Covert manipulation of dietary fat and energy density: effect on substrate flux and food intake in men eating ad libitum[1–3]

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ABSTRACT This study assessed whether human food intake is regulated by negative feedback, directly or indirectly, from carbohydrate stores (glycogenostatic model). Six men were studied on three occasions during 7 d of whole-body indirect calorimetry, throughout which they had ad libitum access to one of three covertly manipulated diets: low fat (20% of energy as fat, 67% of energy as carbohydrate, and 13% of energy as protein; 4.80 kJ/g; LF), medium fat (40% of energy as fat, 47% of energy as carbohydrate, and 13% of energy as protein; 5.59 kJ/g; MF), or high fat (60% of energy as fat, 27% of energy as carbohydrate, and 13% of energy as protein; 7.04 kJ/g; HF). Energy intakes increased with percent fat ($F_{[92, 60]} = 36.7$; $P < 0.001$), producing average daily balances of − 0.27, 0.77, and 2.58 MJ/d during the LF, MF, and HF diets, respectively. Changes in carbohydrate stores were attenuated by autoregulatory changes in carbohydrate oxidation. Carbohydrate balance showed a negative relation to the subsequent day's energy balance ($t = 2.696$; $P = 0.0082$) but explained only 5.5% of the variance. The relation for fat was positive ($t = 5.245$; $P < 0.0001$), accounting for 19.9% of the variance (stepwise regression). LF, lower-energy diets are more satiating than are HF-higher-energy diets, but carbohydrate stores per se did not entirely account for the change that diet composition had on energy intake. This study suggests that protein and carbohydrate have potential to reduce subsequent energy intake whereas there was no apparent reductive effect due to fat. *Am J Clin Nutr* 1995;62:316–29.

KEY WORDS Carbohydrate, fat, energy, food intake, appetite, whole-body calorimetry, humans

INTRODUCTION

Many workers have recently concluded that interindividual differences in the capacity to dispose of an excess energy intake by adaptive thermogenesis are not primarily responsible for differences in body-weight regulation, or for the increased prevalence of obesity in Western society (1). Four recent human overfeeding studies that measured the components of energy balance found no experimental support for the concept of adaptive thermogenesis (2–5). Therefore, major changes in energy balance are likely to be primarily due to differences in the regulation of energy intake (6–8), together with alterations in physical activity. The composition of the diet may be important in influencing regulation of energy intake (7). The data derived from diet-survey studies suggest a positive correlation between energy intakes and body mass index (BMI, or wt/ht^2) (9, 10). Some studies suggest a correlation between dietary fat intakes and percent body fat (9–15). Other studies have demonstrated a relation between reduced dietary fat intake and reduced energy intake (16–19). The importance of dietary macronutrients in bringing about different energy intakes has been highlighted by several laboratory-based studies in humans. Most commonly, high-fat, high-energy density (HF-HE) diets have been found to bring about hyperphagia relative to low-fat, lower-energy density (LF-LE) diets (13, 17, 20–23).

To explain these phenomena, Flatt (7, 24) proposed an elegant model of energy balance regulation. The model views body-weight regulation as a dynamic equilibrium between diet composition [represented by the food quotient (FQ)], the composition of the fuel mix being oxidized [represented by the respiratory quotient (RQ)] and stored, and the amount of energy ingested. Flatt emphasizes that the body's capacity to store carbohydrate is approximately equal to the amount that is oxidized per day, whereas the storage capacity for fat is far greater than that oxidized per day. Although carbohydrate oxidation is highly responsive to intake (25–27), the same is not necessarily true for fat. On a mixed diet, excess energy in the form of fat tends to promote fat storage, and this may predispose people to obesity (25, 28). However, this can only be so if macronutrients have different effects on food intake.

In support of the glycogenostatic model for the regulation of energy balance, Flatt observed a significant negative correlation between a given day's carbohydrate balance and the change in the subsequent day's energy intake in ad libitum feeding of mice. The correlations between the previous day's fat and energy balance and change in food intake relative to the previous day's intake were significant and negative, but the slope for carbohydrate was steeper (7, 24). Flatt argued that these correlations amount to causation and are consistent with

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[2] Supported by a Pfizer Scholarship awarded by Churchill College, Cambridge, UK (to RJS).

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Received February 14, 1994.

Accepted for publication March 20, 1995.

the concept of carbohydrate stores (ie, glycogen) acting as a major lever that feeds back to influence day-to-day food intake in rodents and in humans, although Flatt (personal communication, 1994) suspects that the time period for feedback to occur in humans may be > 24 h. He suggested that, over time, people tend to eat to maintain a given range of glycogen stores and because HF diets displace carbohydrate as a proportion of dietary energy, HF diets will promote hyperphagia.

Studies that have demonstrated a relative hyperphagia in humans feeding on HF diets all give circumstantial support to the concept of a glycogenostatic mechanism driving energy intake (13, 20–23). However, none have mechanistically examined whether carbohydrate balance has a large negative feedback effect on subsequent energy intake. It is not justified to assume that people are eating to maintain a given range of carbohydrate stores or some habitual level of intake (and hence oxidation) simply on the basis of the hyperphagia observed on HF diets. This is particularly the case in view of the fact that the balance of some macronutrients (protein and carbohydrate) is more tightly regulated than fat. To fully examine the glycogenostatic hypothesis, it is suggested here that measured changes in macronutrient balances should be carried out in relation to changes in energy balance.

Recent work (29) aimed at testing the above hypothesis has suggested that manipulating carbohydrate stores by 153 ± 42 g relative to a control diet (2.45 ± 0.67 MJ) (absolute = − 47 compared with 106, respectively), while controlling energy balance, did not affect the ad libitum energy intake of nine men on a subsequent day relative to the control diet. Instead, carbohydrate balance was reestablished by directing more carbohydrate toward storage, and maintaining high rates of fat oxidation throughout the ad libitum day. This study questioned the notion that carbohydrate balance exerts a negative feedback effect on the subsequent day's energy intake.

The present study was designed to examine the effects of increasing dietary fat and energy density on the ad libitum feeding behavior and substrate balance of six men during 7 d of continuous whole-body indirect calorimetry. This allowed an examination of the patterns of feeding behavior in relation to covert changes in diet composition. Thus, it was possible to assess whether, under the experimental conditions imposed, subjects regulated energy intake by monitoring energy balance, carbohydrate balance, fat balance, or none of these variables. It was also possible to determine the strength of potential negative feedback from nutrient balance onto subsequent energy balance.

SUBJECTS AND METHODS

Subjects and experimental protocol

Six healthy male volunteers with a mean (± SD) age of 41.8 ± 10.63 y, weight of 75.1 ± 4.18 kg, and height of 1.76 ± 0.02 m were recruited by advertisement. They were normal-weight, nondepressed, nontrained, healthy men who were not taking any medication. They were given a medical examination before beginning the study. The men were not informed of the true purpose of the study, but were told that the study objective was to examine the repeatability of substrate oxidation and storage measurements in subjects eating identical diets ad libitum. The study was approved by the Dunn Nutrition Unit Ethical Committee and subjects gave written consent.

Each subject was studied three times in 9-d experiments that involved 170 h (7 d) of whole-body indirect calorimetry. This long period in a relatively confined space was made possible by the large respiration chamber, which allowed subjects to comfortably tolerate their experimental environment for long periods. Such considerations are important when ad libitum food intake is being measured. The chamber is described in detail elsewhere (30, 31).

On days 1 and 2 (the equilibration period) subjects were fed a diet designed to match maintenance energy requirements, estimated at 1.4 × basal metabolic rate (BMR). This may have led to a slight underestimate of requirements in this group because all were moderately active men whose likely free-living energy expenditure would be closer to 1.6 × BMR. Indeed, a couple of subjects reported feeling quite hungry during these 2 d. Nevertheless, the dietary protocol was standardized for all subjects. The diet used in the equilibration period comprised 47% of energy as carbohydrate, 40% as fat, and 13% as protein, served in three isoenergetic meals. This composition was identical to the medium-fat (MF) diet of this experiment (**Table 1**). On day 3 of the protocol, subjects entered the calorimeter at 0800, having previously emptied their bladders. During the next 7 d in the calorimeter, they had access to one of the three covertly manipulated diets described in Table 1. The macronutrient composition of each item of the diet, the actual recipes, and the details of the 3-d rotating menu can be obtained by contacting the corresponding author. Dietary energy density increased with percent fat. These diets were referred to as LF (20% of energy as fat), MF (40% of energy as fat), and HF (60% of energy as fat). The order of diets was randomized among subjects.

