are from experiment #2. The DEV_{part} was calculated from the values obtained by regression analysis (Livesey, 1990b): $DEV_{part} = dH_{c,s} \times (1 - \mathbf{S})$, where the partial undigestibility of the diet (**S**) is given by: **S** = 1 – (intercept + slope). See the Materials and Methods section for more details about the calculations.

The apparent digestibility of HWB fiber does not give a complete picture of dietary energy availability during HWB supplementation. This is provided by measuring the DEV_{part} which describes the influence of the supplement on overall dietary energy digestibility. It is possible to determine the DEV_{part} for the fiber component of HWB by assuming that the non–fiber components of HWB had an energy digestibility similar to that of the basal diet. As expected, addition of HWB to a bran-free (basal) diet decreased the overall energy digestibility of the diet since HWB fiber is relatively poorly digested. Figure 4 shows a plot of *D* versus the fraction of dietary energy derived from HWB fiber for weeks 6–8 and 16–18 and Table 6 presents the results. These data show that the DEV_{part} was significantly lower than the apparent DEV for HWB fiber. These calculations rely on the assumption that the non-fiber portion of the HWB has a *D* similar to that of the basal diet. If the non-fiber component *D* was lower than the basal diet, the DEV_{part} would be overestimated. It is possible to estimate the extent of this overestimation by assuming a *D* of 0.9 instead of 0.97 for the non-fiber component. In this case, the DEV_{part} of the HWB

fiber would have been 0.17 kcal/g too high. This shows that the DEV_{part} for the bran component is sensitive to the digestibility of the non–fiber component. This should not be a problem because the non-fiber component is composed mostly of carbohydrate with a small proportion of protein (<8%). We could not, however, measure the DEV_{part} of fiber directly because it was impossible to separate the two components in our experiments.

The DEV_{part} was also calculated for the complete HWB supplement (fiber plus non-fiber components) to give values that ranged from 2.02 to 2.55 kcal/g (Table 6). These values are similar to those obtained by Lockhart (1980) but were significantly higher than those calculated for the fiber component of the HWB. The difference was due to the high digestibility of the non-fiber components of HWB (that make up 50–60% of the total HWB). The HWB DEV_{part} values also differed depending on the source of bran (either bran #1 or bran #2) and on the age of the animals. Thus, the DEV_{part} for HWB-1 in animals exposed for 6–8 weeks was significantly higher than that of animals exposed for 16–18 weeks. No differences were noted in the DEV_{part} during HWB-2 supplementation. No age-related effects were apparent when the fiber DEV_{part} values were compared suggesting that differences may be associated with the non-fiber component only.

The diets of the present study were formulated to contain equal weight densities of vitamins and minerals. This was done to ensure the animals did not suffer from deficiencies over the course of the 18 weeks. Because the vitamin/mineral mix was held constant, a small variation in the basal diet composition occurred when supplemented and non-supplemented diets were compared. The DEV_{part} analysis of the preceding paragraphs assumed that the non-mineral and non-vitamin components (mostly sucrose and citrate) of the mineral/vitamin mix had a D identical to that of the basal diet. It is possible to check the validity of this assumption by manually changing the D of the diet and re-calculating DEV_{part}. Following this procedure, and knowing that the $dH_{c,s}$ value for the vitamin/mineral mixture was 1.871 kcal/g (measured directly), one can calculate that the DEV_{part} will be 0.01 kcal/g lower if the D of the vitamin/mineral mix was 0.9 (versus the assumed 0.97). This shows that the DEV_{part} would not be greatly affected by changes in the digestibility of the carbohydrate component of the vitamin/mineral mix.

3.4. Net Energy Value

Net energy values provide a more realistic estimate of the energy available to the animal since they include all the additional losses that may occur during digestion. For example, loss of energy to: urine, fermentation gasses, the heat of fermentation, increased fecal bacterial mass, potential extra energy lost during digestion and inefficient use of the fermentation products by the animal are all components that must be accounted for in a complete analysis of energy availability. While it is possible to directly measure some of these losses (losses to urine and fermentation gasses), measurements of H_2 and CH_4 require special equipment to ensure complete collection. Other losses are difficult to measure directly. A method for estimating the metabolic energy available from a supplement has been proposed by Livesey (1993). This method estimates the metabolic energy for maintenance (XE) from body composition data and metabolic energy intakes (see Materials and Methods for calculation methods). Application of these equations gave the results shown in Figure 5 (for fiber) and presented in Table 6. The procedure incorporates significantly more variation since accurate gains in body energy must be measured. The large standard deviations of the NEVs illustrate the increased variability of this method (Table 6). Because of the large variation in the results, no statistical differences were noted between the diet groups although the NEVs of older animals were significantly higher than those of younger animals.

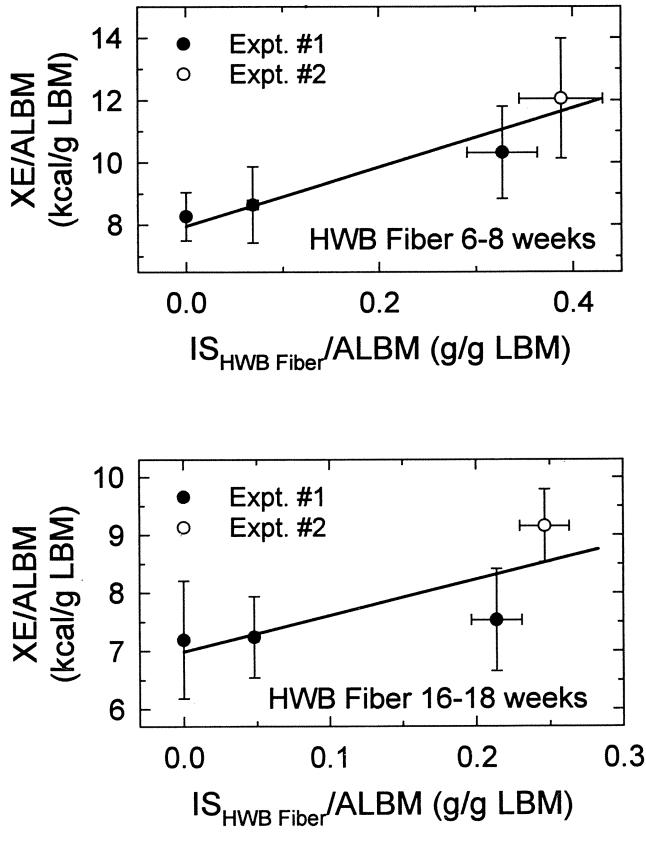

Figure 5. Calculation of NEV for hard wheat bran fiber. The metabolic energy for maintenance (XE) was plotted as a function of the dietary HWB fiber intake ($IM_{HWB\ Fiber}$). Both XE and $IM_{HWB\ Fiber}$ were normalized by dividing by the average lean body mass (ALBM). The points represent the mean ± one standard deviation for N = 8 animals. The closed circles represent data from experiment #1, the open circles are from experiment #2. NEV is calculated as $dH_{c.s}$ – slope (see the Materials and Methods section).

The negative NEVs obtained with bran and corn starch supplementation were unexpected. Especially puzzling was the large negative NEV obtained for corn starch since this substance has a high digestibility and metabolizability. A closer examination of the energy use distribution in the rats gave a partial answer. The data in Table 7 show energy intake, energy deposition and energy expenditure (EE) for rats fed the five diets during the early 6–8 week and later 16–18 week periods. Significantly higher digestible energy intake was noted for rats fed 10% DF2 and 10% CS diets at 6–8 weeks only. This increased energy intake was completely accounted for by changes in body energy (Body energy gain, Table 7) and energy expenditure (including losses to urine and gas production; Table 7) since no statistical differences were noted after analyzing the data with dietary energy as a covariate. Contrary to younger rats, no change in digestible energy intake was noted in older rats. However, in older rats fed the 10% DF2 and 10% CS diets, body energy gain was lower despite equivalent total dietary energy intakes. This reduction in body energy was due to lower fat and LBM energy gains and could be accounted for by an increased EE value.

It is possible to partition the energy derived from the supplement into body energy gains and EE by subtracting changes due to differences in basal dietary energy consump-

Table 7. Energy intake, energy deposition and energy expenditure in rats fed basal and supplemented diets over two week periods (mean values for N = 8 animals)

Week	Diet	Total DE intake (kcal)	Body energy gain (kcal)	Body fat gain (kcal)	LBM gain (kcal)	EE + losses to urine and gas (kcal)	IM_S(g)	DEV_{part} (kcal)	ΔBody energy (kcal/g S)	ΔBody fat (kcal/g S)	ΔLBM (kcal/g S)	ΔEE + losses to urine and gas (kcal/g S)
6–8	Basal	1204.7[a]	406.1[a]	270.5[a]	135.6[a]	798.6[a]	0.00[a]	—	—	—	—	—
	2%DF1	1222.9[a]	436.7[a]	316.1[a]	120.6[a]	786.2[a]	6.49[b]	0.45	3.36[a]	6.00[a]	−2.64[a]	−2.91[a]
	10%DF1	1194.1[a]	357.8[a]	249.6[a]	108.2[a]	836.3[a]	30.06[c]	0.45	−1.45[a]	−0.59[a]	−0.86[a]	1.91[a]
	10%DF2	1433.4[b]	414.7[a]	284.5[a]	130.2[a]	1018.7[a]	37.37[d]	0.45	−1.94[a]	−1.10[a]	−0.84[a]	2.39[a]
	10%CS	1440.2[b]	454.6[a]	319.3[a]	135.3[a]	985.7[a]	34.50[c]	3.84	−0.94[a]	−0.16[a]	−0.78[a]	4.78[a]
16–18	Basal	1236.3[v]	263.5[v]	200.4[v]	63.0[v]	972.8[v]	0.00[v]	—	—	—	—	—
	2%DF1	1260.6[v]	280.7[v]	231.7[v]	49.0[v]	979.8[v]	6.68[w]	0.51	1.77[v]	4.02[v]	−2.25[v]	−1.26[a]
	10%DF2	1124.9[v]	223.6[v]	182.0[v]	41.7[v]	901.3[v]	28.30[x]	0.51	−0.41[v]	0.10[v]	−0.51[v]	0.92[a]
	10%DF2	1239.8[v]	152.6[w]	115.7[w]	36.8[w]	1087.2[w]	32.19[y]	0.51	−3.44[v]	−2.63[v]	−0.80[v]	3.94[a]
	10%CS	1347.5[v]	174.7[w]	132.7[w]	42.0[w]	1172.8[w]	32.19[y]	3.83	−3.56[v]	−2.72[v]	−0.84[v]	7.40[a]

Energy, fat and LBM gains were calculated from regression equations relating body weight to changes in the percentage body composition of each parameter. These regression values were determined from actual measurements of body composition at 6 and 16 weeks. The energy values for body fat and LBM include the cost of fat and LBM deposition (1.36 and 2.25 times the energy value, see Pullar and Webster, 1977). EE represents energy expenditure. ΔBody energy, ΔBody fat, ΔLBM and ΔEE represent the change in body energy, fat, LBM and in EE per gram dietary HWB fiber. Mean values in a column within a single time period with different letters were significantly different ($P < 0.05$). Tests were conducted with "Total DE intake" as a covariate. Adjusted means were then tested by ANOVA (one way) followed by Student Neuman-Keuls test when differences were indicated. Differences are noted for values reported during the same 2 week period only. Significant differences between 6–8 and 16–18 week periods were also noted for "Body energy gain", "Body fat gain", "LBM gain", "IM_S", "ΔBody Energy/g S" and "ΔBody fat/g S". See Table 2 for index definitions.

tion. These measurements provide an estimate of the change in body energy (fat and LBM) as well as the change in EE per gram of supplement consumed. The standard deviations of these measurements were large so that no statistical differences were observed between diet groups although differences between 6–8 and 16–18 week values were noted for body energy gain/g supplement and for body fat gain/g supplement. Despite the lack of statistical significance, the results suggest that a considerable difference existed between supplemented and non–supplemented animals with respect to energy utilization. Supplementation with 10% fiber or 10% corn starch appeared to increase EE and decrease body fat and LBM deposition per gram added supplement. For animals fed on 2%DF1, a different pattern was apparent. These animals appeared to accumulate body fat but lose LBM in response to supplementation. The difference between these two opposing effects resulted in a positive energy gain per gram supplement and a negative EE per g supplement.

