

Plasma fatty acid composition and incidence of diabetes in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) Study¹⁻³

Lu Wang, Aaron R Folsom, Zhi-Jie Zheng, James S Pankow, and John H Eckfeldt for the ARIC Study Investigators

ABSTRACT

Background: The results of some epidemiologic studies conducted by using questionnaires suggest that dietary fat composition influences diabetes risk. Confirmation of this finding with use of a biomarker is warranted.

Objective: We prospectively investigated the relation of plasma cholesterol ester (CE) and phospholipid (PL) fatty acid composition with the incidence of diabetes mellitus.

Design: In 2909 adults aged 45–64 y, plasma fatty acid composition was quantified by using gas-liquid chromatography and was expressed as a percentage of total fatty acids. Incident diabetes ($n = 252$) was identified during 9 y of follow-up.

Results: After adjustment for age, sex, baseline body mass index, waist-to-hip ratio, alcohol intake, cigarette smoking, physical activity, education, and parental history of diabetes, diabetes incidence was significantly and positively associated with the proportions of total saturated fatty acids in plasma CE and PL. The rate ratios of incident diabetes across quintiles of saturated fatty acids were 1.00, 1.36, 1.16, 1.60, and 2.08 ($P = 0.0013$) in CE and 1.00, 1.75, 1.87, 2.40, and 3.37 ($P < 0.0001$) in PL. In CE, the incidence of diabetes was also positively associated with the proportions of palmitic (16:0), palmitoleic (16:1n-7), and dihomo- γ -linolenic (20:3n-6) acids and inversely associated with the proportion of linoleic acid (18:2n-6). In PL, incident diabetes was positively associated with the proportions of 16:0 and stearic acid (18:0).

Conclusions: The proportional saturated fatty acid composition of plasma is positively associated with the development of diabetes. Our findings with the use of this biomarker suggest indirectly that the dietary fat profile, particularly that of saturated fat, may contribute to the etiology of diabetes. *Am J Clin Nutr* 2003;78:91–8.

KEY WORDS Atherosclerosis Risk in Communities Study, prospective study, diabetes, fatty acids, saturated fatty acids

INTRODUCTION

The results of both animal and human studies indicate that a high intake of total dietary fat is associated with impaired insulin sensitivity and an increased risk of developing type 2 diabetes, independent of obesity and body fat localization. The effect of dietary fatty acid composition on glucose metabolism and diabetes etiology is less well understood than the effects of total fat (1). In experimental animals, chronic feeding of saturated fat, relative to that of monounsaturated and polyunsaturated fat, is more deleterious to insulin sensitivity, and some of the induced effects are reduced by feeding with $n-3$ fatty acids (2, 3). Human in vitro metabolic studies suggest that the fatty acid profile of peripheral

tissue membranes influences the sensitivity of the tissue to insulin (1). Epidemiologic data, based on dietary questionnaires, support a positive association between intake of saturated fatty acids (SFAs) and risk of impaired glucose tolerance, insulin resistance, and diabetes. The association for polyunsaturated fatty acids (PUFAs) is less clear: both positive and inverse associations have been reported (4). In human feeding trials, substitution of unsaturated fat for saturated fat appears to improve glucose metabolism in patients with type 2 diabetes (1). In healthy subjects, however, change of dietary fat quality does not affect insulin sensitivity (5).

The methods used to estimate dietary fat composition among free-living subjects are far from perfect (5). In the search for reliable biomarkers, the fatty acid composition of serum lipid esters or of adipose tissue triacylglycerols has been shown to mirror the fatty acid pattern of the diet over several preceding weeks (serum) or months (adipose tissue) (5). In the Atherosclerosis Risk in Communities (ARIC) Study, plasma fatty acid composition was shown to be a reasonably accurate biochemical marker of long-term proportionate fatty acid intake, especially for PUFAs and essential fatty acids (6). The present study investigated the relation of plasma cholesterol ester (CE) and phospholipid (PL) fatty acid composition with the incidence of diabetes mellitus during 9 y of follow-up. We hypothesized that higher concentrations of SFAs and lower concentrations of PUFAs in plasma would be associated with increased risk of developing type 2 diabetes.