Diets

Diets were fed as a 3-d rotating menu in which every item was of the same composition but not of the same energy density. This was done to better approximate, within each dietary treatment, the situation encountered in real life. In the calorimeter, food was brought to each subject on request. Food was served within three meal-choice periods: 0800–1130 (breakfast), 1130–1730 (lunch), and 1730–2230 (supper). Within this loose framework subjects determined their own meal times. If the meal request was within 30 min of changeover time, then the next meal could be given. Subjects could forego or request repeats of meals and eat as much or as little of each as they wished. The diets were presented to the subjects in excess, in the form of large casserole dishes that

TABLE 1
Macronutrient composition and energy density of the three manipulated diets[1]

	Low fat	Medium fat	High fat
Fat (% of energy)	19.97 ± 0.002	38.97 ± 0.27	58.70 ± 0.32
Carbohydrate (% of energy)	67.00 ± 0.28	48.61 ± 0.50	29.39 ± 0.06
Protein (% of energy)	13.33 ± 0.22	12.51 ± 0.34	11.96 ± 1.02
Energy density (kJ/g)	4.80 ± 0.48	5.60 ± 0.58	70.40 ± 0.72

[1] $\bar{x}$ ± SEM; n = 15.

typically contained 1.0 kg food. All food items were homogeneous and of a known composition, which was calculated from food tables (32), and were presented in separate containers. This allowed each item to be weighed before and after the meal to the nearest 1 g. The energy content of each item of the diets was checked by bomb calorimetry (Autobomb CBA-301 Series; Gallenkamp, Sussex, UK), which agreed with the food tables to 1–2%. The subjects entirely determined the time, frequency, and quantity of all meals and snacks by ordering food on an intercom system. In this way, the size, frequency, and composition of every meal was continuously monitored. All subjects began on day 2 of the 3-d rotating menu.

The daily routine followed by the subjects was as follows. Subjects rose at 0800 and had 30 min to prepare for the day (wash, shave, dress, etc). Lights were turned off at 2300, with 30 min to prepare for bed beforehand. Subjects cycled at 24.5 W for 30 min from 1000 to 1030 and 1600 to 1630, to increase the level of exercise to a level more similar to that encountered during a relatively sedentary routine outside of the calorimeter. Urine samples were collected every 3 h for Kjeldahl nitrogen analysis.

In addition to the use of energy intake as an index of satiety, a visual analogue rating form was completed by subjects every hour, on the hour, to assess rated hunger, appetite, and fullness and 15 min after each meal to assess the palatability of the food. These questionnaires followed the methodology of Blundell (33) and Hill and Blundell (34).

Indirect calorimetry

Oxygen consumption and carbon dioxide production were estimated by using the rapid-response calculations of Brown et al (35). Energy expenditure was calculated from Weir's equation (36). Substrate oxidation rates were calculated from oxygen and carbon dioxide exchanges and urinary nitrogen excretion by using the values of Livesey and Elia (37) for volumes of oxygen consumed per oxidized gram of protein, fat, and carbohydrate and the associated RQs. The calorimeter and its general running were described elsewhere (30, 31). The gas analyzers were calibrated before every run. The precision and accuracy of the calorimeters were periodically checked by using controlled infusions of pure 80% N_2 and 20% CO_2 and were typically $\pm$ 0.09, $\pm$ 0.04 and $\pm$ 0.008 kJ/min over 0.5-, 1- and 24-h periods, respectively. The analytical precision of 30-min measurements is $\pm$ 0.266 g for fat, 0.500 g for carbohydrate, and 0.405 g for protein (38). The corresponding error values over 24 h would be 9.5 g/d for fat (0.37 MJ) and 20 g/d (0.32 MJ) for carbohydrate oxidation. If a subject were to expend 9.6 MJ/d in the proportions assumed above, this would produce an error of $\pm$ 8.5% of the true value for fat and $\pm$ 7.4% for carbohydrate. The estimates of errors for energy expenditure are much lower, 1–2% (33, 38–40). The major source of calorimetric error originates from calibration of calorimeter ventilation rate, linearity of the carbon dioxide analyzers, and the composition of the carbon dioxide calibration gas. These remained unchanged throughout the course of the study and the same calorimeter was used for all runs. Additional errors may arise from the choice of calorimetric coefficients and the use of average gross-to-metabolizable conversion factors. Thus, whereas errors in the calculation of substrate balance plots may not be insignificant they would have been primarily systematic and would have had little impact on the relative comparison across diets.

Statistics

Analysis of variance was conducted on the intakes, oxidations, and balances of energy, fat, carbohydrate, and protein for the individual 24-h results from each subject by using the GENSTAT 5 statistical program (Rothampstead Experimental Station, Harpenden, UK). The analysis treated menu day, diet, and subject as independent variables and the intake, oxidation, and balance of energy, fat, carbohydrate, and protein as dependent variables. In this context "subject" refers to interindividual variation in response. Regression analysis was conducted on individual 24-h results. Energy intake (dependent variable) was regressed against the previous day's balance of fat, carbohydrate, and protein.

Covert manipulation

The 3-d rotating menu was designed so that there would be three versions of each dish, which corresponded to the three macronutrient manipulations. The success of the covert manipulation was then tested by presenting the three versions at once to members of the Dunn Clinical Nutrition Centre staff. They were then told that one of the three was LF, MF, or HF and asked to evaluate which was which. It was sometimes possible to discern ($\approx$30% of occasions) under these conditions that the HF diet seemed higher in fat. All diets were served to volunteers $\geq$ 1 wk apart, and so a direct comparison by subjects was never possible. None of the volunteers were aware that the diets had been systematically manipulated. This was confirmed by a poststudy interview with each volunteer. During the course of the study, three volunteers made occasional comments about the apparent freshness or lightness of an individual dish, which was different for each subject. One volunteer (subject 4) commented that his chicken and rice stew was more greasy on one occasion (this was indeed a high-fat version). They were asked about this after their study or studies. None of these volunteers had ascribed these differences to the macronutrient or energy contents of the diet. The covert manipulation of the macronutrient content and energy density of the diets appeared to be completely successful.

RESULTS

Intakes

Table 2 gives the mean 24-h intakes of energy and macronutrients for the six subjects on the 7 d of each diet. **Figure 1** shows that the weight of food eaten on each diet was virtually identical and accumulated at a constant rate over time. The mean daily food intakes were 2.10, 2.05, and 2.00 kg/d on the LF, MF, and HF diets, respectively. Subjects differed from each other significantly in the amount of food eaten ($F_{[5, 57]}$ = 27.13; $P < 0.001$). This effect was therefore also apparent for energy and macronutrients.

The simultaneous manipulation of the energy density and fat content of the diet, inherent in the study design, led to a highly significant response in energy intake ($F_{[2, 60]}$ = 43.36; $P <$ 0.001) producing mean daily intakes of 9.03, 10.22, and 12.36 MJ/d on the LF, MF, and HF diets, respectively. There was a significant day effect of the 3-d rotating menu on energy intake

TABLE 2
Average 24-h intakes of energy and macronutrients by the six subjects during the 7 d of each diet[1]

Day	20% Fat				40% Fat				60% Fat			
	Energy	Fat	Carbohydrate	Protein	Energy	Fat	Carbohydrate	Protein	Energy	Fat	Carbohydrate	Protein
						MJ/d						
1	7.88 ± 0.44	1.63 ± 0.08	5.20 ± 0.31	1.09 ± 0.05	9.48 ± 1.08	3.55 ± 0.42	4.58 ± 0.53	1.29 ± 0.14	11.47 ± 0.89	6.79 ± 0.53	3.34 ± 0.28	1.34 ± 0.09
2	9.82 ± 0.52	2.07 ± 0.11	6.42 ± 0.35	1.36 ± 0.07	10.69 ± 0.57	4.18 ± 0.22	5.15 ± 0.28	1.38 ± 0.07	12.54 ± 1.59	7.33 ± 0.95	3.66 ± 0.43	1.56 ± 0.21
3	9.34 ± 1.00	1.90 ± 0.20	6.18 ± 0.67	1.26 ± 0.13	10.31 ± 0.82	3.98 ± 0.3	4.94 ± 0.41	1.37 ± 0.11	11.89 ± 0.91	6.99 ± 0.53	3.53 ± 0.3	1.38 ± 0.09
4	8.75 ± 0.49	1.80 ± 0.09	5.79 ± 0.35	1.19 ± 0.06	9.97 ± 0.74	3.74 ± 0.27	4.79 ± 0.36	1.36 ± 0.11	11.27 ± 0.78	6.58 ± 0.46	3.32 ± 0.23	1.38 ± 0.1
5	9.65 ± 0.75	1.99 ± 0.15	6.33 ± 0.50	1.36 ± 0.10	10.53 ± 0.97	4.1 ± 0.37	5.09 ± 0.47	1.35 ± 0.13	15.55 ± 0.85	9.17 ± 0.52	4.54 ± 0.25	1.84 ± 0.9
6	9.52 ± 0.73	1.94 ± 0.15	6.32 ± 0.49	1.28 ± 0.09	10.55 ± 0.59	4.08 ± 0.22	5.07 ± 0.3	1.39 ± 0.08	11.66 ± 1.08	6.79 ± 0.63	3.35 ± 0.43	1.41 ± 0.13
7	8.24 ± 0.34	1.68 ± 0.07	5.47 ± 0.23	1.13 ± 0.05	10.02 ± 0.89	3.75 ± 0.32	4.84 ± 0.45	1.33 ± 0.11	12.11 ± 0.68	7.15 ± 0.41	3.57 ± 0.2	1.41 ± 0.08
$\bar{x}$ ± SEM	9.03 ± 0.25	1.86 ± 0.05	5.96 ± 0.17	1.24 ± 0.03	10.22 ± 0.29	3.91 ± 0.12	4.92 ± 0.14	1.36 ± 0.04	12.36 ± 0.41	7.26 ± 0.24	3.62 ± 0.12	1.47 ± 0.05
Total	63.39	13.01	41.71	8.68	71.55	27.38	34.46	9.51	86.49	50.80	25.37	10.32