It is also possible to estimate the NEV of a supplement by a factorial approach that attempts to model digestion. This model can be simplified by assuming that virtually all of the ingested DF is digested only by fermentation in the lower GI tract (Table 6; A = 0.0, B = 0.0). Studies with other fiber sources, such as oat bran, indicate that this may not be true for all fiber sources (Bach Knudsen *et al.*, 1993; Marlett, 1990; McBurney and Thompson, 1989). However, no direct measure of small intestinal HWB fiber degradation and absorption of degradation products has been done. Knowing the amount of fiber in the feces, it is possible to calculate the energy fraction of fermented fiber that is lost to the feces. Figure 2 shows the increase in fermentation-related bacterial energy as a function of the increase in the fiber energy available for fermentation. The slope of this line (designated *c*; Table 8) can be used to estimate the net energy value of HWB fiber relative to glucose (REV) as shown in Table 8. These calculations gave REVs of 0.35 kcal/g for week 6–8 and 0.31 kcal/g for week 16–18.

3.5. Metabolic Parameters

HWB or corn starch supplementation caused significant reductions in plasma levels of triglycerides but only during weeks 8 and 16 (Figure 6). Diet had no effect on plasma HDL

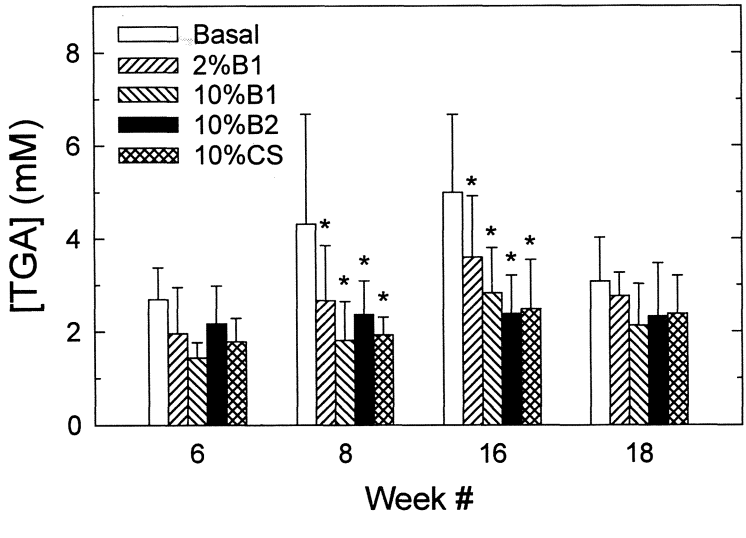

Figure 6. Plasma triglyceride concentrations as a function of diet and duration of time on the diet. Bars represent the mean ± one standard deviation for N = 8 animals.

Table 8. Factorial calculation of the net energy value relative to glucose (REV) for dietary fiber supplementation (mean values for N = 8 animals)

Week	Fraction absorbed by small intestine (A)	Fraction of absorbed energy that is metabolically available (B)	Fraction of DF energy lost to feces (D)	Fraction of ingested DF energy fermented (1 – A – D)	Loss to heat of fermentation (***a***)	Loss to H_2 and CH_4 (***b***)	Loss to increased bacterial mass (***c***)	Relative efficiency of SCFA utilization (***d***)	(C)	Hard wheat bran fiber REV (kcal/g)
6–8	0.0	0.0	0.560	0.440	0.07	0.02	0.674	0.825	0.195	0.35
16–18	0.0	0.0	0.610	0.390	0.07	0.02	0.674	0.825	0.195	0.31

All values are reported as kcal/kcal. The calculation of the net energy value relative to glucose is based on the equation: REV = $[(A \times B) + (1 - A - D) \times C] \times \Delta H_c$ Where A is the fraction absorbed from the small intestine and B is the fraction of absorbed substance that is metabolically available. The variable D represents the fraction of energy lost to feces directly in the form of fiber. The value (1 – A – D) is, therefore, the fraction that is fermented and C is the efficiency with which the host can utilize the energy resulting from fermentation. The value of C can be broken down into components by: $C = (1 - \boldsymbol{a} - \boldsymbol{b} - \boldsymbol{c}) \times \boldsymbol{d}$. The variable ***a*** represents the energy lost to heat of fermentation, and ***b*** represents the energy lost to the formation of fermentation gasses such as H_2 and CH_4. The ***c*** variable represents the energy lost to feces (usually associated with increased fecal bacteria) and was obtained from Figure 2. The ***d*** variable is the efficiency with which the host utilizes the products of fermentation (short chain fatty acids). See the Materials and Methods section for more details on the calculations.

Table 9. Metabolic parameters that did not change with diet

Parameter	Week 6	Week 16
Plasma HDL cholesterol (mM)	88.5 ± 21.3	109.2 ± 21.6[a]
Plasma glucose (mM)	192.0 ± 22.9	177.3 ± 17.8[a]
Liver AspAT (U/gww)	103.9 ± 13.6	110.1 ± 19.1
Liver AlaAT (U/gww)	6.34 ± 2.04	10.70 ± 3.9[a]
Liver SDH (U/gww)	0.06 ± 0.04	0.17 ± 0.11[a]
Liver PEPCK (U/gww)	0.29 ± 0.09	0.29 ± 0.11

Abbreviations: AspAT, aspartate amino transferase; AlaAT, alanine aminotransferase; SDH, serine dehydratase; PEPCK, phosphoenolpyruvate carboxykinase. Values are mean ± one standard deviation for N = 20 (liver values) or N = 40 (plasma values) animals.
[a]Significantly different from week 6 value as determined by the two tailed Student's t-test ($P < 0.01$).

cholesterol or on glucose (Table 9). Older animals had significantly higher levels of HDL cholesterol (1.2 times higher) and significantly lower levels of plasma glucose (1.1 times lower).

Changes in metabolism are often associated with changes in enzyme levels since these are the catalysts that drive metabolic reactions. In general, increasing DF lowered glucose-6-phosphate dehydrogenase (G6PDH) and fatty acid synthesis enzyme (FAS) activities but this

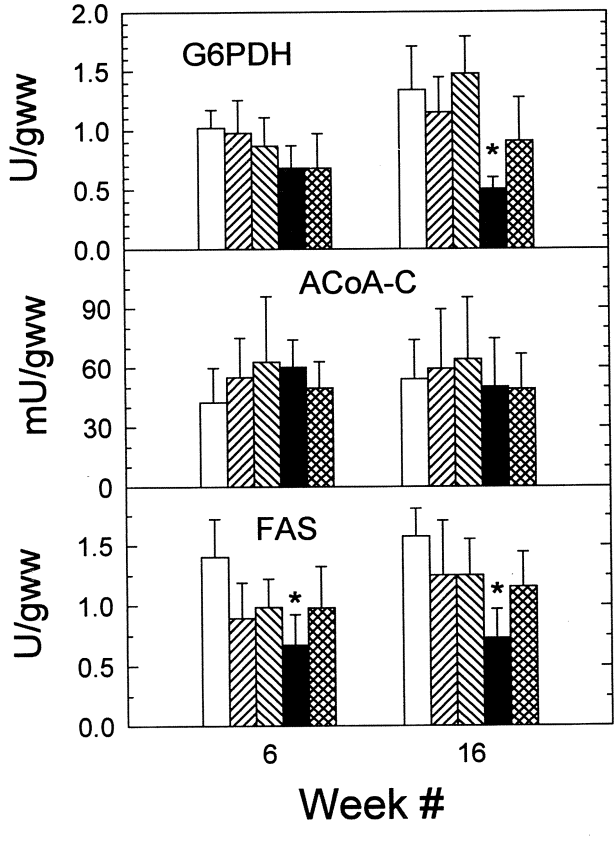

Figure 7. Fatty acid synthesis enzyme activities in animals fed different diets for 6 and 16 weeks. Symbols as defined in Figure 6. Bars represent the mean ± one standard deviation for N = 8 animals.

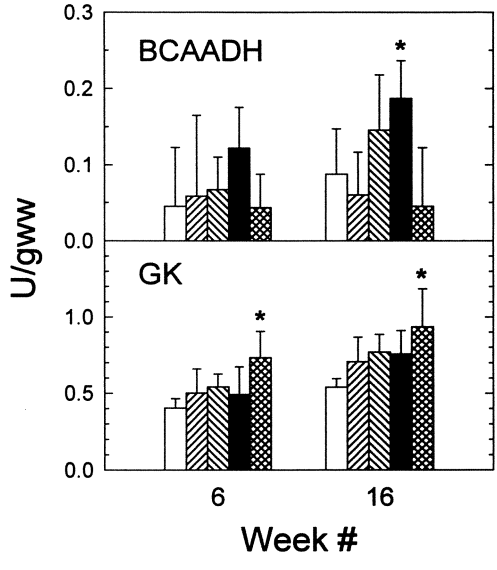

Figure 8. Branch chain amino acid dehydrogenase and glucokinase activities in rats fed different diets for 6 and 16 weeks. Symbols as defined in Figure 6. Bars represent the mean ± one standard deviation for N = 8 animals.

effect was only significant when rats fed the basal diet were compared to rats fed the 10% DF2 diet (Figure 7). No changes in acetyl CoA carboxylase activity were noted. Increasing DF also appeared to increase branched-chain amino acid dehydrogenase (BCAADH) activity although this was only significant in older rats fed 10% DF2 (Figure 8). Glucokinase (GK) was significantly higher than basal in animals fed corn starch at both 6 and 16 weeks (Figure 8). Diet had no effect on aspartate aminotransferase (AspAT), alanine aminotransferase (AlaAT), succinate dehydrogenase (SDH) or phosphoenolpyruvate carboxykinase (PEPCK) activities (Table 9). Older animals had significantly higher AlaAT (1.7 times) and SDH (2.8 times) activities than did younger animals.

4. DISCUSSION

4.1. Weight of GI Tract

The absolute weight of the small intestine suggests that the effect of a high bran diet may be interpreted differently depending on the choice of the control diet. Small intestine weight was 32% lower than basal in animals fed 10% DF at week 6 ($P<0.05$) but was not different from basal at week 16. On the other hand, when compared with animals fed 2% DF, small intestine weight was 13% lower in week 6 but was 14% higher in week 16 ($P<0.05$). Thus, the comparison between animals fed the Basal and 2% DF1 diets indicates that the effect of a fiber-free diet on the small intestine was not necessarily similar to that of a low-fiber diet. The weight of the large intestine increased with increasing levels of dietary hard wheat bran (HWB) in week 6 but in week 16 it was not higher than basal in animals fed 2% DF. Dietary fiber fermentation has been reported to increase the weights of cecum and large intestine at the muscular and mucosal levels after two weeks feeding in rats (Goodlad *et al.*, 1995).

4.2. Digestible and Net Energy Value

The method adopted to measure energy digestibility has a high degree of precision (Livesey, 1990b) and this was evident in the final results (low standard deviations). The present paper also demonstrates that the DEV_{part} value for HWB fiber was fairly robust to small changes in experimental conditions since results with different batches of animals could be combined to provide a single estimate (Figure 4). In the present paper, we followed energy consumption and excretion for 2 week periods only. The decision to monitor energy over a shorter time period (four weeks is more common for these types of measurements) followed from earlier experiments that suggested fiber metabolism changed as a function of time of exposure (Mongeau *et al.* 1991). The shorter 2 week period employed here may introduce a higher degree of variability by reducing the mass of feces produced, the mass of the diet consumed and the body energy accumulated by the rats. Any increase in variability should not be due to diet adaptation. Faulks *et al.* (1989) showed that approximately 3 days were required for the appearance of supplement in the feces and for adjustment to the new diets. However, our animals were maintained on the supplemented diet for 6 or 16 weeks prior to the collection period.