SUBJECTS AND METHODS

Study participants

The ARIC Study is a population-based prospective cohort study of the etiology and natural history of atherosclerosis and atherosclerotic diseases in middle-aged adults (7). In brief, a probability sample of 45–64-y-old persons was recruited to the ARIC cohort

¹ From the Division of Epidemiology, School of Public Health (LW, ARF, and JSP), and the Department of Laboratory Medicine and Pathology (JHE), University of Minnesota, Minneapolis, and the Cardiovascular Health Program, Centers for Disease Control and Prevention, Atlanta (Z-JZ).

² Supported by grant RO1-HL-40848 and contracts NO1-HC-55015, NO1-HC-55016, NO1-HC-55018, NO1-HC-55019, NO1-HC-55020, NO1-HC-55021, and NO1-HC-55022 from the National Heart, Lung, and Blood Institute, National Institutes of Health.

³ Address reprint requests to AR Folsom, Division of Epidemiology, School of Public Health, University of Minnesota, 1300 South Second Street, Suite 300, Minneapolis, MN 55454-0315. E-mail: folsom@epi.umn.edu.

Received November 1, 2002.

Accepted for publication January 10, 2003.

from Forsyth County, NC; the city of Jackson, MS; selected suburbs of Minneapolis; and Washington County, MD. A total of 15 792 ARIC participants underwent a comprehensive baseline examination for cardiovascular disease risk factor assessment in 1987–1989. Participants were followed up annually by telephone. Reexaminations were performed every 3 y, up to 9 y. In the Minneapolis field center only ($n = 4009$), plasma was saved at baseline and was analyzed for fatty acids. The ARIC Study was approved by the institutional review boards of each participating center.

Plasma fatty acid measurement

Fasting blood was collected into 10-mL vacuum tubes containing EDTA. The blood was centrifuged at $800 \times g$ for 10 min at 4°C . Plasma was then separated and dispensed into two 1.5-mL aliquots and frozen at -70°C for ≈ 2 y until analyzed for fatty acid content by a single technician.

A detailed description of the methods used to analyze plasma fatty acids was published previously (6). Briefly, after thawing, 0.5 mL plasma was extracted with 0.5 mL methanol followed by 1.0 mL chloroform under a nitrogen atmosphere. The lipid extract was filtered to remove protein. The CE and PL fractions were separated by thin-layer chromatography with a silica gel plate (Silica Gel H; Analtech, Newark, DE) and 2-stage mobile phase development, which consisted of solvents of petroleum ether, diethyl ether, and glacial acetic acid in ratios of 80:20:1 (by vol) and 40:60:1 (by vol), respectively. The plate was dried between development solvents and the second mobile phase was allowed to migrate for only one-half of the plate length. After re-drying, one lane was sprayed with dichlorofluorescein to visualize the CE, PL, triacylglycerol, and free fatty acid bands under ultraviolet light. The CE and PL bands were scraped into separate test tubes, and the lipids were converted to methyl esters of fatty acids by boron trifluoride catalysis. The methyl esters were then separated and measured on a Hewlett-Packard 5890 gas chromatograph (Hewlett-Packard, Avondale, PA) equipped with a 50 m FFAP WCOT glass capillary column (J&W Scientific, Folsom, CA) and a flame ionization detector. The identity of each fatty acid peak was ascertained by comparison of the peak's retention time with the retention times of fatty acids in synthetic standards of known fatty acid composition. The relative amount of each fatty acid (% of total fatty acids) was quantified by integrating the area under the peak and dividing the result by the total area for all fatty acids.

Plasma SFAs, monounsaturated fatty acids (MUFAs), and PUFAs were calculated by summing the respective fatty acids with 12–24 carbon atoms. Test-retest reliability coefficients (individuals sampled 3 times, 2 wk apart) for various plasma fatty acids ranged from 0.50 to 0.93 for CE and from 0.31 to 0.89 for PL (8).