[1] $\bar{x}$ ± SEM.

($F_{[6, 60]} = 3.75$; $P < 0.003$). This was almost entirely due to a larger intake on day 3 of the menu relative to either of the other 2 d ($F_{[1, 60]} = 15.82$; $P < 0.001$). Because the composition of each diet was fixed, this day effect was apparent for each of the macronutrients and will not be discussed further. Mean energy intake, on each diet increased cumulatively at a constant rate (Figure 1), but the slope was different for each dietary manipulation. Within each dietary treatment, however, the rate of cumulative energy intake was virtually constant. Because of this and because the macronutrient composition of each diet was constant, the intake of each individual macronutrient increased cumulatively at a constant rate for each diet. The difference in rates between diets was greater for fat than for carbohydrate (**Figure 2**), because the absolute difference in dietary energy from fat varied by threefold whereas the absolute difference in energy derived from carbohydrate only varied by twofold.

Dietary treatment produced a very large effect on fat intake, which averaged 1.86, 3.91, and 7.26 MJ/d on the LF, MF, and HF diets, respectively ($F_{[2, 60]} = 499$; $P < 0.001$). This was due to both a graded increase in the amount of fat on the different diets and also to a graded increase in energy intake as the fat content increased. Intersubject variation in response to the diets was significant ($F_{[10, 60]} = 2.55$; $P < 0.012$), and subjects tended to treat each diet differently for the same day of the menu ($F_{[12, 60]} = 2.10$; $P = 0.03$). Indeed, diet-day and diet-subject interactions were significant for intake, oxidation, and balances of energy and all macronutrients.

Carbohydrate intake was not tightly regulated. Mean daily carbohydrate intakes were significantly different at 5.96, 4.92, and 3.62 MJ/d on the LF, MF, and HF diets, respectively ($F_{[2, 60]} = 102.35$; $P < 0.001$). Because protein was a fixed proportion of the energy content of the diet, the variance in intake also followed the same trends as for energy. Thus, dietary manipulation produced a mean protein intake of 1.24, 1.36, and 1.47 MJ/d on the LF, MF, and HF diets, respectively ($F_{[2, 60]} = 12.40$; $P < 0.001$).

The relation between the energy density of the food and the energy intakes of the subjects was examined by expressing the average energy density of the three diets relative to the LF diet, having taken account of the proportion in which the different foods were typically eaten. This gave ratios of 1.00, 1.14, and 1.43 for the LF, MF, and HF diets, respectively. These ratios were then compared with the ratios of energy intakes on each diet, relative to the energy intake on the LF diet, to assess whether any compensation for the energy-density difference during the three diets occurred. This gave ratios of 1.00, 1.13, and 1.37 for the LF, MF, and HF diets, respectively. Subjects had an energy intake that was almost directly proportional to the energy density of the diet. Only during the HF diet was the relative energy intake actually lower and only by some 5%.

Energy expenditure and substrate oxidation

Table 3 gives the mean 24-h energy expenditure and macronutrient oxidation rates for the six subjects during the 7 d of each diet. The main factor influencing energy expenditure was interindividual variation in metabolic body size ($F_{[5, 60]} = 295.83$; $P < 0.001$). Energy expenditure increased slightly as subjects overfed themselves with increasing fat content of the diet ($F_{[2, 60]} = 48.79$; $P < 0.001$). Mean daily energy expen-

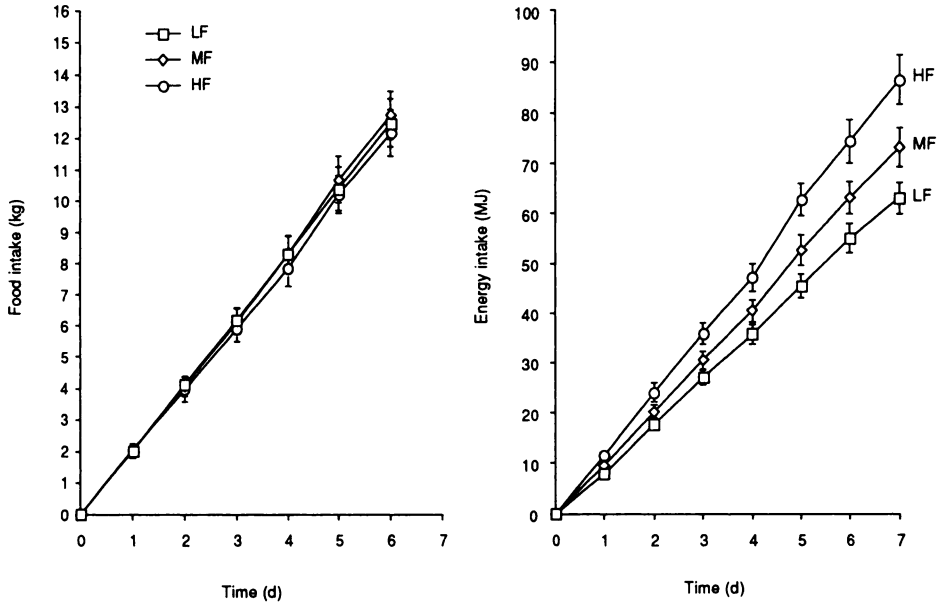

FIGURE 1. Mean (± SEM) cumulative food (kg wet wt) and energy intakes for the six men during the three dietary treatments: LF, low-fat diet; MF, medium-fat diet; HF, high-fat diet.

diture was 9.32, 9.43, and 9.77 MJ/d on the LF, MF, and HF diets, respectively.

As fat intake increased (and carbohydrate intake correspondingly decreased), when subjects switched from the LF to the HF diet, fat oxidation increased ($F_{[2, 60]} = 693.18$; $P < 0.001$). Mean values were 2.72, 3.52, and 5.02 MJ/d on the LF, MF, and HF diets, respectively. This is illustrated in **Figure 3**, which shows the mean (± SEM) cumulative 24-h fat and carbohydrate oxidation for the group on each of the diets. Fat oxidation progressively decreased from days 1 to 7 of the study on all diets (Table 3). This can be related to the increase in glycogen stores and changes in carbohydrate metabolism that occurred on each diet between days 1 and 7.