In contrast to the DEV_{part} measurements, the NEV measurements were much more variable in our hands. The precision of the NEVs would have been significantly reduced by a longer collection period since larger measurements of body energy gain, ingested diet and fecal mass would have been observed. The data in Table 6 suggest that longer collection periods will not affect the DEV_{part} for DF since rat digestion appears to remain stable with respect to DF over the long term (6–16 weeks). Thus a 4 week collection period would have been more desirable. Some of the variability in the NEV could also have been reduced if the animals had similar body weights and compositions at the start of the collection period. Inter-animal differences in body weights and compositions were readily apparent by the start of the first collection period (6 weeks) and were even larger at the start of the second collection period (16 weeks). The use of younger animals may also decrease the variability of the NEV measurement since younger animals accrue body energy at higher rates than do older animals. The higher rate of body energy gain is illustrated by the data in Table 7 that show younger rats gained body energy at rates approximately 2 times those of the older rats. Another potential source of variation may have come from the experimental protocol followed in the present paper. The NEVs reported in Table 6 were calculated using the data from Experiments 1 and 2. This introduced a significant variability since differences in body energy gain and in energy expenditure could be observed, even after allowing for the differences in food consumption (Table 7). The NEV measurement may be more susceptible to small changes in experimental protocols (placement of cages, room temperature, seasonality, ...; see Livesey, 1993) requiring all measurements to be made within a single experiment.

The results of the digestibility measurements show that HWB and HWB fiber are relatively poor sources of dietary energy. This is true despite the fact that between 39–44% of the DF was apparently fermented. The relatively high fiber digestibility gave an apparent digestible energy value between 1.6 and 1.8 kcal/g but DEV_{part} analysis showed that only 0.5 kcal/g of this energy was potentially available to the rat. Because DF is fermented in the lower GI tract and not absorbed by the small intestine (at least theoretically), a fraction of the 0.5 kcal/g will be lost during fiber fermentation and so the amount of dietary energy available to the rat will be less than 0.5 kcal/g. We attempted to measure the actual amount of energy available to the rat by calculating the NEV for HWB fiber. This value proved to be unreliable because of a high degree of experimental variability. We, therefore, adopted a factorial approach to

estimate the amount of metabolic energy available from DF. Our calculations suggest that 0.31–0.35 kcal/g of DF was available to the rat. This value is significantly lower than the apparent DEV calculated from fiber digestibility (approximately 1.7 kcal/g). The major factor accounting for the difference between the apparent DEV and the REV calculated from the factorial model is the loss to fecal energy via the fermentation process (*c*). This "extra" fecal energy is presumably due to increased bacterial mass. We did not, however, measure fecal bacteria directly and so cannot definitely attribute the energy loss to bacteria. In other studies on large–intestine digested carbohydrate, increased fermentation resulted in increased bacterial growth and some of these bacteria were found in the feces. In the case of sugar alcohols and other non–absorbed carbohydrates, values for *c* were on the order of 20–35% of the fermented energy (Bär, 1990; Livesey, 1990b, 1992). HWB fiber appears to promote bacterial growth to a much greater extent since approximately 67% of the fermented energy was lost to feces (Figure 2). Part of the difference between our value and those reported earlier may be due to the fact that we subtracted fecal DF from ingested DF in calculating the quantity of fiber fermented:

$$\text{fermented fiber} = \text{ingested DF} - \text{fecal DF}$$

Other values may have reported fermentation–associated bacterial losses as a function of ingested DF only. Omitting the loss of fecal fiber from the calculation would give a value for *c* of 39 ± 1% (N = 64). This value is much closer to the values reported for mixed diets (Livesey, 1990b) and is similar to the value of 34% reported for Isomalt fermentation (Livesey, 1990d). The calculation of fermented fiber also assumes that the DF is not absorbed by the small intestine. The close agreement between the DEV_{part} and the calculated REV suggests that this assumption is valid for HWB fiber even though it may not hold for other dietary fibers.

Our results suggest that the metabolic energy available from dietary HWB fiber is approximately 0.35 kcal/g. This value is dependent on the measurement of DF in the diet. Because lignin constitutes approximately 9% of the DF in HWB, one g of total DF represents 0.91 g NSP (non-starch polysaccharides) as measured by the Englyst method. Using 0.9 in the denominator above would not substantially change the calculated energy value (0.35/0.9 = 0.39).

4.3. Metabolic Parameters

The present results suggest that increased DF would represent a poor method for reducing caloric intake. Although the REV for fiber is low (approximately 0.35 kcal/g), the rats tended to eat isocalorically so that an equivalent or higher total dietary energy intake was recorded for supplemented animals (Table 7). Analysis of body weight gain and body energy gain showed minimal effects of dietary bran supplementation. No change in body composition was noted. Plasma triglyceride levels were significantly lower at 8 weeks and at 16 weeks but this may have arisen because the rats were not fasted prior to sacrifice: lower plasma triglyceride levels may have resulted from lower dietary fat ingestion. Interestingly, HWB appeared to decrease the levels of fatty acid synthesis enzymes in the liver. This effect was only statistically significant for the highest level of bran supplementation but suggests that bran may lower the rates of fatty acid synthesis, since fatty acid synthesis enzymes generally parallel the rate of fatty acid synthesis (Herzberg, 1983). This effect remains to be verified by direct measurement of fatty acid synthesis rates.

4.4. Energy Value and Energy Intake

The non-fiber components of wheat bran may be quite nutritious since HWB supplementation significantly improved the gain/food ratio of a diet deficient in essential amino acids (Mongeau *et al.*, 1995). Therefore, unless energy intake is also reduced, low-energy supplements are not helpful in body weight control. Stevens *et al.* (1987) reported a reduction in energy intake when wheat bran was given with a gum supplement that has an appetite suppressant effect. Further work is needed to understand the long-term effects of DF and foods on the physiological mechanisms underlying appetite/satiety.

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THE PROTECTIVE ROLE OF DIETARY FIBER IN DIVERTICULAR DISEASE

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INTRODUCTION

Diverticular disease is one of the most common disorders of the colon among the elderly in Western societies (1). In North America diverticular disease is estimated to occur in one third of all persons older than 45 years of age, and in two thirds of all persons older than 85 years of age (2). In the great majority of cases the condition is symptomless (3), and only between 10 to 25 percent of affected individuals develop symptoms (2). Symptomatic diverticular disease results in 200,000 hospitalizations in the USA annually (4).

Earlier this century diverticular disease was widely believed to be extremely rare, and was regarded as a pathological curiosity. This prompted Burkitt and Painter to call it "a 20th century problem" or "a disease of Western civilization" (5,6), in contrast to its rarity in many developing countries (7). This sharp contrast was largely attributed to dietary changes, mainly in the decline of dietary fiber intake from cereal grains (5). The dietary fiber hypothesis is supported by human (8–13) and animal studies (14–16). Previous case-control studies have consistently found that patients with diverticula report lower fiber intake than controls. However, the findings of case-control studies were related to the diet mainly after the onset of symptoms, which may have been altered as a result of the disease (12). In addition, failure to control for potential risk factors, such as physical activity, alcohol, smoking, coffee drinking, and other dietary component, limits the interpretation of most case-control studies (17). Therefore, we studied, prospectively, the association between crude fiber, total dietary fiber and sources of fiber (vegetable, cereal, fruit, soluble, insoluble, cellulose, hemicellulose, and lignin) other dietary component (eg. red meat, fats, and different dietary sources of fat), nondietary factors (eg. physical activity, body composition, and smoking) and the diagnosis of diverticular disease among U.S men participating in the Health Professionals Follow-up Study [HPFS] (18–20).

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METHODS

Study Population

The HPFS is a prospective study of heart disease and cancer among 51,529 U.S male health professionals, residing in 50 U.S states and aged 40–75 years in 1986. The study population consists of 29,683 dentists, 3745 optometrists, 2218 osteopathic physicians, 4185 pharmacists, 1600 podiatrists, and 10,098 veterinarians. The study began in 1986 when cohort members completed a detailed food-frequency questionnaire and provided information about medical history, age, weight, height, smoking, alcohol consumption, physical activity, and history of professionally diagnosed medical conditions. Every two years (1988, 1990, 1992) follow-up questionnaires have been sent to update information on potential risk factors and to identify newly diagnosed cases of various diseases. The 1990 and 1992 follow-up questionnaires contained specific questions regarding diverticular disease.

Dietary Assessment

To assess dietary intake, we used a semiquantitative food-frequency questionnaire that was validated in this cohort (21). In our validation study, the correlation between the diet records and the dietary questionnaire was 0.68 for total dietary fiber. Also, there was a high degree of validity of those foods that were the major contributors to total fiber intake (22). The dietary questionnaire included 131 food items plus vitamins and mineral supplements that collectively account for over 90 percent of the absolute intake of the major nutrients consumed by this population. The intake of dietary fiber was computed by multiplying the consumption frequency of each food item by the fiber content of the specified portion. The values for dietary fiber we used, were based on the work of Southgate and colleagues (23,24). We have also expanded our dietary fiber database to include dietary fiber content of foods derived from food composition data tables based on the Association of Official Analytical Chemists (AOAC) method (25–28), as well as Englyst method (29). Data on soluble fiber, insoluble fiber, cellulose, hemicellulose, and lignin was available directly from some manufacturers, but was otherwise based on calculations from ingredients. The composition values for dietary lipids and crude fiber were obtained from U.S. Department of Agriculture sources (30).

We adjusted dietary intake values for total energy intake, using regression analysis (17,31) to focus on the composition of diets rather than on absolute intake which is a combination of composition and overall energy intake. This procedure also reduces variation caused by underreporting or overreporting of intake on the food-frequency questionnaire, thus improving the precision of nutrient measurement (17,21).

Physical activity was assessed using a validated (32) self-administered questionnaire that assesses the average weekly recreational physical activity. Eight moderate or vigorous activities were listed on the questionnaire and participants were asked to report the average time per week spent at each activity (20). The contribution of each activity was based on its energy expenditure requirements in metabolic equivalents (METs) (33), multiplied by the duration of the activity. The contributions from each activity was summed to give a physical activity index in total METs expressed as hours/week. We further classified sports and other recreational activities into vigorous and nonvigorous activities according to their intensity (20).

Identification of Diverticular Disease Cases

Follow-up questionnaires were sent in 1988, 1990, and 1992 to all study participants. On the forms mailed in 1990 and 1992, we asked whether diverticular disease had been diagnosed during the previous two years. Diverticular disease was not a specified end point in the 1988 follow-up questionnaire. After up to six mailings for each follow-up period, the response rate was 96 percent in 1990 and 94 percent in 1992. When diverticular disease was reported on the follow-up questionnaire, we mailed the cohort member a supplementary questionnaire to confirm the reporting, and to ascertain the date of diagnosis, symptoms, procedure performed to confirm the diagnosis (e.g. barium study or endoscopy), treatment, and any dietary changes prior to the diagnosis. We obtained 182 medical records from a sample of participants reporting diverticular disease to assess the validity of self-reporting, and to ascertain the site of the diverticula. The records confirmed the self-reports in 95 percent of the cases. We therefore accepted the self-report of diverticular disease. In 96 percent of the cases, diverticula were located in the left colon (sigmoid, descending, or mid transverse colon), as expected in a predominantly caucasian population (34). We considered the cases ascertained during the four years of follow-up — between February 1, 1988 and January 31, 1992.

We excluded from this analysis men who did not report an average daily energy intake between 3.35 to 17.6 MJ (800 to 4,200 Kcal), and who left blank 70 or more food items on the dietary questionnaire (0.5 percent of the participants). We also excluded men who reported previous cancer (other than nonmelanoma skin cancer), colon or rectal polyp, ulcerative colitis, and diverticular disease prior to 1988. After these exclusions, the baseline population consisted of 47,888 men eligible for this analysis, and 503 newly diagnosed cases of diverticular disease were identified. Of the 503 new cases, 385 were classified as symptomatic (presenting with pain, change in bowel habits, or bleeding) (35), and 118 cases were classified as asymptomatic (the diverticula were discovered during a routine screening). To reduce the possibility of detection bias, we used symptomatic diverticular disease as our primary end point.