Other measurements

Serum glucose was assessed by the hexokinase–glucose-6-phosphate dehydrogenase method. Diabetes was defined at baseline, for exclusion, by 1) self-reported history of physician-diagnosed diabetes, 2) current use of diabetes medications, 3) 8-h fasting serum glucose concentration ≥ 126 mg/dL (9), or 4) nonfasting serum glucose concentration ≥ 200 mg/dL. Incident diabetes was identified in the 3 ARIC follow-up visits among those who were free of diabetes at baseline. Time of diabetes diagnosis was unknown and therefore imputed. For participants who reported diabetes diagnosed by a physician or who were taking

diabetes medication, the diagnosis date was assigned to the midpoint between the last visit without diabetes and the next visit with diabetes. For diabetes defined by serum glucose concentration, the diagnosis date was estimated as the point when the serum glucose concentration crossed the diabetes cutoffs (126 mg/dL fasting or 200 mg/dL nonfasting) on a regression line of glucose concentrations by visit date. For participants who did not develop diabetes, the censoring date for analysis was the last completed follow-up visit.

Information about education level, sports activity, smoking status, alcohol intake, and family history were obtained through interview. Cigarette-years of smoking was defined as the average number of cigarettes smoked per day multiplied by the number of years of smoking. The sports index, derived from the survey of Baecke et al (10), ranged from 1 (low) to 5 (high) for physical activity from sports during leisure time. Height, weight, and circumferences of the waist (at the umbilicus) and hips (maximum) were measured during the clinic visit. Quetelet's body mass index (BMI; in kg/m^2) and waist-to-hip ratio (WHR) were calculated.

Statistical analysis

Participants were excluded from the analysis if they had prevalent diabetes at baseline ($n = 301$), had unknown prevalent or incident diabetes status ($n = 104$), were missing fatty acid measurements ($n = 74$), were taking cholesterol-lowering medications ($n = 130$), were consuming a special diet ($n = 597$), or had prevalent cardiovascular disease ($n = 222$). The exclusion for baseline clinical cardiovascular disease was made because symptomatic patients may change their diets. Nonwhites were also excluded as a result of their small number ($n = 37$). After all the nonmutual exclusions, 2909 subjects remained.

SAS 6.0 (SAS Institute Inc, Cary, NC) was used for the analysis. Proportionate plasma fatty acid composition was compared between participants with and without incident diabetes by using a t test. The association of plasma fatty acid quintiles with other risk factors was analyzed by using analysis of covariance. Age-adjusted mean values for baseline risk factors were compared across quintiles of plasma fatty acids, after the assumptions of normal distribution and constant variance were confirmed. To reduce problems related to conducting multiple comparisons, $P < 0.01$ was set as the significance level. Cox proportional hazards regression models were used to estimate the rate ratios (RRs) and 95% CIs for incident diabetes in relation to the quintiles of plasma fatty acid composition. Time at risk was calculated from baseline to the time of diabetes diagnosis or censoring. There was no multiplicative interaction between sex and plasma fatty acids in association with diabetes, so the data for men and women were combined. Baseline age (continuous) and sex (dichotomous) were adjusted for in model 1. In model 2, additional adjustment was made for BMI (continuous), WHR (continuous), cigarette-years of smoking (continuous), alcohol intake (continuous), sports index (continuous), education (\leq high school, college or vocational school, or graduate school), and parental family history of diabetes (yes, no, or unknown). The trend of RRs across quintiles of fatty acids was tested by using equal weight for each quintile. Analyses were conducted for grouped fatty acids and for the most common individual fatty acids, for CE and PL separately.

Several supplemental analyses were performed. First, analyses were repeated with those participants having prevalent

TABLE 1

Baseline characteristics of participants with or without incident diabetes mellitus, Atherosclerosis Risk in Communities Study, Minneapolis Field Center, 1987–1989

Baseline characteristic	Incident diabetes (n = 252)	No diabetes (n = 2657)
Men (%)	58.3 ¹	45.8
≤High school education (%)	46.4	39.4
Current smoking (%)	25.4	22.5
Parental history of diabetes (%)	22.6 ¹	12.9
Age (y)	54.0 ± 5.5 ²	53.5 ± 5.5
BMI (kg/m ²)	30.6 ± 4.8 ¹	26.3 ± 4.2
Waist-to-hip ratio	0.966 ± 0.071 ¹	0.902 ± 0.084
Fasting serum glucose (mmol/L)	6.05 ± 0.51 ¹	5.46 ± 0.45
Fasting serum insulin (pmol/L)	106 ± 65 ¹	62 ± 43
Alcohol intake (g/wk)	71.9 ± 137	60.0 ± 101
Cigarette-years of smoking ³	395 ± 449 ¹	305 ± 384
Sports index ⁴	2.50 ± 0.80	2.60 ± 0.81

¹ Significantly different from participants without diabetes, $P < 0.01$.