An increase in the carbohydrate content of the diet increased the rate of carbohydrate intake and hence oxidation (Figure 3), giving values of 5.34, 4.56, and 3.47 MJ/d on the LF, MF, and HF diets, respectively (Table 3). This effect was independent of the amount of energy ingested. Carbohydrate oxidation progressively increased from days 1 to 7 of the study on all diets. The increased carbohydrate oxidation and progressive suppression of fat oxidation on all three diets was probably due to the interactions between the amounts of energy and macronutrients ingested on the three diets. The continually high carbohydrate intakes on the LF diet would probably have saturated carbohydrate stores and led to increased oxidative disposal of carbohydrate, thus suppressing fat oxidation. This can be seen

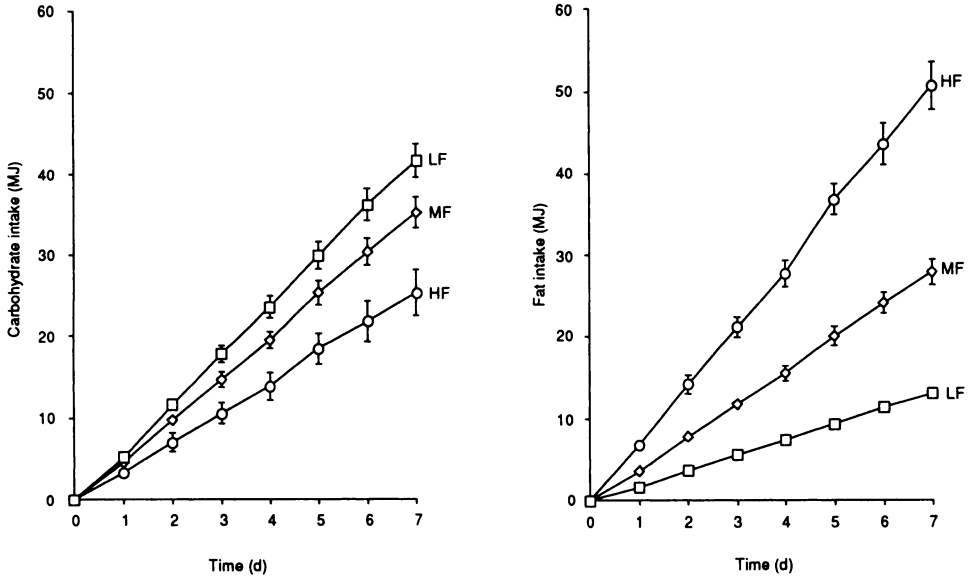

FIGURE 2. Mean (± SEM) cumulative carbohydrate and fat intakes for six men during the three dietary treatments: LF, low-fat diet; MF, medium-fat diet; HF, high-fat diet.

TABLE 3
Average energy expenditure and macronutrient oxidation rates for the six subjects during the 7 d of each diet.[1]

Day	20% Fat				40% Fat				60% Fat			
	Energy	Fat	Carbohydrate	Protein	Energy	Fat	Carbohydrate	Protein	Energy	Fat	Carbohydrate	Protein
						MJ/d						
1	9.34 ± 0.38	3.73 ± 0.26	4.26 ± 0.37	1.35 ± 0.03	9.35 ± 0.39	4.33 ± 0.27	3.68 ± 0.33	1.32 ± 0.07	9.64 ± 0.36	5.48 ± 0.33	2.91 ± 0.15	1.25 ± 0.04
2	9.38 ± 0.29	3.02 ± 0.23	5.07 ± 0.30	1.29 ± 0.09	9.48 ± 0.32	4.00 ± 0.24	4.09 ± 0.40	1.39 ± 0.07	9.68 ± 0.44	5.22 ± 0.34	3.17 ± 0.18	1.29 ± 0.08
3	9.33 ± 0.32	2.86 ± 0.21	5.22 ± 0.31	1.25 ± 0.06	9.43 ± 0.29	3.46 ± 0.34	4.44 ± 0.35	1.53 ± 0.23	9.71 ± 0.35	5.03 ± 0.28	3.37 ± 0.25	1.31 ± 0.07
4	9.26 ± 0.38	2.61 ± 0.24	5.43 ± 0.42	1.22 ± 0.08	9.37 ± 0.30	3.39 ± 0.36	4.72 ± 0.36	1.26 ± 0.09	9.79 ± 0.32	5.12 ± 0.27	3.49 ± 0.26	1.18 ± 0.04
5	9.40 ± 0.38	2.41 ± 0.18	5.68 ± 0.37	1.31 ± 0.07	9.50 ± 0.31	3.31 ± 0.31	4.94 ± 0.28	1.25 ± 0.08	10.07 ± 0.37	5.06 ± 0.40	3.68 ± 0.32	1.33 ± 0.07
6	9.38 ± 0.31	2.37 ± 0.21	5.78 ± 0.33	1.23 ± 0.05	9.47 ± 0.25	3.01 ± 0.29	5.15 ± 0.22	1.31 ± 0.07	9.76 ± 0.40	4.69 ± 0.39	3.81 ± 0.24	1.26 ± 0.12
7	9.17 ± 0.30	2.03 ± 0.27	5.94 ± 0.45	1.20 ± 0.05	9.34 ± 0.30	3.13 ± 0.30	4.93 ± 0.27	1.28 ± 0.08	9.75 ± 0.27	4.53 ± 0.31	3.84 ± 0.31	1.38 ± 0.05
$\bar{x}$ ± SEM	9.32 ± 0.12	2.72 ± 0.11	5.34 ± 0.15	1.26 ± 0.03	9.43 ± 0.11	3.52 ± 0.13	4.56 ± 0.14	1.33 ± 0.04	9.77 ± 0.13	5.02 ± 0.13	3.47 ± 0.10	1.29 ± 0.03
Total	65.26	19.04	37.38	8.85	65.99	24.62	31.95	9.34	68.40	35.13	24.27	9.00

[1] $\bar{x}$ ± SEM.

from the cumulative plots of carbohydrate oxidation (Figure 3) and balance (**Figure 4**). During the HF diet, the high energy intakes would have also led to an elevation of carbohydrate stores, increased oxidative disposal of carbohydrate, and progressively suppressed fat oxidation. This was less pronounced because of the smaller increase in carbohydrate stores on this diet. The extent to which this effect occurred appears not to have been influenced by energy balance, but primarily by carbohydrate balance. This must be the case because the same patterns occurred on all diets, but to an increasing degree as the carbohydrate intake increased. These trends in substrate oxidation may have also been enhanced by the fact that subjects were probably in a mild degree of negative energy balance on the equilibration diet. Thus, at the beginning of the study rates of fat oxidation would have been high. As subjects fed ad libitum and carbohydrate stores increased, these relatively high rates of fat oxidation would have been suppressed.

Protein oxidation was not significantly affected by diet. The average daily protein oxidation rates were 1.26, 1.33, and 1.29 MJ/d on the LF, MF, and HF diets, respectively. However, subjects differed significantly from one another in their protein oxidation rates ($F_{[5, 60]} = 19.25$; $P < 0.001$). Much of this variation is probably due to differences in metabolic body size, because dividing the 24-h protein oxidation by the 24-h energy expenditure produced ratios of 0.13, 0.13, 0.12, 0.16, 0.13, and 0.13 for subjects 1–6, respectively. The high ratio for subject 4 was due to the large amounts of protein (and energy) he ingested and so oxidized.

Table 4 gives the mean 24-h energy and macronutrient balances for the six men on the 7 d of each diet. Energy expenditure was virtually constant from days 1 to 7 on each diet, differing by 0.4 MJ/d between the LF and HF diets. Energy intake increased with increasing percent fat and energy density of the diet. Energy balance was therefore profoundly affected by increasing dietary energy density in the form of fat. Mean daily energy balance was − 0.27, 0.77, and 2.58 MJ/d on the LF, MF, and HF diets, respectively ($F_{[2, 60]} = 36.7$; $P < 0.001$). **Figure 5** illustrates how the dietary manipulation led to a dramatic response in cumulative energy balance. Whereas all subjects showed a relative hyperphagia in relation to increased fat and energy contents of the diet, their absolute values for energy balance varied considerably ($F_{[5, 60]} = 11.08$; $P < 0.001$).

Fat balance (Figure 4) increased markedly with increased fat and energy contents of the diet ($F_{[2, 60]} = 176.59$; $P < 0.001$). The fat balances of the subjects differed markedly from each other as a result of the three diets ($F_{[5, 60]} = 34.43$; $P < 0.001$). Mean cumulative carbohydrate balance (Figure 4) decreased ($F_{[2, 60]} = 3.78$; $P = 0.028$) in response to decreased carbohydrate ingestion when subjects switched from the LF to the HF diet. Average daily carbohydrate balance was 0.62, 0.35, and 0.15 MJ/d on the LF, MF, and HF diets, respectively. Interestingly, the cumulative balance plots for carbohydrate given in Figure 4 show a curvilinear trajectory. Intake accumulated at a constant rate. This shows that for the group, carbohydrate oxidation increased over the first 4 d of the study, and was maintained at a new, elevated level.