Statistical Analysis

Participants were categorized according to quintiles of intake of dietary variables which included crude fiber, dietary fiber, and sources of fiber (vegetable, cereal, fruit, soluble, insoluble, cellulose, hemicellulose, and lignin). These dietary variables were computed using the food frequency questionnaire completed by the participants in 1986. Follow-up time was calculated as the number of months between February 1, 1988 and the date of diagnosing diverticular disease, death, or to January 31, 1992, whichever came first. The relative risk — the incidence among the men in different exposure categories divided by the corresponding rate in the reference category — was used as the measure of association (36). Age-adjusted rates were calculated with the use of five-year categories. The Mantel extension test (37) was used to evaluate linear trends across categories of different dietary variables. In addition to the dietary variables mentioned, other potentially confounding variables were modelled with multiple logistic regression. These variables included body mass index (weight in kilograms divided by the square of height in meters), physical activity (in metabolic equivalents per week computed on the basis of the reported frequency and duration of various forms of exercise), smoking, alcohol consumption, coffee intake, and dietary intake of potassium, magnesium, vitamin C, and beta-carotene. The P-values are all two tailed, and for all relative risks, we calculated 95 percent confidence interval (38).

RESULTS

During 188,252 person-years of follow-up over a period of four years, 385 cases of symptomatic diverticular disease were documented in this cohort. The predominant symptoms were abdominal pain (63%), change in bowel habits (8%), and bleeding (18%). Energy-adjusted intakes of crude and total dietary fiber were both inversely associated with the risk of diverticular disease [p for trend = <0.00001 for crude fiber, and p for trend = 0.0002 for dietary fiber] (see Table 1).

We computed the contribution of age and energy-adjusted dietary fiber from fruits, vegetables, and cereals. Intake of fruit and vegetable fiber were inversely associated with risk of diverticular disease [for higher versus lowest quintile, relative risk (RR) for fruit fiber = 0.62, 95 percent confidence interval (CI), 0.45 to 0.86; RR for vegetable fiber = 0.55, 95 percent CI, 0.37 to 0.84]. Cereal fiber was not associated with a decreased risk of diverticular disease (see Table 1). When we analyzed separately the sources of dietary fiber (fruits, vegetables, or cereals) in multivariate models including age, total energy intake, total fat, and physical activity, our findings for each source of dietary fiber did not change appreciably from those in the age and energy-adjusted model (see Table 1). When we included these three sources of dietary fiber (fruits, vegetables, and cereals) simultaneously in the multivariate model with age, total energy intake, total fat, and physical activity, our findings did not change appreciably [RR for fruit fiber = 0.66, 95 percent CI, 0.46 to 0.95; RR for vegetable fiber = 0.68, 95 percent CI, 0.46 to 0.99; RR for cereal fiber = 1.18, 95 percent CI, 0.85 to 1.65 for the highest quintile].

Age and energy-adjusted intake of soluble fiber, insoluble fiber, cellulose, hemicellulose, and lignin were inversely associated with risk of diverticular disease (Table 3 and Table 4). This association was particularly stronger for cellulose, lignin and insoluble fiber (Table 3 and Table 4). When we analyzed separately these sources of dietary fiber in multivariate models including age, energy-adjusted total fat, and physical activity, our findings for each source of dietary fiber did not change appreciably from those in the age and energy-adjusted model (Table 3, Table 4). When we included soluble and insoluble fiber simultaneously in the multivariate model, the association with insoluble fiber did not change appreciably, but that with soluble fiber was eliminated [RR for insoluble fiber = 0.53, 95 percent CI, 0.33 to 0.85; RR for soluble fiber = 1.34, 95 percent CI, 0. 83 to 2.18, for the highest quintile]. Similarly when we included hemicellulose, cellulose, and lignin in the multivariate model, the association with cellulose did not change appreciably, but that with hemicellulose and lignin was attenuated [RR for hemicellulose = 1.26, 95 percent CI, 0.81 to 1.96; RR for cellulose = 0.57, 95 percent CI, 0.34 to 0.94; RR for lignin = 0.80, 95 percent CI, 0.47 to 1.38 for the highest quintile].

It has been suggested that the interpretation of epidemiologic studies of fiber-disease association is limited, because different analytic techniques have been used to define dietary fiber. However, in our data, results using total fiber intake calculated from the Association of Official Analytical Chemists standards (AOAC) or Englyst method were not appreciably different from results from Southgate (Table 1) [for AOAC, RR = 0.60, 95 percent CI, 0.41 to 0.87; for Englyst, RR = 0.60, 95 percent CI, 0.42 to 0.87, for the highest quintiles].

For energy-adjusted total fat intake, the relative risks for men in each of the upper quintiles were elevated compared to men in the lowest quintile (Table 4). After energy-adjustment, saturated and monounsaturated fat, were both positively associated with the risk of diverticular disease (Table 4). Positive associations were also seen with *trans* fatty acids and polyunsaturated fat whereas a weak inverse association was seen for the omega-3 fatty acid intake. However, when we also adjusted for physical activity and dietary fiber, the associations with

Table 1. Relative risk (RR) of symptomatic diverticular disease by quintiles of energy-adjusted total dietary fiber, crude fiber, and by dietary fiber from fruits, vegetables, cereals and potatoes

Variable	Quintile					P value for trend*
	1	2	3	4	5	
Total dietary fiber (median g/d)	13.0	18.0	21.0	24.0	32.0	
Cases	99	75	73	83	55	0.0002
RR† (95% CI)	1.0	0.76 (0.57–1.03)	0.73 (0.54–0.99)	0.78 (0.58–1.05)	0.51 (0.37–0.71)	0.01
Multivariate RR‡ (95% CI)	1.0	0.79 (0.58–1.07)	0.77 (0.57–1.05)	0.87 (0.64–1.17)	0.58 (0.41–0.83)	
Total crude fiber (median g/d)	3.3	4.5	5.4	6.4	8.3	
Cases	101	72	86	72	54	<0.00001
RR† (95% CI)	1.0	0.78 (0.58–1.06)	0.72 (0.54–0.96)	0.71 (0.52–0.96)	0.48 (0.35–0.67)	0.001
Multivariate RR‡ (95% CI)	1.0	0.81 (0.59–1.10)	0.76 (0.57–1.02)	0.78 (0.57–1.06)	0.54 (0.38–0.76)	
Fruit fiber (median g/d)§	1.1	2.3	3.8	5.5	8.8	
Cases	85	98	84	57	61	<0.00001
RR† (95% CI)	1.0	1.03 (0.77–1.38)	0.85 (0.63–1.15)	0.54 (0.39–0.76)	0.62 (0.45–0.86)	<0.00001
Multivariate RR‡ (95% CI)	1.0	1.10 (0.82–1.47)	0.90 (0.66–1.23)	0.60 (0.43–0.85)	0.61 (0.43–0.87)	
Vegetable fiber (median g/d)‖	3.4	5.1	6.6	8.6	12.4	
Cases	84	82	87	78	54	0.003
RR† (95% CI)	1.0	0.93 (0.68–1.28)	0.98 (0.71–1.35)	0.90 (0.64–1.26)	0.55 (0.37–0.84)	0.003
Multivariate RR‡ (95% CI)	1.0	0.96 (0.70–1.31)	1.0 (0.73–1.36)	0.87 (0.63–1.20)	0.59 (0.41–0.86)	
Cereal fiber (median g/d)	1.7	3.7	5.7	8.2	13.5	
Cases	77	91	78	61	78	0.23
RR† (95% CI)	1.0	1.21 (0.89–1.66)	1.10 (0.79–1.54)	0.85 (0.58–1.24)	1.09 (0.77–1.54)	0.72
Multivariate RR‡ (95% CI)	1.0	1.23 (0.90–1.67)	1.08 (0.78–1.49)	0.87 (0.61–1.22)	1.06 (0.76–1.47)	

*Test for trend was calculated by using the median intake of nutrients in each quintile as a continous variable in a multiple logistic regression.

†Adjusted for age and total energy. CI denotes confidence interval.

‡Adjusted for age, physical activity, and energy-adjusted total fat.

§Includes raisins, avocados, bananas, cantaloupes, watermelon, apples, pears, oranges, grapefruits, strawberries, blueberries, peaches, apricots, plums, and fruit juices.

‖Includes string beans, broccoli, sauerkraut, coleslaw, cauliflower, brussels sprouts, carrots, corn, peas, mixed vegetables, beans, cabbage, lentils, alfalfa sprouts, celery, mushrooms, yellow squash, eggplant, yams, spinach, iceberg or romain lettuce, green pepper, garlic, tomatoes, tomato juice and sauce, red chili sauce, and kale or chard greens.

¶Includes cold breakfast cereal, cooked oatmeal, other cooked breakfast cereal, white bread, dark bread, English muffins, bagels, rolls, brown rice, white rice, pasta, other grains, pancakes, crackers, potatoes, and added bran.

Table 2. Relative risk of symptomatic diverticular disease by quintiles of soluble and insoluble fiber components

	Quintile					
	1	2	3	4	5	P-value for trend
Soluble fiber (g/d)	4.10	5.10	5.90	6.90	8.60	
RR* (95% CI)	1.00	1.03 (0.75–1.43)	1.00 (0.72–1.38)	0.83 (0.59–1.17)	0.82 (0.59–1.16)	0.05
Multivariate RR** (95% CI)	1.00	1.05 (0.76–1.45)	1.04 (0.75–1.44)	0.91 (0.65–1.29)	0.90 (0.62–1.29)	0.40
Insoluble fiber (g/d)	10.10	13.0	15.20	17.90	22.70	
RR* (95% CI)	1.00	0.89 (0.65–1.22)	0.78 (0.57–1.07)	0.80 (0.59–1.10)	0.55 (0.39–0.78)	0.001
Multivariate RR** (95% CI)	1.00	0.91 (0.66–1.24)	0.80 (0.58–1.10)	0.87 (0.63–1.20)	0.63 (0.44–0.91)	0.02

*Adjusted for age and total energy.
**Adjusted for age, physical activity, and energy-adjusted total fat.

Table 3. Relative risk of symptomatic diverticular disease by quintiles of hemicellulose, cellulose, and lignin intake

	Quintile					
	1	2	3	4	5	P-value for trend
Hemicellulose (g/d)	4.40	6.00	7.30	8.90	11.70	
RR* (95% CI)	1.00	1.27 (0.94–1.71)	0.77 (0.55–1.07)	0.78 (0.56–1.09)	0.78 (0.56–1.08)	0.01
Multivariate RR** (95% CI)	1.00	1.29 (0.95–1.74)	0.79 (0.56–1.10)	0.84 (0.60–1.18)	0.88 (0.62–1.25)	0.11
Cellulose (g/d)	3.40	4.40	5.30	6.20	8.00	
RR* (95% CI)	1.00	0.75 (0.55–1.03)	0.71 (0.52–0.97)	0.78 (0.57–1.06)	0.47 (0.33–0.66)	0.0002
Multivariate RR** (95% CI)	1.00	0.76 (0.56–1.05)	0.74 (0.54–1.01)	0.83 (0.61–1.14)	0.52 (0.36–0.75)	0.002
Lignin (g/d)	1.00	1.20	1.50	1.80	2.40	
RR* (95% CI)	1.0	0.82 (0.60–1.12)	0.87 (0.64–1.19)	0.65 (0.47–0.90)	0.53 (0.38–0.76)	0.0002
Multivariate RR** (95% CI)	1.0	0.83 (0.61–1.14)	0.89 (0.65–1.21)	0.71 (0.51–1.00)	0.62 (0.43–0.89)	0.01

*Adjusted for age and total energy.
**Adjusted for age, physical activity, and energy-adjusted total fat.