² $\bar{x} \pm$ SD.

³ Defined as the average number of cigarettes smoked per day multiplied by the number of years of smoking.

⁴ Derived from the survey of Baecke et al (10); values ranged from 1 (low) to 5 (high).

cardiovascular disease or consuming special diets at baseline included. Second, analyses were repeated with the time of incident diabetes assigned for everyone as the midpoint between visits without and with diabetes. Third, analyses were repeated with adjustment for baseline fasting serum glucose and insulin concentrations. Fourth, analyses were repeated stratified on baseline fasting glucose concentrations (<110 compared with 110–125 mg/dL). Finally, analyses were repeated with adjustment for time-dependent body weight at all 4 ARIC examinations. None of these supplemental analyses altered the conclusions; thus, for the most part, these are not presented.

RESULTS

Among the 2909 participants at risk, 252 developed incident diabetes over a mean of 8.1 y of follow-up. The remaining 2657 participants stayed free of diabetes.

Compared with those without diabetes, participants with incident diabetes had significantly ($P < 0.01$) higher mean baseline values for BMI, WHR, fasting serum glucose and insulin, and cigarette-years of smoking (Table 1). Diabetes incidence was greater in men and in those with at least one parent with diabetes. Baseline age, alcohol intake, sports activity, education, and smoking status were not significantly different between participants who did or did not develop diabetes.

For the CE fraction, Pearson correlations between baseline fatty acid concentrations were $r = 0.51$ for SFAs with MUFAs, $r = -0.68$ for SFAs with PUFAs, and $r = -0.96$ for MUFAs with PUFAs. For the PL fraction, these respective correlations were $r = 0.00$, $r = -0.36$, and $r = -0.84$. Compared with those who remained free of diabetes, persons who developed incident diabetes had significantly ($P < 0.01$) higher proportions of total SFAs in both the CE and PL fractions (Table 2). The percentage of SFAs in total fatty acids was 12.0% in CE and 41.3% in PL for those who developed incident diabetes compared with 11.6% in CE and 40.5% in PL for those who did not. In CE only, total MUFAs were

TABLE 2

Unadjusted baseline plasma fatty acid composition by participants' incident diabetes status, Atherosclerosis Risk in Communities Study, Minneapolis Field Center, 1987–1989¹

Fatty acid composition	Incident diabetes (n = 252)	No diabetes (n = 2657)
<i>% of total fatty acids</i>		
Cholesterol esters		
SFAs	12.0 ± 1.0 ²	11.6 ± 1.0
16:0	10.3 ± 0.8 ²	9.95 ± 0.8
18:0	0.93 ± 0.19 ²	0.90 ± 0.21
MUFAs	19.1 ± 2.7 ²	18.6 ± 3.0
16:1n-7	2.90 ± 1.21 ²	2.51 ± 1.21
18:1n-9	16.2 ± 1.8	16.0 ± 2.1
PUFAs	64.8 ± 3.6 ²	65.8 ± 3.9
18:2n-6	52.9 ± 4.4 ²	54.3 ± 4.8
18:3n-3	0.40 ± 0.11	0.42 ± 0.11
18:3n-6	1.11 ± 0.39 ²	1.01 ± 0.37
20:3n-6	0.83 ± 0.17 ²	0.75 ± 0.16
20:4n-6	8.42 ± 1.71	8.16 ± 1.64
20:5n-3	0.59 ± 0.36	0.54 ± 0.28
22:6n-3	0.43 ± 0.15	0.43 ± 0.15
Phospholipids		
SFAs	41.3 ± 1.2 ²	40.5 ± 1.4
16:0	25.7 ± 1.5 ²	25.3 ± 1.6
18:0	13.7 ± 1.2 ²	13.2 ± 1.1
MUFAs	9.8 ± 1.2 ²	10.0 ± 1.3
16:1n-7	0.66 ± 0.18	0.63 ± 0.18
18:1n-9	8.44 ± 1.11 ²	8.66 ± 1.17
PUFAs	42.5 ± 1.7	42.7 ± 1.7
18:2n-6	21.4 ± 2.4 ²	22.1 ± 2.7
18:3n-3	0.13 ± 0.05 ²	0.15 ± 0.05
18:3n-6	0.11 ± 0.06	0.11 ± 0.06
20:3n-6	3.62 ± 0.79 ²	3.28 ± 0.75
20:4n-6	11.6 ± 2.0	11.4 ± 1.9
20:5n-3	0.58 ± 0.33	0.56 ± 0.31
22:6n-3	2.71 ± 0.83	2.76 ± 0.84