Protein balance was tightly regulated over the course of the study and across dietary treatments, although there was a slight tendency for protein balance to increase in parallel with the positive energy balance during the HF diet. Analysis of vari-

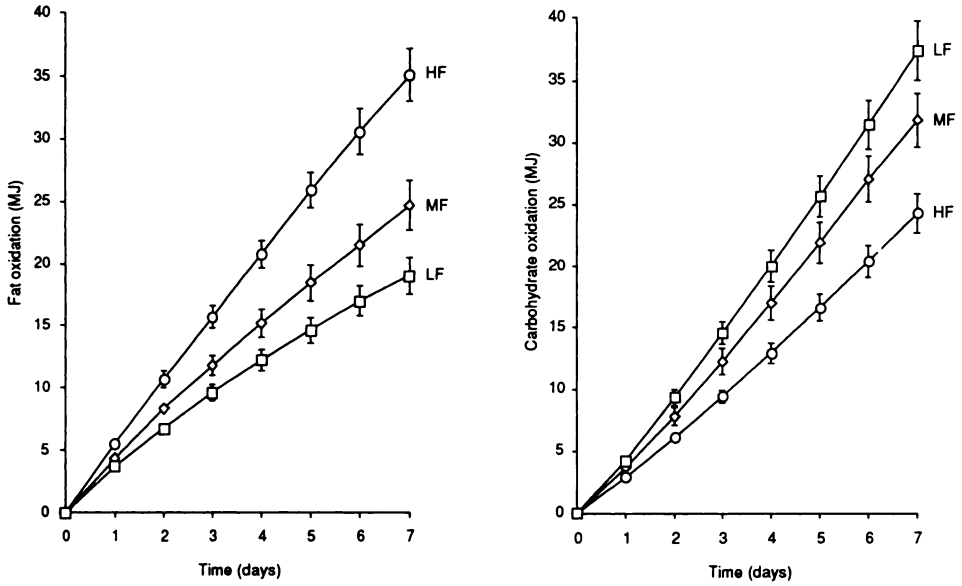

FIGURE 3. Mean (± SEM) cumulative carbohydrate and fat oxidation for six men during the three dietary treatments: LF, low-fat diet; MF, medium-fat diet; HF, high-fat diet.

ance of the 7-d cumulative energy balance showed large differences between the three diets ($F_{[2, 10]} = 80.51$; $P < 0.001$), with mean cumulative energy balances of − 1.89 MJ for the LF diet, 5.36 MJ for the MF diet, and 18.07 MJ for the HF diet; SE = 1.13 MJ.

An analysis of variance of the cumulative carbohydrate balance over the 7 d showed a significant difference between diets ($F_{[2, 10]} = 7.28$; $P = 0.011$), with mean cumulative carbohydrate balances of 4.32, 2.44, and 1.07 MJ (SE = 0.55) for the LF, MF, and HF diets, respectively. Thus, the LF and MF diets produced balances significantly different from zero, whereas the cumulative balance for the HF diet was not significant. Likewise, the cumulative balances for only the LF and HF diets were significantly different from each other ($t_{10} = 3.81$; $P = 0.003$). Thus, here was an unusual situation in which a dietary manipulation had produced an inverse relation between carbohydrate balance and energy balance. Large positive carbohydrate balances were found under conditions of slight negative energy balance.

Body weights

There was a modest, graded increase in body weight of 0.2, 0.5, and 0.9 kg by day 7 on the LF, MF, and HF diets, respectively. The LF diet induced an increase in weight of nearly 0.5 kg by day 3, which decreased to a steady 0.2 kg after day 4. This trend parallels the trend in cumulative carbohydrate balance itself that occurred on each diet. Thus, subjects gained 0.2 kg on average, despite a slight negative energy balance.

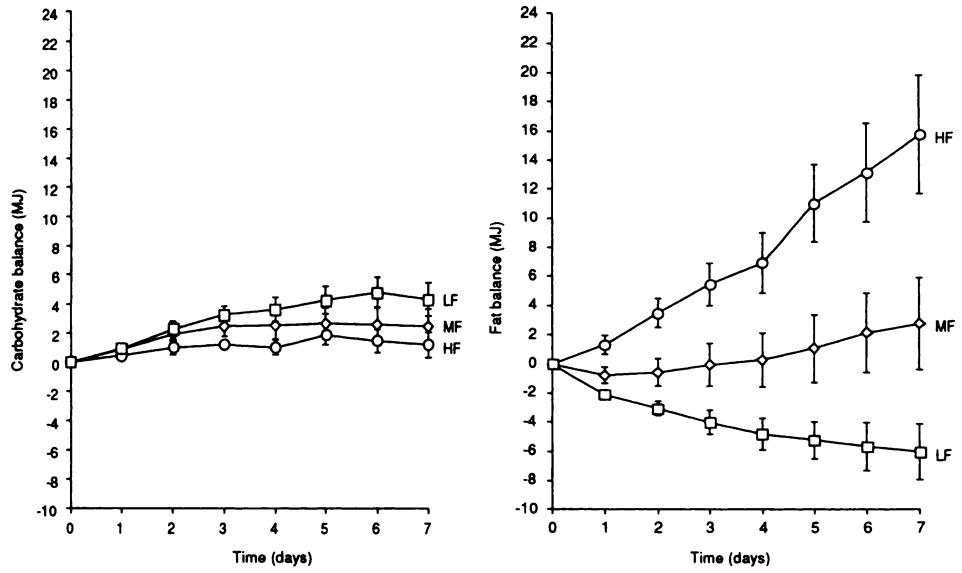

FIGURE 4. Mean (± SEM) cumulative carbohydrate and fat balances for the six men during the three dietary treatments: LF, low-fat diet; MF, medium-fat diet; HF, high-fat diet.

TABLE 4
Average 24-h balances of energy and macronutrients for the six subjects during the 7 d of each diet[1]

Day	20% Fat				40% Fat				60% Fat			
	Energy	Fat	Carbohydrate	Protein	Energy	Fat	Carbohydrate	Protein	Energy	Fat	Carbohydrate	Protein
						MJ/d						
1	− 1.43 ± 0.17	− 2.11 ± 0.25	0.94 ± 0.26	− 0.26 ± 0.04	0.07 ± 2.15	− 0.78 ± 1.39	0.88 ± 1.01	− 0.03 ± 0.24	1.83 ± 0.87	1.31 ± 0.66	0.43 ± 0.17	0.09 ± 0.07
2	0.46 ± 0.57	− 0.95 ± 0.30	1.34 ± 0.38	0.07 ± 0.13	1.24 ± 1.37	0.19 ± 0.95	1.06 ± 0.94	− 0.01 ± 0.19	2.87 ± 1.22	2.11 ± 0.71	0.49 ± 0.42	0.26 ± 0.16
3	− 0.01 ± 0.81	− 0.96 ± 0.34	0.96 ± 0.44	0.01 ± 0.10	0.87 ± 1.86	0.53 ± 1.54	0.50 ± 0.85	− 0.16 ± 0.39	2.20 ± 0.97	1.97 ± 0.71	0.16 ± 0.30	0.68 ± 0.09
4	− 0.48 ± 0.40	− 0.81 ± 0.29	0.36 ± 0.33	− 0.03 ± 0.06	0.52 ± 1.89	0.34 ± 1.28	0.07 ± 1.05	0.10 ± 0.32	1.47 ± 0.87	1.46 ± 0.65	− 0.18 ± 0.28	0.19 ± 0.08
5	0.28 ± 0.47	− 0.42 ± 0.23	0.65 ± 0.35	0.05 ± 0.09	1.04 ± 2.17	0.79 ± 1.22	0.15 ± 1.12	0.10 ± 0.24	5.48 ± 0.80	4.12 ± 0.70	0.85 ± 0.30	0.51 ± 0.06
6	0.16 ± 0.72	− 0.43 ± 0.36	0.54 ± 0.35	0.05 ± 0.06	1.07 ± 1.66	1.07 ± 1.17	− 0.08 ± 0.53	0.08 ± 0.17	1.84 ± 0.96	2.10 ± 0.79	− 0.40 ± 0.30	0.15 ± 0.11
7	− 0.89 ± 0.28	− 0.36 ± 0.27	− 0.47 ± 0.41	− 0.06 ± 0.06	0.56 ± 1.66	0.62 ± 1.17	− 0.142 ± 0.53	0.08 ± 0.17	2.38 ± 0.76	2.62 ± 0.69	− 0.03 ± 0.20	0.30 ± 0.09
$\bar{x}$ ± SEM	− 0.27 ± 0.69	− 0.86 ± 0.61	0.62 ± 0.58	− 0.02 ± 0.11	0.77 ± 0.37	0.39 ± 0.59	0.35 ± 0.47	0.02 ± 0.10	2.58 ± 1.34	2.24 ± 0.94	0.15 ± 0.46	0.19 ± 0.16
Total	− 1.89	− 6.04	4.32	− 0.17	5.36	2.76	2.44	0.16	18.07	15.69	1.07	1.31

[1] $\bar{x}$ ± SEM.