Table 4. Relative risk (RR) of symptomatic diverticular disease by quintiles of energy-adjusted total fat and specific dietary fats

Fats	Quintile					P for trend*
	1	2	3	4	5	
Total fat (median g/d)	47.0	60.0	70.0	76.0	82.0	
Cases	53	76	100	58	98	
RR† (95% CI)	1.0	1.27 (0.90–1.81)	1.57 (1.13–2.19)	1.25 (0.86–1.83)	1.71 (1.0–2.37)	0.005
Multivariate RR‡ (95% CI)	1.0	1.17 (0.82–1.67)	1.40 (1.0–1.98)	1.09 (0.74–1.60)	1.38 (0.96–1.96)	0.20
Animal fat (median g/d)	22.0	31.0	37.0	43.0	52.0	
Cases	60	88	80	83	74	
RR† (95% CI)	1.0	1.66 (1.20–2.03)	1.39 (0.99–1.94)	1.57 (1.13–2.19)	1.43 (1.02–2.01)	0.12
Multivariate RR‡ (95% CI)	1.0	1.50 (1.08–2.10)	1.20 (0.85–1.69)	1.27 (0.90–1.80)	1.08 (0.75–1.56)	0.80
Vegetable fat (median g/d)	18.0	25.0	30.0	35.0	43.0	
Cases	70	73	85	70	87	
RR† (95% CI)	1.0	1.06 (0.76–1.47)	1.28 (0.93–1.75)	1.06 (0.76–1.48)	1.28 (0.94–1.76)	0.13
Multivariate RR‡ (95% CI)	1.0	1.07 (0.77–1.49)	1.30 (0.94–1.79)	1.08 (0.77–1.51)	1.32 (0.96–1.81)	0.10
Saturated fatty acids (median g/d)	15.1	20.9	24.3	26.7	30.3	
Cases	59	82	82	80	82	
RR† (95% CI)	1.0	1.45 (1.04–2.02)	1.54 (1.10–2.15)	1.51 (1.08–2.13)	1.61 (1.16–2.25)	0.01
Multivariate RR‡ (95% CI)	1.0	1.30 (0.92–1.83)	1.34 (0.94–1.89)	1.27 (0.89–1.81)	1.22 (0.85–1.76)	0.47
Monounsaturated fatty acids (median g/d)	16.5	22.4	26.0	28.6	31.2	
Cases	61	73	86	79	86	
RR† (95% CI)	1.0	1.26 (0.90–1.77)	1.45 (1.04–2.01)	1.41 (1.01–1.97)	1.53 (1.10–2.11)	0.01
Multivariate RR‡ (95% CI)	1.0	1.15 (0.81–1.62)	1.29 (0.92–1.81)	1.19 (0.84–1.68)	1.24 (0.87–1.75)	0.26
Polyunsaturated fatty acids (median g/d)	8.6	11.3	12.8	14.6	17.4	
Cases	73	79	66	79	88	
RR† (95% CI)	1.0	1.14 (0.83–1.57)	0.95 (0.68–1.32)	1.14 (0.83–1.57)	1.25 (0.92–1.71)	0.19
Multivariate RR‡ (95% CI)	1.0	1.15 (0.83–1.58)	0.95 (0.68–1.32)	1.14 (0.83–1.58)	1.28 (0.94–1.76)	0.16
Omega-3 fatty acids (median g/d)	<0.1	0.1	0.2	0.3	0.5	
Cases	72	86	83	77	67	
RR† (95% CI)	1.0	1.08 (0.79–1.47)	1.17 (0.86–1.61)	0.92 (0.67–1.27)	0.86 (0.61–1.19)	0.20
Multivariate RR‡ (95% CI)	1.0	1.11 (0.81–1.52)	1.25 (0.91–1.71)	1.02 (0.74–1.41)	0.97 (0.69–1.36)	0.38
Trans fatty acids (median g/d)	1.9	2.7	3.2	3.7	4.6	
Cases	62	64	106	76	77	
RR† (95% CI)	1.0	1.28 (0.90–1.81)	1.73 (1.27–2.35)	1.70 (1.22–2.37)	1.49 (1.06–2.09)	0.002
Multivariate RR‡ (95% CI)	1.0	1.17 (0.82–1.67)	1.52 (1.10–2.10)	1.46 (1.03–2.07)	1.29 (0.91–1.84)	0.25

*Test for trend was calculated by using the median intake of the nutrient in each quintile as a continous variable in a multiple logistic regression.
†Adjusted for age and total energy. CI denotes confidence interval.
‡Adjusted for age, energy-adjusted dietary fiber, and physical activity.

total fat and types of fat were substantially attenuated and no longer significant (Table 4). This attenuation was primarily due to the influence of dietary fiber and to a lesser extent to physical activity. After adjusting for age, dietary fiber, physical activity, and total energy, intake of red meat was positively associated with the risk of diverticular disease, however the association was non-linear and the trend not statistically significant [RR = 1.48, 95 percent CI, 1.00 to 2.19 for the highest quintile, P value for trend = 0.24]. Little suggestion of an association was seen with combined intake of chicken and fish, and with dairy fat.

We also examined specific foods in relation to the risk of diverticular disease (Table 5). Of the 131 foods included in the questionnaire, fourteen foods were significantly (P for trend $\leq$ 0.01) associated with diverticular disease, either positively or inversely. These food items, mainly discriminated between cases and noncases particularly for their crude and dietary fiber, total fat, and red meat intake. Among the strong positive associations were those with beef eaten as a main dish, and with processed meat (Table 5). Significant inverse trends for several fruits and vegetables largely account for the inverse associations of fruit and vegetable fiber with the risk of diverticular disease (Table 1). After adjusting for age and total energy, intake of bran added to food was inversely associated with the risk of diverticular disease [RR for five or more tablespoons/week = 0.59, 95 percent CI, 0.36 to 0.98], however the association was not statistically significant (P for trend = 0.13).

Using a multivariate model controlling for age, physical activity, and total energy, we examined the risk of diverticular disease among the subjects classified jointly according to their intake of total dietary fiber and total fat or red meat. For men in the highest quintile of total fat intake and the lowest quintile of total dietary fiber intake (as compared with those in the opposite extreme), the relative risk of diverticular disease was 2.35, (95 percent CI, 1.38 to 3.98) (Fig. 1). Similarly for those in the highest category of red meat and the lowest quintile of total dietary fiber intake, the relative risk was 3.22, (95 percent CI, 1.46 to 7.54) (Fig. 2). We further analyzed groups described in Figures 1 & 2 to characterize the dietary patterns of the extreme categories. In general, there was about a three fold difference in the median intake of the high dietary fiber/low total fat or red meat group compared to the low dietary fiber/high total fat or red meat group. Although differences were greater for total dietary fiber, rather than for any specific dietary fiber source, fruit fiber was the only fiber source which significantly discriminated between the high dietary fiber and the low dietary fiber group. Fruit fiber constituted about 33 percent of the total dietary fiber component in the high dietary fiber/low red meat or total fat group compared to about 10 percent in the low dietary fiber/high red meat or total fat group.

We examined the relationships of age-adjusted total physical activity, vigorous activity, and non-vigorous activity with the risk of diverticular disease. Total physical activity was inversely associated with the risk of diverticular disease. Compared to men in the lowest quintile of physical activity, those in the highest quintile had a relative risk of 0.57, (95 percent CI, 0.41 to 0.79). Nearly all of this inverse association was attributable to the vigorous activities. The RR comparing extreme categories of vigorous activity was 0.53, (95 percent CI, 0.37 to 0.75). In contrast non-vigorous activity was not appreciably associated with risk of diverticular disease [RR = 0.93, 95 percent CI, 0.67 to 1.30] (Table 6). When we analyzed separately total physical activity, vigorous activity and non-vigorous activity in multivariate models with age, energy adjusted dietary fiber, and energy-adjusted total fat, our findings did not change appreciably from those in the age-adjusted model (Table 6). When we included vigorous activity and non-vigorous activity simultaneously in the multivariate model with age, energy-adjusted dietary fiber, and energy-adjusted total fat, our findings did not change appreciably [RR for vigorous activity = 0.60, 95 percent CI, 0.41 to 0.87; RR for non-vigorous activity = 0.93, 95 percent CI, 0.67 to 1.29].

Table 5. Age and energy-adjusted relative risks for foods associated with symptomatic diverticular disease

Food	Frequency						P value* for trend
	<1/mo	1–3+/mo	1/wk	2–4/wk	5–6/wk	≥1/day	
Beef, pork or lamb as a main dish (4–6 oz)							†
Cases	23	62	136	137	25		
RR (95% CI)	1.0	1.35 (0.38–2.19)	1.96 (1.25–3.09)	1.96 (1.18–3.27)	3.23 (1.47–7.08)		0.01
Processed meat (piece or slice)							
Cases	86	107	85	74	27		
RR (95% CI)	1.0	1.17 (0.87–1.55)	1.41 (1.03–1.94)	1.56 (1.13–2.17)	1.90 (1.20–3.00)		0.0008
Beef, pork or lamb as a sandwich or mixed dish							
Cases	40	105	141	78	9		
RR (95% CI)	1.0	1.77 (1.23–2.55)	2.13 (1.48–3.08)	2.0 (1.27–3.16)	1.98 (0.75–5.21)		0.002
Bacon (2 slices)							
Cases	122	108	91	49	9		
RR (95% CI)	1.0	1.19 (0.92–1.55)	1.48 (1.13–1.96)	1.49 (1.05–2.10)	1.07 (0.52–2.19)		0.009
Hot dogs (1)							
Cases	124	160	73	18			
RR (95% CI)	1.0	1.53 (1.21–1.94)	1.86 (1.38–2.51)	1.38 (0.84–2.25)			0.001
Romain or leafy lettuce (serving)							
Cases	143	101	57	38	24		
RR (95% CI)	1.0	1.03 (0.80–1.33)	0.87 (0.64–1.18)	0.63 (0.44–0.90)	0.73 (0.47–1.12)		0.008
Peaches, apricots or plums (1 fresh or ½ cup canned)							
Cases	132	154	61	23	3		
RR (95% CI)	1.0	0.95 (0.75–1.20)	0.85 (0.63–1.15)	0.56 (0.36–0.87)	0.23 (0.08–0.70)		0.002

(continued)

Table 5. *(Continued)*

Food	Frequency						P value* for trend
	<1/mo	1–3+/mo	1/wk	2–4/wk	5–6/wk	≥1/day	
Oranges (1)							
Cases	87	98	61	75	35		
RR (95% CI)	1.0	0.88 (0.66–1.16)	0.75 (0.54–1.04)	0.76 (0.56–1.03)	0.70 (0.48–1.02)		0.01
Apples (1)							
Cases	59	70	171		46	12	
RR (95% CI)	1.0	0.59 (0.42–0.83)	0.66 (0.49–0.88)		0.48 (0.33–0.70)	0.77 (0.41–1.44)	0.01
Blueberries (½ cup)							
Cases	263	79		18			
RR (95% CI)	1.0	0.75 (0.59–0.96)		0.54 (0.33–0.87)			0.002
Ready-made cookies (1)							
Cases	99	87	51	71	65		
RR (95% CI)	1.0	1.12 (0.84–1.49)	1.23 (0.87–1.74)	1.65 (1.20–2.26)	1.69 (1.22–2.33)		0.0002
Potato or corn chips (small bag or 1 oz)							
Cases	132	99	82	52	12		
RR (95% CI)	1.0	1.06 (0.82–1.37)	1.42 (1.08–1.88)	1.25 (0.89–1.76)	1.16 (0.61–2.18)		0.007
French fried potatoes (4 oz)							
Cases	125	128	82		41		
RR (95% CI)	1.0	1.20 (0.93–1.55)	1.49 (1.09–2.02)		1.51 (0.97–2.33)		0.009
White bread (slice)							
Cases	100	79	81		61	51	
RR (95% CI)	1.0	1.22 (0.91–1.65)	1.60 (1.19–2.14)		1.35 (0.98–1.86)	1.51 (1.06–2.16)	0.001

*Values add to less than the total number of subjects because of missing responses for specific foods. CI denotes confidence interval.