¹ $\bar{x} \pm$ SD. SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids.

² Significantly different from participants without diabetes, $P < 0.01$.

also significantly higher, whereas total PUFAs were significantly lower, among persons with incident diabetes than among those who did not develop diabetes. In the PL fraction, total MUFAs were significantly lower among those who developed diabetes, whereas total PUFAs did not differ significantly by incident diabetes status.

With respect to the individual fatty acids, the proportions of palmitic (16:0), stearic (18:0), and dihomo- γ -linolenic (20:3n-6) acids in CE and PL and the proportions of palmitoleic (16:1n-7) and γ -linolenic (18:3n-6) acids in CE were significantly higher in participants with incident diabetes than in those without. Proportions of linoleic (18:2n-6) acid in CE and PL and of oleic (18:1n-9) and linolenic (18:3n-3) acids in PL were significantly lower in participants with incident diabetes than in those without. In neither fraction did the mean proportions of arachidonic (20:4n-6), eicosapentaenoic (20:5n-3), or docosahexaenoic (22:6n-3) acids differ according to diabetes development. Considering the small proportions of 20:5n-3 and 22:6n-3 in the total fatty acids and their null association with diabetes incidence in most regression models, the results for these 2 fatty acid constituents are not shown in the remaining tables and figures.

TABLE 3

Mean (or percentage) age-adjusted baseline risk factors by extreme quintiles (Q) of plasma cholesterol ester fatty acids, Atherosclerosis Risk in Communities Study, Minneapolis Field Center, 1987–1989^f

Fatty acid quintile ²	Age	Men	Sports index	BMI	WHR	Fasting serum glucose	Fasting serum insulin	Alcohol intake	Cigarette-years	≤High school education	Parental history of diabetes
	y	%		kg/m ²		mmol/L	pmol/L	g/wk		%	%
SFAs											
Q1	53.0	30.3	2.61	25.7	0.877	5.41	56.2	31.5	246	40.8	13.0
Q5	53.9	63.1 ³	2.58	27.4 ³	0.933 ³	5.602 ³	74.7 ³	92.8 ³	383 ³	37.8	15.4
<i>P</i> for trend ⁴	0.07	<0.0001	0.86	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.16	0.53
MUFAs											
Q1	53.1	38.8	2.68	25.7	0.884	5.42	58.0	28.5	192	35.9	13.1
Q5	53.7	49.0 ³	2.45 ³	27.1 ³	0.927 ³	5.56 ³	68.4 ³	118.5 ³	467 ³	45.2 ³	14.9
<i>P</i> for trend ⁴	0.21	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	<0.0001	<0.0001	0.018	0.91
PUFAs											
Q1	53.8	52.8	2.47	27.3	0.930	5.56	70.3	112.3	445	41.8	13.8
Q5	53.0	35.0 ³	2.68 ³	25.8 ³	0.881 ³	5.44 ³	58.5 ³	28.9 ³	204 ³	37.3	12.9
<i>P</i> for trend ⁴	0.16	<0.0001	0.0004	<0.0001	<0.0001	0.0002	<0.0001	<0.0001	<0.0001	0.43	0.40

¹WHR, waist-to-hip ratio; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids.

²Quintile cutoffs were as follows: SFAs, 10.80%, 11.31%, 11.79%, and 12.35%; MUFAs, 16.33%, 17.57%, 18.89%, and 20.58%; and PUFAs, 62.95%, 65.11%, 66.93%, and 68.82%.