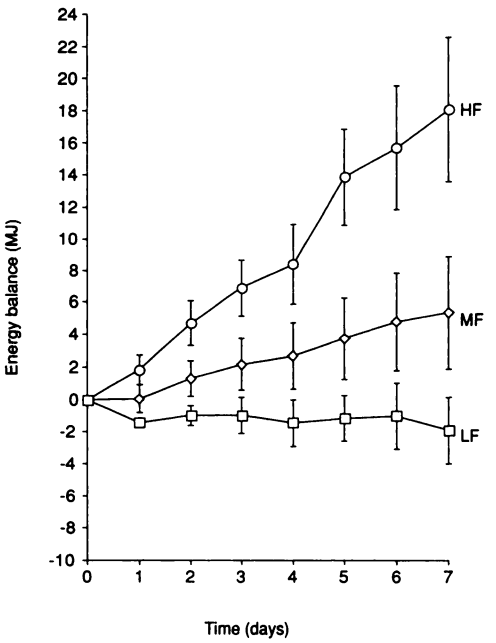

FIGURE 5. Mean (± SEM) cumulative energy balance for six men during the three dietary treatments: LF, low-fat diet; MF, medium-fat diet; HF, high-fat diet.

This gain in body weight is consistent with the water that is retained in association with carbohydrate storage.

Subjective sensations of hunger, fullness, appetite, pleasantness, and satisfaction of food

Mean subjective hunger was not affected by dietary treatment, although there were diet-time interactions for rated hunger ($F_{[30, 1637]} = 1.87$; $P = 0.003$). The mean value for each diet was 27 (pooled SEM = 0.6). The greatest influence on rated hunger was time ($F_{[15, 1637]} = 75.29$; $P < 0.001$). Subjective hunger was elevated at three main time points in the day corresponding to the three main meal times at which subjects most frequently requested food. These times were 0800–0900, 1200–1300, and 1800–1900. Another major source of variation was the way in which subjects individually completed the questionnaires. Subject-diet interactions were also significant ($P < 0.001$) for each of the questions answered and shall not be discussed further.

Diet significantly influenced rated fullness ($F_{[2, 1673]} = 11.41$; $P < 0.001$). The mean rated fullness was 56, 56, and 60 mm for the LF, MF, and HF diets, respectively (pooled SEM = 0.6 mm). Time was also a major factor influencing rated fullness ($F_{[15, 1673]} = 66.34$; $P < 0.001$).

Dietary manipulation had a significant effect on desire to eat ($F_{[2, 1673]} = 5.02$; $P = 0.007$). The mean values for rated desire to eat by diet were 24, 24, and 26 mm on the LF, MF, and HF diets, respectively (pooled SEM = 0.5 mm). Time influenced desire to eat in the same manner as did hunger ($F_{[15, 1673]} = 71.31$; $P < 0.001$).

Although rated hunger did not show significant differences across diets, the HF diet produced an elevated subjective sensation of the desire to eat ($F_{[2, 1673]} = 2.74$; $P = 0.008$). The mean values for dietary treatment were 28, 27, and 29 mm for the LF, MF, and HF diets, respectively (pooled SEM = 0.5

mm). These effects were, however, very small and may have been quantitatively unimportant.

Diet also had significant effects on the subjectively rated urge to eat ($F_{[2, 1673]} = 10.87$; $P < 0.001$). The mean values by diet were 21, 22, and 24 mm on the LF, MF, and HF diets, respectively (pooled SEM = 0.5 mm). Thoughts of food were not influenced by diet but, as might be expected, were influenced by time of day. Diet significantly affected rated pleasantness ($F_{[2, 267]} = 12.71$; $P < 0.01$). The mean rated pleasantness by diet was 81, 87, and 84 mm on the LF, MF, and HF diets, respectively. Subjectively rated satisfaction was affected by diet but not as greatly as was pleasantness ($F_{[2, 270]} = 3.72$; $P = 0.025$). The mean values for diet were 85, 88, and 86 mm on the LF, MF, and HF diets, respectively (pooled SEM = 0.8).

DISCUSSION

Effect of manipulation of dietary fat and energy density on energy balance

Simultaneously increasing the energy density and fat content of the diet led to a dramatic response in energy intake. By day 7 the positive energy balance on the HF diet (+ 18.07 MJ) was much greater than the negative energy balance on the LF diet (− 1.89 MJ). It is possible that normal-weight people will defend against a negative energy balance to a greater extent than against a positive energy balance (41–44). Indeed, studies that have examined the effects of LF diets on body-weight change usually only report a modest decrease in energy balance over time (18, 19), although Lissner et al (21) showed an almost linear change in energy balance in response to altered fat and energy contents of the diet over 2 wk. Alternatively, the tendency toward a positive energy balance observed in this study may have been due to the sedentary nature of the calorimeter environment. This effect also occurred in the study reported by Thomas et al (23).

It is clear from this result that energy intake per se was not tightly regulated over 7 d and was grossly perturbed by simultaneously altering the energy density and ratio of fat to carbohydrate. This agrees with previous studies (13, 21–23). It is also of interest to note that nutrient and energy intakes were remarkably constant compared with those found in real life. Subjects recording their food intakes by diary records typically exhibit within-person CVs of 20–30% for energy intake. **Table 5** gives the CVs for food and energy intakes for each subject on each diet. It can be seen that the within-person CVs in this study were considerably lower than the 20–30% that is normally found (45). This is not surprising because the range of food items was considerably smaller than that which is available to free-living people.

TABLE 5
CVs (expressed as percentages) for food (kg/d) and energy intakes (MJ/d) for each subject during each dietary treatment of the study

	Low fat		Medium fat		High fat	
Subject	Food intake (kg/d)	Energy intake (MJ/d)	Food intake (kg/d)	Energy intake (MJ/d)	Food intake (kg/d)	Energy intake (MJ/d)
1	15	20	16	22	17	21
2	16	15	11	13	15	28
3	6	5	7	15	12	15
4	12	18	5	11	6	5
5	17	12	21	18	28	25
6	10	12	7	9	7	11
Average $\bar{x}$ ± SD	13 ± 4	14 ± 5	11 ± 6	15 ± 5	14 ± 8	18 ± 9

Regulation of energy and fat balance

Over the course of the 7 d of each dietary manipulation, subjects did not regulate energy or fat balance. Because the negative energy balance was very modest during the LF diet, the increase in energy intake induced by the HF diet was considered to be particularly remarkable. Equally remarkable was the dose response in energy intake and energy balance that occurred in response to covert manipulation of diet composition. Ostensibly, these results support Flatt's differential storage capacity model (7). However, it should be remembered that this experimental design, although relevant to every day life, tends to favor the Flatt hypothesis. This is because hyperphagia during the LF diet in this context would be more difficult than during the HF diet. In order to consume the same amount of energy as provided by the HF diet, subjects would have had to eat 40% more food on a wet-weight basis. Because mean daily food intake (wet weight) on the HF diet was ≈2.1 kg, this would have amounted to an additional 0.8 kg food/d. Thus, a critical question in relation to this and similar studies is not why did subjects not consume more of the LF diet, but why did subjects not consume less of the HF diet?

Because the energy density of the least-energy-dense diet (the LF diet) was not unusually low, 4.8 kJ/g on average, a primary conclusion of this study is that subjects do not compensate for a covertly increased energy density derived from fat when a fixed diet composition is given and, therefore, diet selection is prohibited. This is an important point because other short-term studies (41–43) found compensatory changes in energy intake for covert manipulation of the energy density and the macronutrient content of a luncheon meal. However, in those studies, subjects were subsequently able to freely select from a wide range of familiar food items, after consumption of a meal in which the energy and macronutrient contents had been covertly manipulated. Diet selection may be of considerable importance in compensating for short-term energy and macronutrient imbalances, although this does not appear to be primarily due to any compensatory change in macronutrient selection (41–43). Furthermore, there was little evidence of macronutrient-specific compensation in the studies of Foltin et al (46), Rolls et al (47), Burley and Blundell (48), and Mattes et al (43), when subjects had access to a variety of familiar food items from which they could freely select. When familiar foods are ingested, energetic compensation may rely on subtle effects with a large learned component, and may not necessarily be macronutrient specific. This component is, of course, absent on a fixed-diet-composition regimen. Indeed, in the present study learned effects were further controlled by randomizing the order of diets.