†Denotes collapsing adjacent categories.

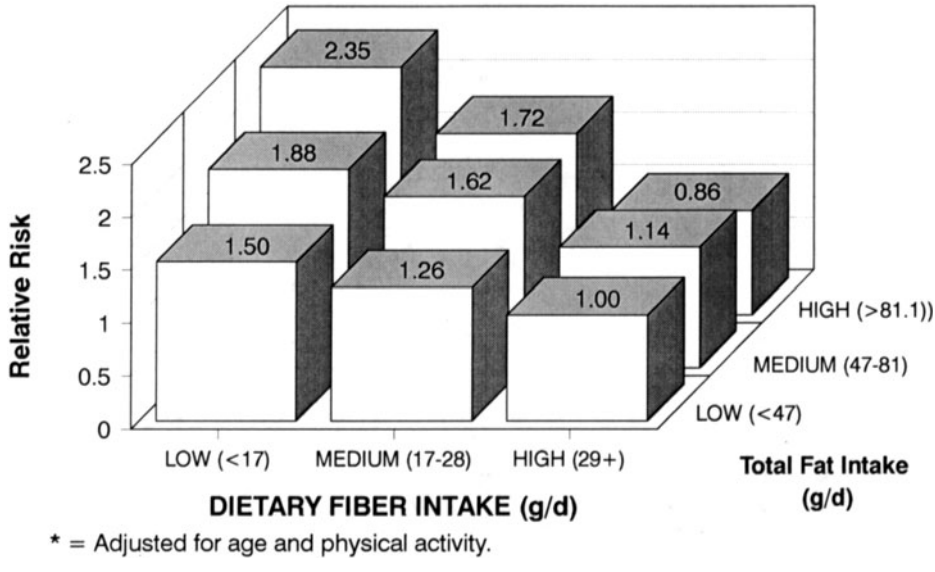

Figure 1. Relative risk* of symptomatic diverticular disease by levels of energy-adjusted dietary fiber and total fat intake.

Using a multivariate model controlling for age and energy-adjusted total fat, we examined the risk of diverticular disease among the subjects classified jointly according to their intake of dietary fiber, and physical activity level. For men in the lowest quintile for both dietary fiber and physical activity (as compared with those in the opposite extreme), the relative risk of diverticular disease was 2.56, 95 percent CI, 1.36 to 4.82 (Fig. 3). The

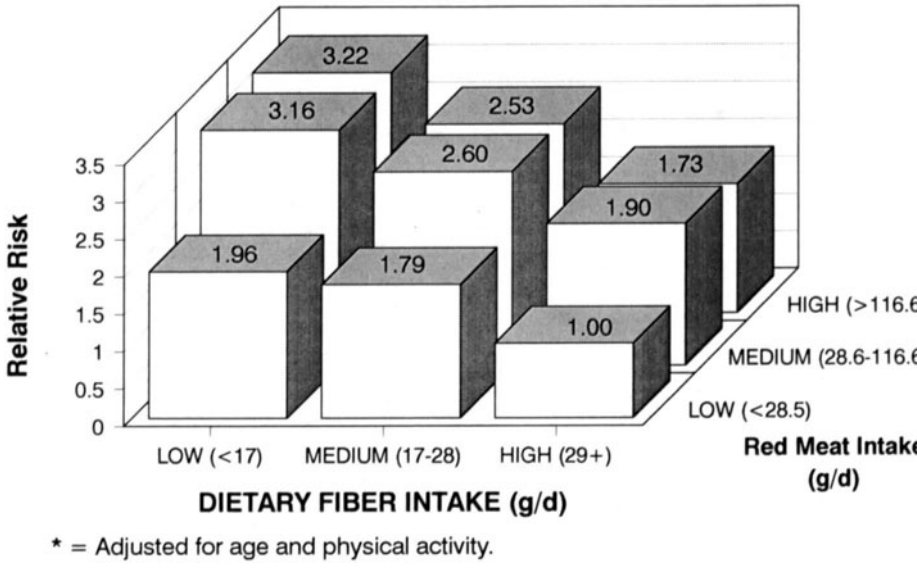

Figure 2. Relative risk* of symptomatic diverticular disease by levels of energy-adjusted dietary fiber and red meat intake.

Table 6. Relative risk (RR) of symptomatic diverticular disease by physical activity, non-vigorous, vigorous activity levels

	Category					
Variable	1	2	3	4	5	P value for trend[†]
Total physical activity (median of total MET)[¶]	0.9	4.8	11.3	22.6	46.8	
RR[*] (95% CI)	1.0	0.89 (0.67–1.18)	0.67 (0.49–0.91)	0.68 (0.50–0.92)	0.57 (0.41–0.79)	0.0002
Multivariate RR[‡] (95% CI)	1.0	0.91 (0.68–1.21)	0.71 (0.52–0.97)	0.74 (0.54–1.01)	0.63 (0.45–0.88)	0.008
Non-vigorous activity (median of total MET)[§]	0.1	1.4	3.4	7.6	20.8	
RR[*] (95% CI)	1.0	1.13 (0.83–1.55)	0.78 (0.56–1.10)	1.06 (0.77–1.46)	0.93 (0.67–1.30)	0.41
Multivariate RR[‡] (95% CI)	1.0	1.15 (0.84–1.58)	0.79 (0.56–1.12)	1.09 (0.79–1.49)	0.93 (0.67–1.69)	0.65
Vigorous activity (median of total MET)[‖]	0	3.5	15.0	41.0		
RR[*] (95% CI)	1.0	0.74 (0.57–0.97)	0.79 (0.61–1.03)	0.53 (0.37–0.75)		0.001
Multivariate RR[‡] (95% CI)	1.0	0.78 (0.60–1.02)	0.88 (0.67–1.15)	0.60 (0.41–0.87)		0.01

[*]Adjusted for age. CI denotes confidence interval.

[†]Test for trend was calculated by using the median total MET value of exercise in each category as a continuous variable in a multiple logistic regression.

[‡]Adjusted for age, energy-adjusted dietary fiber and energy-adjusted total fat.

[§]Included flight of stairs climbed, and walking or hiking outdoors (including walking at golf).

[‖]Included running (10 minutes/mile or faster), jogging (slower than 10 minutes/mile), lap swimming, tennis, squash or racquetball, calisthenics or rowing, bicycling (including stationary machines).

[¶]Total MET = sum of the average time/week spent in each activity × MET value of each activity.

$$\text{MET value} = \frac{\text{caloric need/kg body wt/hour during activity}}{\text{caloric need/kg body wt/hour at rest}}$$

[**]Adjusted for age, physical activity, energy-adjusted dietary fiber and energy-adjusted total fat.

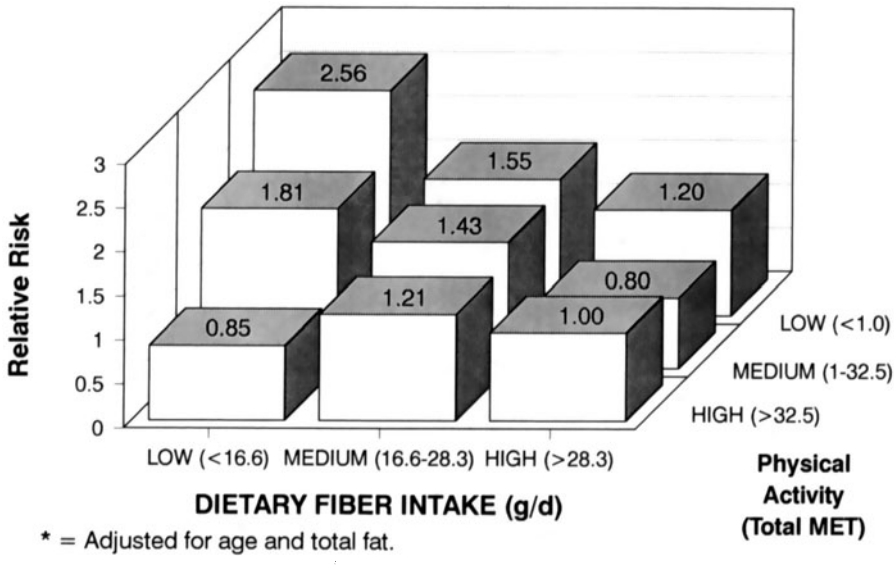

Figure 3. Relative risk* of symptomatic diverticular disease by levels of energy-adjusted dietary fiber intake and physical activity.

association between low level of physical activity and risk of diverticular disease was strongest among men with low intake of fiber. However, the interactions between total or vigorous activity and dietary fiber were not statistically significant when examined in a multivariate model that also included age and energy-adjusted total fat (for interaction, $p = 0.33$ with total physical activity; $p = 0.38$ with vigorous activity).

DISCUSSION

These prospective data support the hypothesis that high intake of dietary fiber reduces the risk of diverticular disease. These data also suggest, that physical activity in general, and vigorous activity, in particular, reduce the risk of diverticular disease. Risks were particularly elevated among men with the combination of high intake of total fat or red meat, low physical activity and low consumption of dietary fiber. To our knowledge, total intake of fat or its different dietary sources, and the intake of red meat have not been considered in previous studies, except in a study in Greece (10). Also, a prospective assessment of physical activity has not been considered in previous studies of diverticular disease.

In our data, fiber from fruit and vegetable sources, and not from cereal sources, was associated with a reduced risk of diverticular disease. Fiber from unprocessed cereal grains (mainly bran) is used in the treatment of diverticular disease, because it increases water holding capacity, decreases fecal transit time, and increases the fecal output, and hence reducing constipation which is commonly associated with diverticular disease. In our study several of the dietary sources of cereal fiber were highly processed and are expected to have a minimal mechanical effect on colonic transit or pressure. Coarse milled bran was repeatedly shown to be more beneficial for colonic functions than finely milled bran (39–41). This observation appears to be related to coarse bran physicochemi-

cal properties on fecal bulk, fecal water and its influence on colonic transit and pressure. Despite being chemically identical, fine bran provided none of those benefits (39–41). Dietary fiber is often a mixture of soluble and insoluble fiber (42). In our data, the insoluble component of fiber was strongly associated with a decreased risk of diverticular disease, and this association was particularly strong for cellulose. Insoluble fiber is the major dietary fiber fraction, and overall, fruits and vegetables tend to be higher in cellulose than cereal (43,44). This might partially explain the observed beneficial effect of fiber from fruits and vegetables in our study. Cellulose comprise approximately one third or less of the total fiber in most foods except for legumes, in which it is about half (44). In fruits and vegetables, cellulose represented an average of 30+% and >50% of the insoluble fraction respectively (44).

Soluble fiber is metabolized by colonic bacteria to a greater extent than insoluble fiber, and hence have little effect on stool weight (42,43). However, ferementable fiber (mainly from fruits and vegetables) can increase fecal output by stimulating microbial growth. As a result of the stimulation of bacterial growth, dietary fiber is degraded to short-chain fatty acids (SCFA) and other products, such as methane, hydrogen, and carbon dioxide. Luminal SCFA are recognized now as an important fuel source for the colon (45), particularly in the distal colon, which is the most common site for diverticular disease. However, some 60+% of ingested cellulose appears in human feces (46), which indicates that insoluble fiber is fermented to some degree since SCFA are not abolished when the diet contains insoluble fiber such as cellulose or wheat bran (47). Also, in an animal study, it was observed that insoluble fiber may modify the proportion of SCFA that are produced (48).

Fiber intake from its different dietary sources is correlated with consumption of several micronutrients that may also be associated with lower risk of diverticular disease. Indeed, several such nutrients (e.g. potassium, beta-carotene, vitamin C, and magnesium) were all inversely related to the risk of diverticular disease. However, these inverse associations were no longer significant when each of these nutrients and energy-adjusted dietary fiber were entered in a multivariate model with age, energy-adjusted total fat, and physical activity, while, the inverse association of dietary fiber and diverticular disease was not appreciably altered.