Carbohydrate balance regulation

Carbohydrate intake was not tightly regulated across diets, relative to the body's capacity to store it, because the mean intake of carbohydrate was 1.7 times greater with the LF diet than with the HF diet (5.96, 4.92, and 3.62 MJ/d on the LF,

MF, and HF diets, respectively). Thus, subjects exhibited mean intakes ranging from 373 (LF) to 225 g/d (HF). Cumulative carbohydrate balance showed mean values of 4.32, 2.44, and 1.07 MJ by day 7 of each run. Thus, there was a fourfold difference in carbohydrate balance between the HF and LF diets. Although measurement errors for carbohydrate balances may have been considerable, they would have been predominantly systematic and so the relative difference between diets would have held. Thus, subjects did not apparently eat to maintain a tight range of carbohydrate stores. Furthermore, although there was an increased rate of carbohydrate oxidation on all three diets from days 1 to 7, the rate of energy intake was virtually constant within each diet. The increased rates of carbohydrate oxidation due to increased carbohydrate intake did not therefore decrease the rate of energy intake as the study progressed, although this elevated rate of carbohydrate oxidation may be associated with the lower energy intakes (ie, greater satiety per unit of energy ingested) that occurred during the LF diet.

Flatt's glycogenostatic model (7) predicts that when feeding ad libitum on a diet of a given composition, people will eat to maintain a fixed range of carbohydrate stores. This suggests that people will become hyperphagic on HF diets. Flatt argues that in subjects consuming HF diets, fat ingested in excess of energy requirements will tend not to be oxidized but stored until the fat mass increases to an extent that the amount of circulating free fatty acids increases. It is predicted that the increased fat oxidation that ensues will lower the RQ to a value similar to the FQ, and a new diet composition–energy balance equilibrium will occur. Flatt also observes that the lower the carbohydrate intake and stores, the higher the amount of fat oxidation. As can be seen from the cumulative oxidation plots, fat oxidation increased dramatically during the HF diet. Subjects oxidized a total of 35.13, 24.62, and 19.04 MJ over 7 d on the HF, MF, and LF diets, respectively. Thus, as carbohydrate stores dropped during the HF diet relative to the other diets, fat oxidation correspondingly increased and reached an amount that was twice that on the LF diet. Griffiths et al (49) recently showed that in the short term, extremely HF-HE diets can elevate fat oxidation and spare carbohydrate oxidation. Eight fasted, normal men were given two separate meals on two occasions. The first was a 4.26-MJ meal comprising 30% carbohydrate, 69.5% fat, and 8% protein. The second was a 1.58-MJ meal made up of 81.6% carbohydrate, virtually no fat (1 g), and 18.4% protein. In both meals carbohydrate was fixed at 80 g (1.28 MJ). Over the subsequent 6 h, energy expenditure was very similar on both occasions. However, after the HF meal fat oxidation was twice that after the LF-LE meal, which was high in carbohydrate. Carbohydrate oxidation was correspondingly spared. Inhibition of glucose oxidation appeared to have been due to higher circulating FFA concentrations produced by the HF meal. This is an example of a situation in which fat intake can produce effects that reduce the postprandial rise in carbohydrate oxidation. Furthermore, it further illustrates that macronutrients physiologically interact at different levels of diet composition and energy intake to produce different profiles of fuels in the substrate mixture being oxidized.

Flatt's model predicts that there will be an inverse relation between carbohydrate balance and energy intake (7). In our present study the highest energy intake occurred on the diet with the lowest carbohydrate intake. However, as discussed above, the experimental design tended to favor such a relation because it would have been difficult for subjects to ingest sufficient food on the LF diet to match the intakes on the HF diet. The key question is whether the relation between prior carbohydrate balance and subsequent energy intake is causative. Energy balance (dependent variable) was regressed against the previous days balance of fat, carbohydrate, and protein by using multiple-regression analysis on individual daily values. Parenthetically, the use of energy intake as the dependent variable produced a near-identical result because of the constancy of energy expenditure. As predicted by Flatt, the regression for fat suggested that for every megajoule of positive fat balance there was a 0.54-MJ positive energy balance on the subsequent day ($t = 5.245$; $P = 0.0001$). The effect accounted for 19.9% of the variance in the subsequent day's energy balance. Carbohydrate balance on the previous day showed a negative relation with the subsequent day's energy balance. For every megajoule of increased carbohydrate stores, the subject's energy balance was 0.63 MJ less on the subsequent day ($t = -2.696$; $P = 0.0082$). This effect accounted for 5.5% of the variance in energy balance. For every megajoule of increased protein stores, the subject's energy balance was -1.49 MJ less on the subsequent day ($t = -1.83$; $P = 0.071$). This model accounted for 27.8% of the variance. There are, however, potential problems with these regressions when net 24-h nutrient balance is used. First, they do not account for the change in physiological state of the subjects over time during the study because they represent the intake minus the oxidation of each macronutrient for that particular day only. For example, the average net 24-h balance of carbohydrate for day 7 of the LF diet was -0.47 MJ/d, whereas the actual or cumulative carbohydrate balance for day 7 (accounting for changes in carbohydrate balance on days 1–7) amounted to + 4.32 MJ. Second, these regressions do not account for the large intersubject differences in dependent and independent variables. For example, some subjects consistently tended to exhibit larger balances than other subjects. Thus, regression of subsequent balance against today's macronutrient balance will create spurious regression coefficients that may be more reflective of intersubject variation than the actual phenomena under scrutiny. Third, in a similar manner these regressions may be driven largely by differences between diets. For example, during the HF diet both fat and energy balances will be consistently higher than during the other two diets. Therefore, to fully observe putative mechanisms underlying the change in energy balance during the LF, MF, and HF diets, further regressions were conducted by using the following model:

$$BAL_{s,r,d} = R_{s,r} + b_F\,FABAL_{s,r,d-1} + b_C\,CABAL_{s,r,d-1} + b_P\,PABAL_{s,r,d-1}$$

where $BAL_{s,r,d}$ is the energy balance of subject s on the dth day of run r; $R_{s,r}$ is the mean balance of subject s on run r; $FABAL_{s,r,d-1}$, $CABAL_{s,r,d-1}$, and $PABAL_{s,r,d-1}$ are the actual (cumulative) fat, carbohydrate, and protein balances of subject s on the $d-1$th day of run r; and b_F, b_C, and b_P are the estimated regression coefficients. This model accounts for the above considerations by removing the effects of subject and diet and also by using actual (cumulative) nutrient balances.

The regression of change in energy balance against cumulative energy balance suggested that an increase in energy balance on 1 d did not lead to a compensatory decrease in energy balance on the subsequent day. When the same regression was performed for the macronutrients there were significant effects. For every megajoule of increased protein stores on 1 d there would tend to be a negative change in energy balance on the subsequent day, amounting to − 2.12 MJ ($t = -2.54$, $P = 0.013$); for carbohydrate this relation was also negative, producing a change in the subsequent day's energy balance of − 0.79 MJ ($t = -4.60$, $P < 0.001$). There was a nonsignificant positive relation for fat in this multiple regression of 0.06 MJ ($t = 0.75$; $P = 0.46$). This model (ie, the combined effects of fat, protein, and carbohydrate) accounted for 16.3% of the variance previously unexplained by the subject and diet effects. Use of the subsequent days' absolute energy balance instead of change in energy balance in the regressions yielded virtually identical results. For every megajoule of increased protein stores on 1 d there would tend to be a negative energy balance on the subsequent day, amounting to − 0.87 MJ ($t = -1.70$, $P = 0.09$); for carbohydrate this relation was also negative, producing a change in the subsequent day's energy balance of − 0.38 MJ ($t = -3.58$, $P < 0.001$). The relation for fat was positive, for every megajoule of increased fat stores on 1 d, energy balance on the subsequent day would tend to increase by 0.42 MJ ($t = 0.87$, $P = 0.39$). This model (ie, the combined effects of fat, protein, and carbohydrate) accounted for 27.8% of the variance not explained by the diet and subject effects. These data suggest that under the conditions of this study design, positive changes in protein and carbohydrate stores (and attendant changes in their metabolism) have the potential to exert negative feedback on the subsequent energy balance via their potentially reductive effects on energy intake. Fat on the other hand showed no tendency to exert negative feedback on subsequent energy balance. The potential for carbohydrate and protein to exert negative feedback on subsequent energy intake or balance only explained a limited proportion of the variance (≈3% for protein and 10% for carbohydrate when cumulative nutrient balances were used in the regressions). The very small effect for protein is not surprising because protein was a constant, small proportion of each diet. At larger protein intakes the potential negative feedback onto energy intake would presumably be amplified. These data suggest that the macronutrients whose balance is most tightly regulated exert suppressive effects on subsequent energy intake, whereas fat (whose balance is not tightly regulated) does not exert such an effect. Protein and carbohydrate balances were far more tightly regulated on a quantitative basis than was fat balance. This appears to have occurred at the expense of fat balance. This is illustrated by the accelerated rate of carbohydrate oxidation and the reciprocal decrease in the rate of fat oxidation from days 1 to 7 of all diets.