An association of red meat with diverticular disease was also reported in an earlier case-control study in Greece (10). In our study, the intake of chicken and fish meat was not appreciably associated with the risk of diverticular disease nor indeed was animal protein, which have not shown to have any consistent effect on stool weight, or transit time (49). This supports the suggestion that, under the influence of red meat intake, bacteria may produce a "toxic metabolite" or a spasmogen that weakens the wall of the colon and favours the formation of diverticula (50). Whether red meat may influence diverticular disease by stimulating the release of bile acids through mechanisms unrelated to its fat or protein content, and whether there is biological interaction between dietary fiber and red meat needs to be explored. It was shown that patients with diverticular disease had a higher average daily fecal bile acid output than controls, which was reduced to near control levels after the administration of bran (51). These changes led some to believe that diverticular disease is a motility disorder which may be reversed by a higher intake of dietary fiber (52). The finding that red meat might be involved in the etiology of diverticular disease may help to explain why vegetarians have a lower prevalence of diverticulosis than expected on the basis of their fiber intake (12).

A limited number of studies have addressed the effect of physical activity on colonic function, despite the widely held belief of a beneficial effect of exercise on gastrointestinal

functions. A reduction in transit time was the consistent finding in most of the studies addressing the effect of exercise on colonic function (53,54), although some did not observe such an effect (55). An increase in colonic motor activity has been postulated; however, the exact mechanism of this effect is still not clear. Some authors have suggested that the effect might be multifactorial, involving hormonal, vascular, and mechanical aspects (53). Whether physical activity may influence diverticular disease through any of these or other mechanisms, and whether specific exercise (eg. jogging and running) has specific effects on colonic functions needs to be explored (20).

Considering that the majority of diverticular disease cases are asymptomatic, and that diagnosis is made when affected individuals develop symptoms, we explored the possibility that the associations we observed were due to detection bias which might be related to their level of dietary fiber intake or physical activity level. For example, people with low dietary fiber intake or who are not physically active might have more abdominal symptoms, and hence more endoscopies, in which diverticulosis is diagnosed. However, the opposite was found; that is those participants in the HPFS who reported having endoscopies for abdominal symptoms were in fact eating more dietary fiber, less total fat, and were more physically active. We therefore conclude that detection bias is unlikely to have appreciably influenced our results.

Because we relied mainly on self report rather than obtaining complete medical records for all the positive respondents, we could not exclude right-sided diverticulosis from our cases. However, among the 108 cases for whom we obtained medical records, exclusive right sided diverticulosis was present in less than 4 percent, which is what is expected in a US population (34). This is in contrast to the Orient where diverticular disease is predominantly right sided (56–58).

Biased recall of diet or physical activity was unlikely, because the dietary and physical activity data were collected before the diagnosis of diverticular disease. We recognize that as in almost every study some degree of misclassification is inevitable. However, the prospective design of this study means that any misclassification would be random with regard to case status, and hence would tend to attenuate any association. Our findings are most directly generalizable to non-vegetarian US men 40 years and older. Apart from reports of a higher preponderance of diverticular disease in women (2), we have no reason to believe that the association we observed in men would be different for women. However, future studies should explore any possible differences in the relations between dietary factors, physical activity and the occurrence of diverticular disease due to gender. Considering that the majority of diverticular disease cases are asymptomatic, we still need to understand the mechanism of why the diverticula become symptomatic. We further need to assess whether specific factors are important for the formation of diverticula, and whether different factors contribute to the symptomatic presentation.

CONCLUSIONS

Our findings provide evidence for the hypothesis that a diet high in dietary fiber mainly from fruits and vegetables, and low in total fat decreases the risk of diverticular disease. The insoluble component of fiber was strongly associated with a decreased risk of diverticular disease, and this association was particularly strong for cellulose. These data are also compatible with the possibility that other factors in red meat unrelated to its protein or its fat content can increase the occurrence of this disease. Our findings also suggest that increasing levels of physical activity reduce the risk of diverticular disease. Risks

were particularly elevated among men with the combination of high intake of total fat or red meat, low physical activity and low consumption of dietary fiber. In summary, high fiber diet, along with physical activity may be an important factor in the prevention of diverticular disease.

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DIETARY GUIDELINES/RDA/DAILY VALUE WORKSHOP

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This Workshop Developed 5 Recommendations

1. A recommended dietary allowance (RDA) should be established for total dietary fiber (TDF) in the next edition of the National Academy of Sciences Report on recommended intakes. Sufficient scientific evidence is available to warrant advice on, at least, the upper and lower limits of fiber intake consistent with health. An RDA needs to be established for fiber intake to guide the development of Daily Values, used in the Nutrient Facts on food labels as well as the development of Dietary Guidelines for Americans.

   The workshop recommended that criteria such as stool bulk, energy contribution and impact on the colonic environment be considered in establishing an RDA for fiber. Stool bulk relates consumption of dietary fiber to a specific aspect of gastrointestinal function and is useful to define a minimum amount of fiber needed in the diet. Its primary disadvantage is the variability inherent in the measurement. Fermentation of fiber in the large intestine is known to provide energy; however, in most subjects, who consume less than 12 g/day, the amount is probably of little significance. Dietary fiber is the primary substrate for microflora in the large intestine, hence modification of the colonic environment by dietary fiber is an important physiological function. To use this criteria for establishment of an RDA, research relating concentrations of short chain fatty acids and bile acids, populations of bacteria, pH in the large intestine and transit time to dietary fiber consumption and gastrointestinal health is needed. Studies examining disorders of the large bowel, such as diversion colitis, are beginning to provide this type of information.
2. It is inappropriate to establish either an RDA or Daily Value (DV) for soluble and insoluble fiber at the present time. Perhaps the greatest concern regarding our ability to make such recommendations was expressed for soluble dietary fiber. While official methods have been developed for their analysis, not all sol-

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uble fiber fractions have similar physiological functions. Thus the analytical methods, while reliable for measuring a specified fraction of fiber in foods, do not predict physiological response. In the absence of an RDA for soluble fiber intake, Dietary Guidelines need to encourage food choices that will include a variety of dietary fibers.

3. Sufficient scientific evidence is available to classify dietary fiber as a nutrient, which includes compounds that must be consumed in the diet to maintain health. For nutrition labeling purposes the Food and Drug Administration (FDA) includes dietary fiber as one of the mandatory nutrients but considers it as a food component. Elsewhere, it is described as "other compounds" in food that are associated with health. As discussed in recommendation one, fiber can provide calories based on its fermentation in the large intestine. Short chain fatty acids are considered important nutrients for the health of the large intestinal mucosa and polysaccharides are the primary substrate for microflora. In addition, dietary fiber is the main dietary determinant of stool bulk. Recommendations in the UK suggest that a minimum stool bulk of about 125 g/day is needed for normal gastrointestinal function.
4. Educational efforts need to be more effective in communicating to consumers how to increase their fiber intake. In spite of the use of the Daily Value on the food label, most consumers still do not know how to increase their total dietary fiber intake. Estimated fiber intake in the USA for adults is 10–12 g/day, which falls short of the DV set at 25 g/day. Dietary Guidelines can be useful for encouraging consumption of the types of foods that are good sources of dietary fiber. The Food Guide Pyramid, Dietary Guidelines and Daily Values all support an increase in foods that provide fiber and can be used as educational tools.
5. Specific recommendations for fiber intake in children and youth (age 2–18 years) need to be developed. Expressing recommendations for fiber intake relative to caloric intake (i.e. per 1000 kcals) is suitable for the adult population but not for children. A new recommendation, which could serve as a guide for future recommendations for children, was made in a meeting report for a Conference on Dietary Fiber in Childhood, held in New York on May 24, 1994.

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SOLUBLE DIETARY FIBER WORKSHOP

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The participants of the soluble dietary fiber workshop were a diverse group with a wide range of reasons for attending. Two issues of interest to most members of the group were the analysis of all components in the dietary fiber complex (not just the soluble fraction) and the physiological effects of soluble fiber. A major portion of our time was spent reviewing methods of fiber analysis (1,2). We did not have a good discussion of our topic—what is the most meaningful way to measure soluble dietary fiber, nor did we reach a consensus on other questions raised by the participants. These other questions included how to present soluble fiber in data bases, g/100 g or serving, or % of total fiber; and what should be the approach to measuring specific components of the fiber complex, such as polydextroses, inulin, oligosaccharides and undigested starch.

How to measure physiologically-relevant soluble dietary fiber remains a dilemma. This is because analytical methods have been developed without knowing the relationship between values generated by those methods and the fraction of total fiber that is soluble in the stomach and small intestine. Yet, most investigators in the field agree that a physiologically-relevant analysis is what is needed. To date, **no in vitro method has been developed that mimics the exquisite and efficient process of monogastric digestion.** Available data suggest that a portion of the insoluble fraction would be solubilized during movement through the stomach and small intestine, but the extent of solubilization appears to vary among foods (3,4).

Methods of fiber analysis define soluble dietary fiber as the material or carbohydrate that is solubilized during analysis and recovered by precipitation in ethanol (1,2). The dilemma of what is soluble dietary fiber is complicated by the fact that the yield of soluble fiber varies with the method used for fiber analysis, even when the same sample is analyzed (5–9), because fiber solubilization depends on the extent of disruption of the plant cell wall matrix (Table 1). Disruption of the plant matrix is more extensive during those fiber analysis procedures that use more degradative enzymatic steps. A further complication is that the term "soluble dietary fiber" also reflects a concept that delineates a particular set of responses to fiber ingestion. Thus, a physiological definition of soluble dietary fiber generally infers demonstrable effects on the upper gastrointestinal tract and rapid fermentation when it reaches the large intestine.

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Table 1. Analytical measures of soluble dietary fiber

	% of total DF				
	Uppsala[a]	AOAC[b]	Anderson[c]	Mongeau[d]	Englyst[c]
Fruits	19	32	38	43	53
Vegetables	14	24	32	41	49
Grains	23	23	32	56	36
Legumes	11	14	25	33	39

Data sources: [a]ref 10, [b]ref 11, [c]ref 12, [d]ref 13, [e]ref 14.

The following proposal was presented by the workshop chair at the session of workshop summaries. The aims of this proposal are to provide a focus for work on soluble dietary fiber analysis and to integrate efforts that are already underway to develop a scheme for carbohydrate analysis that includes biologically available as well as unavailable carbohydrate. The first aim addresses the purpose of the workshop. The second aim addresses several related issues raised by the workshop participants, e.g., analysis of specific fiber components, oligosaccharides and resistant starch.

As an **interim** solution, it is proposed that soluble fiber be measured analytically using the Mongeau (13) or AOAC (11) method. These two methods appear to yield soluble fiber values that are in the middle of the ranges of data that have been obtained using widely different methods (Table 1). These two methods also are accepted analytical standards in the USA or Canada. While the data obtained by Anderson (12) also appear to be in the middle range, this procedure is not being actively pursued.

It is proposed that the **final** methods for analysis of carbohydrate be developed following the schemes for the analysis of available and unavailable carbohydrates developed by David Southgate (15,16) 25 years ago! These schemes are innovative and conceptually correct. Professor Southgate's approach was difficult to implement at the time it was first proposed because precise methods for measuring specific carbohydrates were not available at that time. Suitable methods have been developed in the past 25 years and it is now possible to fully appreciate the foresight inherent in these fraction schemes.

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WORKSHOP REPORT

Fiber and CHD Management

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There were about 20 participants in this workshop, with 15 being from industry. As an introduction, I suggested a number of areas of potential discussion. The first was to consider the mechanisms by which dietary fiber reduces the risk for coronary heart disease (CHD). Previously in the symposium we were presented with evidence from epidemiologic studies that fiber reduces CHD risk. One of the ways fiber could do this is by reducing blood lipids, and evidence about this had been presented in the symposium earlier. Thus, we could consider the effects of fiber on blood lipids, and particularly the importance of the fall in HDL cholesterol in some studies, or the effects of dietary fiber on serum triglycerides and apolipoproteins. A number of speakers in the symposium presented data about the mechanisms by which fiber lowers serum cholesterol, including altered bile acid metabolism, short chain fatty acid production, insulin or other hormone effects, altered fat absorption or possibly other mechanisms. However, we also heard evidence that fiber reduces CHD risk independent of any effect on serum cholesterol, which suggests that fiber might be acting in other ways, such as reducing blood clotting or altering vascular biology.

Another proposed area of discussion was about practical issues such as the type of fiber to use, whether the fiber should be taken whole or in supplements, whether there are responders and non-responders to fiber and whether there is any difference between the effect of fiber in men and women. Finally, it was suggested that we could discuss regulatory issues such as the nature and type of claims on food packages, the design of studies which would be required to support claims, and whether claims should be product specific or based on an analysis of dietary fiber content.

1. FIBER MECHANISMS

There was not very much enthusiasm for discussion in this area. However, with respect to blood clotting, the point was made that a high fat diet increases the propensity of

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the blood to clot, and this possibly could be related to the concentration of triglycerides in the blood. Thus, if fiber has an effect on hemostatic mechanisms, it may be acting by altering postprandial lipemia. This led us on to talk a little about the fact that there are relatively few studies looking at postprandial fat metabolism in relation to dietary fiber. Most of them have used relatively large amounts of fat in test meals. The effects of fiber at lower, and more realistic levels of dietary fat on postprandial lipemia are not known, but possibly could be different from the effects seen with a high fat test meal.

2. PRACTICAL ISSUES

A number of questions came up here. One was: How is fiber to be given? Participants in the workshop had experience with oat gum, psyllium and mixed fiber diets. In all cases, when fiber lowered serum cholesterol, it was given either twice or three times daily with meals. There has only been one study known to the participants where the method of fiber adminstration was studied, and it showed that psyllium given twice daily with meals reduced serum cholesterol significantly, whereas when the same amount of psyllium was given between meals it had no effect[1]. The total fiber dose varied from about 7 to 20 grams per day. It was not known whether a once daily dose of fiber would be effective. More studies looking at the efficacy of different methods of administration of fiber might be helpful.

The point was also strongly made that there are few data about the effects of fiber on blood lipids in women, and it was generally agreed that more studies are needed. However, it was also recognized that studies in women are difficult because of effects of the menstrual cycle on lipids and gut physiology, the difference between the premenopausal and postmenopausal states, and the effects of hormone replacement therapy on blood lipids. The postprandial lipemic response to a standard oral dose of fat is lower in women than men because lipoprotein lipase activity is higher in women.

It was recognized that there are responders and non-responders to fiber, although in most studies it cannot be determined whether this is due to random variation, or to consistent effects. There are a few studies showing that changes in bile acid excretion are related to the effect of fiber on blood lipids[2] and bile acid kinetics differ in responders and non-responders. Thus, bile acids might provide some of the explanation for inter-individual variation in response of blood lipids to fiber. More studies on the interaction between diet and genetics could also provide data about the sources of variation in response among different individuals.

3. FIBER CLAIMS

Discussion in this area was lively. It was noted that nutrition studies often recieve much publicity and media "hype", and that sometimes this can have a damaging effect, as with the Swain paper[3] showing a lack of effect of oats in young women with normal cholesterol levels. It took a long time for oat bran to regain acceptance as being able to lower serum cholesterol. The proposed regulation for claims about oat bran-containing products is based on a the measurement of oat bran in the product. However, there was concern amongst the group that this kind of definition might not necessarily guarantee effectiveness. Measurement of the quantity of oat bran does not guarantee that the oat bran will be viscous because the analysis does not indicate differences in molecular weight; ony high molecular weight beta-glucan is

viscous. In addition, the solubility and hence viscosity of the beta-glucan can vary depending upon the manufacturing process. Thus, there is a dilemma as to whether every different product needs to be tested. Common sense would dictate that not every new oat bran product needs testing, but common sense may not be that common! It would be good if a surrogate measure of response was available, such as viscosity, but further studies are needed to find if such in vitro measures are useful.

It was pointed out that, in submitting a product for a claim, the FDA is looking for an active ingredient which is well defined and for which a dose response can be demonstrated. The nature of the studies required varies depending upon the nature of the claim sought and how the product is to be marketed. FDA criteria differ for foods versus supplements. For oat bran, out of the 40–50 studies done, only about 3 or 4 were designed in such a way that they met the FDA criteria for substantiating a claim; including features such as a baseline run-in period, being controlled and randomized, and being of sufficient length and number of subjects. Thus, it may be more economical to plan a smaller number of larger but individually more expensive studies, than a larger number of small studies.

An area in which fiber supplements may differ from fiber naturally present in foods is in the area of potential side effects. For example it was pointed out that soluble fiber can reduce postprandial responses of beta-carotene and vitamins A and E which may indicate a reduced bioavailability. On the other hand, fiber rich foods may contain high amounts of beta-carotene and so some reduction of bioavailability would not be critical in terms of the overall amount absorbed. However, the interpretation of reduced serum concentrations of vitamins postprandially is not necessarily straightforward. For example, the reduction in postprandial blood glucose response by fiber is not due to a reduction in the overall amount of glucose absorbed, but appears to be due, rather, to a reduction in the rate of absorption[4]. It was also pointed out that there no adverse effects on serum vitamins and minerals of taking psyllium at 10g per day.

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WORKSHOP ON ANIMAL MODELS USED IN FIBER RESEARCH

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Participants in the animal model workshop discussed the advantages and disadvantages of existing models for studying the fate of dietary fiber. Knowledge of both the pros and cons will allow us to use animal models in a more effective way of learning the details of fiber's effects in the gastrointestinal tract. The choice of an animal model depends entirely on the question being asked. Animal models can answer mechanistic questions and provide tight control of diet and other environmental conditions. Animal models have found widespread use in studies of nutrient absorption and utilization. Other aspects of normal physiology studied in animals and affected by dietary fiber are transit time and gastrointestinal mucosal cytokinetics, hyperlipidemias, and cancer.

1. CANCER

A major use of animal models in research on dietary fiber has been in the study of colon cancer. One weakness of cancer studies in animals is that there are no good spontaneous models of colon cancer. But it should be remembered that even the human would not be considered a good model to study spontaneous colon cancer simply because the incidence rate is too low to conduct a primary prevention trial (19 per 100,000 U.S. males in 1992). The *Min* (multiple intestinal neoplasia) mouse is a transgenic animal that develops hundreds of intestinal polyps, primarily in the small bowel, and dies from intestinal bleeding before six months of age. The key hurdle to developing a genetic model of colon cancer is that there are no known colon-specific promoters to cause expression of an oncogene in that organ. Most other, so called spontaneous colon cancers develop as a result of colitis as seen in the cotton-top Marmoset and the Wistar-Kyoto rat. In the absence of better models, the chemically-induced colon tumor model is the most commonly used. While one can argue that humans are not exposed to large doses of dimethylhydrazine or other colon-specific carcinogens, it is clear that we are exposed to a multitude of chemicals (some of endo-

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genous origin) that are capable of inducing cancer of the colon. A few studies have observed beneficial effects of high levels of wheat bran on chemically-induced mammary cancer in rats but tumors at other sites have not been studied.

2. MONOGASTRICS

It seems self-evident that animals with a stomach that is anatomically close to that of humans would be the best model but there are many factors to consider. The most commonly used rodents — rats, mice, gerbils, hamsters, and guinea pigs — have advantages of small size and cost as well as ready availability. Nyman and Asp showed that the adaptation period to dietary fiber is similar in rats as it is in humans. Coupled with similar transit times, this makes the rat a potentially useful model. There are clear differences in the structure of the large intestine as these animals have a large discrete cecum and the colon holds pelleted feces. Rabbits are also used in fiber studies, particularly when changes in lipids and atherosclerosis are of interest. Swine are sometimes claimed to be "closer" anatomically and physiologically to humans than other animals; sampling size is not a limiting factor in these animals as it may be in small species.

3. RUMINANTS

These species are natural flow-through fermenters. In addition, the large bowel has been likened to a rumen since the microbial processses in each are quite similar. Many of the seminal discoveries on anaerobic microbiology and gut responsiveness to metabolites have come from studies with ruminanats. They have been used extensively to study fiber degradation by bacteria, especially in fistulated animals. However, they have clear limitations in extrapolating to the human situation.

4. RELEVANT ANIMAL CHARACTERISTICS

The size of a particular animal, and its housing requirements, are the biggest determinant of the cost of using that species in experiments; this can be a determining factor in choosing a model. However, the factor that should be the most important is the animal's anatomy and physiology as compared to those of the human. The size and shape of the cecum are factors to consider. For example, it should be obvious that rodents have evolved to consume a high fiber diet do the large, saccular nature of their caeca. The absence of a gall bladder in species such as the rat and Chinese hamster is a factor in some studies of dietary fiber. The fact that fermentation of fiber can occur in the forestomach of the hamster renders this animal less useful for some aspects of fiber studies. Although the gerbil is being touted as a replacement for more commonly used rodents, this species is a desert animal and drinks very little water, which can have an effect of hydration of intestinal contents and subsequent fermentation. The overall colon shape and length determine if a animal excretes feces in pellets or in larger, variable size stools. An additional factor on how an animal adapts to changes in dietary fiber is whether it evolved as an herbivore or omnivore. One should also be aware of the fact that most rodents practice coprophagy and that it is virtually impossible to eliminate this. In fact, rabbits depend on this for proper nitrogen and vitamin assimilation.

There are differences in meal patterns between most species and the way humans usually eat which may be a factor in studies of fiber. There are potential differences in diurnal patterns, gender, genetics (inbred versus outbred strains), and growth. All of these factors have either been demonstrated, or have potential, to modify the response to dietary fiber.

The bacterial flora associated with each species differs and this may be a significant factor in responses to changes in dietary fiber. Germ-free animals have been used for a number of studies but there are several problems with these creatures. Strangulation of the intestine is common, suggesting a lack of neurogenic stimulus in the large bowel that is unrelated to bulk in the colon. Axenic animals, with defined flora such as from humans, have great potential for examining the effects of specific bacterial populations on the fate of dietary fiber in the intestine. In addition, there is considerable potential of learning more about probiotics using animal models.

5. DIET CHARACTERISTICS

The basal diet can make a large difference in observed effects. Clearly the fiber content plays a role as does the type of basal diet. While purified diets are the "gold standard" they are probably the least relevant physiologically. A critical question is what should be the fiber content of control and experimental diets? It seems clear that a fiber-free diet is not optimal for rodents; they eat bedding and/or their hair and their colons atrophy when maintained for months on such a regimen. Undoubtedly, the interactions of macronutrients has an effect on the effects of dietary fiber in the diet.

6. *IN VITRO* MODELS

Although not strictly animal models, the participants in the workshop discussed this category of investigation since many of these studies utilize tissues from animals and the techniques are of potential application for study of human tissues. The categories of in vitro models that were considered are everted intestinal sacs, strips of isolated gut, crypt culture, and cell lines. One limitation of cell culture work is the fact that all intestinal lines are transformed; primary culture is of use only in very short-term studies and it appears that cells in these studies are dying slowly. Although in vitro work allows sophisticated measurement of a variety of parameters that cannot be done in vivo, a limitation of these systems if the absence of the various feedback mechanisms and the fact that a system has not been developed to model the interaction of fiber fermentation by bacteria with healthy gastrointestinal tissue that maintains its original morphologic arrangements.

7. CONCLUSIONS

The participants of the animal model workshop felt that no system reproduced all the conditions of the human intestinal tract but that these models were invaluable for studying specific aspects of dietary fiber effects. Ready access to intestinal contents at different levels of the gastrointestinal tract is best achieved through use of animal models. Measurement of tissue responses to fiber are also far easier in animals than in humans.

The choice of the model may be critical in determining the outcome of the study and one must be aware of the strengths and weaknesses of each experimental system.

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