Of critical interest in this context is whether an HE-LF diet (which is high in carbohydrate) would produce hyperphagia over ≥ 7 d, relative to LE-LF diets, LE-HF diets, and HE-HF diets. Certainly, in a study by Porikos et al (50), normal subjects were hyperphagic on a "platter-style" control diet that contained 55% carbohydrate and 31% fat. The mean daily intake of the men on this diet was 15.18 MJ/d. However, these diets were rather atypical and a substantial energy intake was derived from high-carbohydrate drinks. Furthermore, those diets were designed to prevent obese people from losing weight under experimental conditions (51). When given to lean men under the same conditions, the diets appear to have led to a marked positive energy balance. It is therefore of interest whether HE-LF diets would produce hyperphagia under conditions similar to those in the present study.

There are alternative explanations for the hyperphagia observed on HF diets. First, subjects may have overeaten on the HF diet because of its hedonic qualities. However, the highest values for rated pleasantness were for the MF diets and although the desire to eat and prospective consumption were elevated on the HF diet compared with the other two diets, these effects were very small indeed. It may be concluded that part of the failure to regulate energy intake on the HF diet may be related to sensory factors because indexes of appetite (desire to eat and prospective consumption) were marginally elevated on the HF diet whereas indexes of hunger (hunger and thoughts of food) were, on average, unaffected by the dietary treatments. If this were the case then the fat content of the HF diet may have led to some degree of oversatiation or mild aversion, reflected by the pleasantness ratings on that diet. This is supported by the fact that subjects felt significantly more full on the HF diet compared with the other two diets. The alternative explanation is that subjects rated as most pleasant the diet they were most used to. Thus, although significant, the differences in rated pleasantness were small, did not parallel differences in energy intake, and may have been too subtle to exert much influence on energy intake. These data may then be considered in relation to the assertions of Ramirez et al (52), who argue that palatability and energy intake are not necessarily highly correlated.

Friedman and Stricker (53) and Friedman and Ramirez (54) on the other hand predict that factors favoring storage of substrates promote hyperphagia and that factors promoting their release generate hypophagia. HF-HE diets apparently disturb the regulatory interactions of the enteroinsular axis (55). Furthermore, voluntary hyperphagia during HF diets is reported to be associated with moderate increases in neuropeptide Y concentrations in the paraventricular nucleus and reduced concentrations in the lateral hypothalamus compared with rats fed freely or on an LF diet (56). These effects may be mechanistically involved in the hyperphagia seen on HF diets. If so, these mechanisms may be very different for HE-LF diets.

Macronutrients hierarchically interact at different dietary energy densities to determine rates of utilization and storage. Specifically, a positive balance of protein will be disposed of by oxidation more readily than an energetically equivalent positive carbohydrate balance, which will be disposed of more readily than a positive fat balance. Considering the work of Friedman and Stricker (53) and Friedman and Ramirez (54), Stubbs (57) recently proposed that substrates at the top of the oxidative hierarchy, which are disposed of by obligatory oxidation, may be associated with a stronger metabolic satiety signal. Those most readily stored are possibly the least likely to decrease subsequent energy intakes. He further suggests that ingestion of any substrate in excess of the body's capacity to store or interconvert it may lead to rapid rates of obligatory utilization and that this may be associated with a metabolic satiety signal. Thus, if autoregulation of nutrient balance is involved in the extent to which a given nutrient suppresses subsequent energy intake, there should be a hierarchical rela-

tion between nutrient oxidation and subsequent energy intake such that, per unit of energy, protein suppresses subsequent intake to a greater extent than does carbohydrate, which has a greater effect than fat (57). To test this hypothesis, energy intake (dependent variable) was regressed against the previous day's oxidation of fat, carbohydrate, and protein by using multiple regression with the effects of subject and diet removed as before, ie, fitting the model

$$I_{s,r,d} = R_{s,r} + b_F FOX_{s,r,d-1} + b_C COX_{s,r,d-1} + b_P POX_{s,r,d-1}$$

where $I_{s,r,d}$ is the intake of subject s on the dth day of run r; $R_{s,r}$ is the mean intake of subject s on run r; $FOX_{s,r,d-1}$, $COX_{s,r,d-1}$, and $POX_{s,r,d-1}$ are the fat, carbohydrate, and protein oxidation of subject s on the $d-1$th day of run r; and b_F, b_C, and b_P are the estimated regression coefficients. For every megajoule of protein oxidation on 1 d there would tend to be a decrease in energy intake on the subsequent day of 4.2 MJ ($t = -3.38$; $P = 0.001$); for every megajoule of carbohydrate oxidation on 1 d there would tend to be a decrease in energy intake of 2.3 MJ ($t = -2.98$; $P = 0.004$). Fat exerted a slightly smaller effect at 2.0 MJ ($t = -2.49$; $P = 0.014$). This regression accounted for 13.4% of the variance in subsequent energy intake unaccounted for by subject and diet effects. Thus, within the context of this study, the metabolism of the macronutrients protein, carbohydrate, and fat appeared to exert a hierarchical, suppressive effect on subsequent energy intake. The potential feedback relation between nutrient balance and subsequent energy intake observed in this study may therefore relate to feedback from nutrient stores, via the extent to which those stores effect nutrient metabolism or to some other (unmeasured) variables that are themselves related to the change in protein, carbohydrate, and fat balances.

Overall regulation of substrate balance

The protein content of the diet was designed in such a way as to represent the relatively small contribution (12–13%) to total energy intake that it generally provides in the majority of the world's diets (58). The positive protein balance during the HF diet paralleled energy balance. This positive nitrogen balance was presumably a result of the synthesis of the new tissues associated with the deposition of excess energy. Thus, whereas protein intake was considerably higher on this diet, protein oxidation rates were very similar on all diets. The cumulative balance plots for energy, fat, carbohydrate, and protein clearly illustrate that regulation of carbohydrate balance was given priority over that of fat balance. As the oxidative disposal of carbohydrate increased, that of fat decreased and more was diverted toward storage. However, of considerable interest are the relative substrate imbalances that each of the diets generated. It must be remembered that in this context regulation of substrate balance is dependent on both voluntary energy intakes and on regulation of substrate flux. By the 7th d of the LF diet, subjects were in a negative energy balance of − 1.89 MJ, a negative fat balance of − 6.04 MJ, and a positive carbohydrate balance of ≈4.32 MJ. The 5.36-MJ positive energy balance on day 7 of the MF diet was more evenly shared between fat (+ 2.76 MJ) and carbohydrate (+ 2.44 MJ). On day 7 the group receiving the HF diet had a 1.07-MJ positive carbohydrate balance and a 15.69-MJ positive fat balance.

These data illustrate that the physiological response to the feeding behavior had not achieved a steady state after 7 d. Furthermore, the tightest physiological regulation of substrate balance occurred during the MF diet. Thus, when the macronutrient and energy density of normal foods was covertly manipulated, subjects regulated macronutrient balances most tightly at the diet composition they were most used to eating. Regulation of energy balance did not necessarily parallel regulation of macronutrient balance, as seen in the difference between the LF and MF diets. When all three diets were considered, carbohydrate balance was more tightly regulated than was fat balance. However, on the basis of these data alone it is not possible to conclude that carbohydrate balance per se is the single major factor exerting negative feedback during ad libitum day-to-day food intake. Other studies suggest that protein is also highly satiating (59, 60). These data can be considered in relation to the results of Stubbs et al (29), where reciprocal manipulation of carbohydrate and fat, but not energy balance, had no effect on the subsequent day's ad libitum energy intake. However, carbohydrate and protein do appear to exert suppressive effects on subsequent energy intake, which may be related to autoregulatory changes in their oxidation, or other unmeasured variables. Of considerable interest is the apparent ease with which normal men can, without being aware of it, feed themselves into a large positive energy balance while consuming an HF diet. The fact that the dose response to the dietary manipulation was in direct parallel with the energy density of the diet supports diet survey and epidemiological studies that show that an increase in percent body fat appears to be related to an increased energy intake from dietary fat (10–15).

The extent to which an HE-HF diet can produce a positive energy balance in men feeding ad libitum, without any apparent knowledge on their part, is however, quite remarkable. Under the conditions of this study, carbohydrate and protein balances did exhibit the potential to exert negative feedback on energy intake and hence balance. It would appear that energy balance may be influenced by many more factors than a simple need to eat to maintain stable glycogen concentrations. Identification of the quantitative importance of changes in nutrient balance and metabolism, in influencing appetite and energy balance in humans, remains a considerable challenge for the future.

We are grateful to Marinos Elia, Bruce Bistrian, and Jean-Pierre Flatt for useful discussions concerning the results. We also thank Elaine Collard for preparing the experimental diets.

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