³Significantly different from Q1, *P* < 0.01.

⁴*P* for trend across quintiles.

As presented in **Table 3**, compared with those in the lowest quintile, persons in the highest quintile of CE SFAs and MUFAs were more likely to be male and engaged in less sports activity (MUFAs only), whereas persons in the highest quintile of CE PUFAs were more likely to be female and to be more physically active. Baseline BMI, WHR, and fasting serum glucose and insulin were significantly (*P* < 0.01) higher in the highest than in the lowest quintile of total SFAs and total MUFAs but were significantly lower in the highest than in the lowest quintile of total PUFAs. Alcohol intake and cigarette smoking were significantly greater in the highest than in the lowest quintile of SFAs and MUFAs but were significantly lower in the highest than in the lowest quintile of PUFAs. There were only small differences in education and no significant differences in parental history according to quintiles of the fatty acid constituents of CE. The relations for PL (data not shown) were basically consistent with those for CE, except for an inverse relation of total MUFAs in PL with baseline BMI, WHR, serum glucose, and insulin. Therefore, multivariate models included most of the variables in Table 3 as potential confounding factors.

After adjustment for age and sex (model 1), the incidence of diabetes was significantly (*P* < 0.05 for the RR of the highest quintile compared with the lowest quintile and *P* < 0.05 for trend) and positively associated with total SFAs and MUFAs and significantly and inversely associated with total PUFAs in CE (**Figure 1**). After further adjustment for baseline BMI, WHR, alcohol intake, cigarette-years of smoking, physical activity, education, and parental history (model 2), the associations were attenuated and the trend across quintiles remained significant only for total SFAs. In model 2, the adjusted RRs of diabetes were 1.00, 1.36, 1.16, 1.60, and 2.08 across total SFAs quintiles (*P* for trend: 0.0013). Certain individual fatty acids were also significantly associated with diabetes in model 2. Compared with the lowest quintile of individual fatty acids in CE, the RR of incident diabetes was 2.38 (95% CI: 1.51, 3.73) for the highest quintile of 16:0, 1.72 (95% CI: 1.07, 2.75) for

the highest quintile of 16:1n-7, 1.86 (95% CI: 1.15, 3.01) for the highest quintile of 20:3n-6, and 0.46 (95% CI: 0.28, 0.74) for the highest quintile of 18:2n-6.

In the PL fraction, the positive association between total SFAs and incident diabetes was even stronger than in the CE fraction (**Figure 2**). In model 2, the adjusted RRs of diabetes were 1.00, 1.75, 1.87, 2.40, and 3.37 across PL SFAs quintiles (*P* for trend: <0.0001). Total MUFAs in PL were inversely associated with diabetes risk, with RRs across quintiles of 1.00, 0.93, 0.80, 0.58, and 0.80 (*P* = 0.045) after multivariable adjustment. PL PUFAs were not significantly associated with incident diabetes in any model. In model 2, significant relations of diabetes with individual fatty acids existed in PL for 16:0 (RRs across quintiles: 1.00, 0.80, 1.57, 1.47, and 1.65; *P* = 0.002) and 18:0 (RRs across quintiles: 1.00, 1.21, 1.50, 1.37, and 1.80, *P* = 0.01). A tendency for decreased incidence of diabetes across quintiles was also observed for PL 18:1n-9, 18:2n-6, and 18:3n-3, although the RR corresponding to the highest quintile compared with the lowest quintile was not significant for any of these constituents.

DISCUSSION

The results of the present prospective study showed that a high proportion of SFAs in plasma CE and PL was associated with an increased incidence of type 2 diabetes. The association was moderately strong, displayed a dose-response pattern, and remained statistically significant after adjustment for several diabetes risk factors. Total MUFAs and PUFAs did not show clear relations to diabetes incidence. With respect to the individual fatty acids, 16:0 in plasma CE and PL, 16:1n-7 and 20:3n-6 in CE, and 18:0 in PL showed independent positive associations with incident diabetes. 18:2n-6 in CE was negatively related to incident diabetes.

Previous studies showed that the fatty acid composition of plasma PL and CE reflects dietary intake weeks to months before the collection of the sample and can therefore be used as an objec-

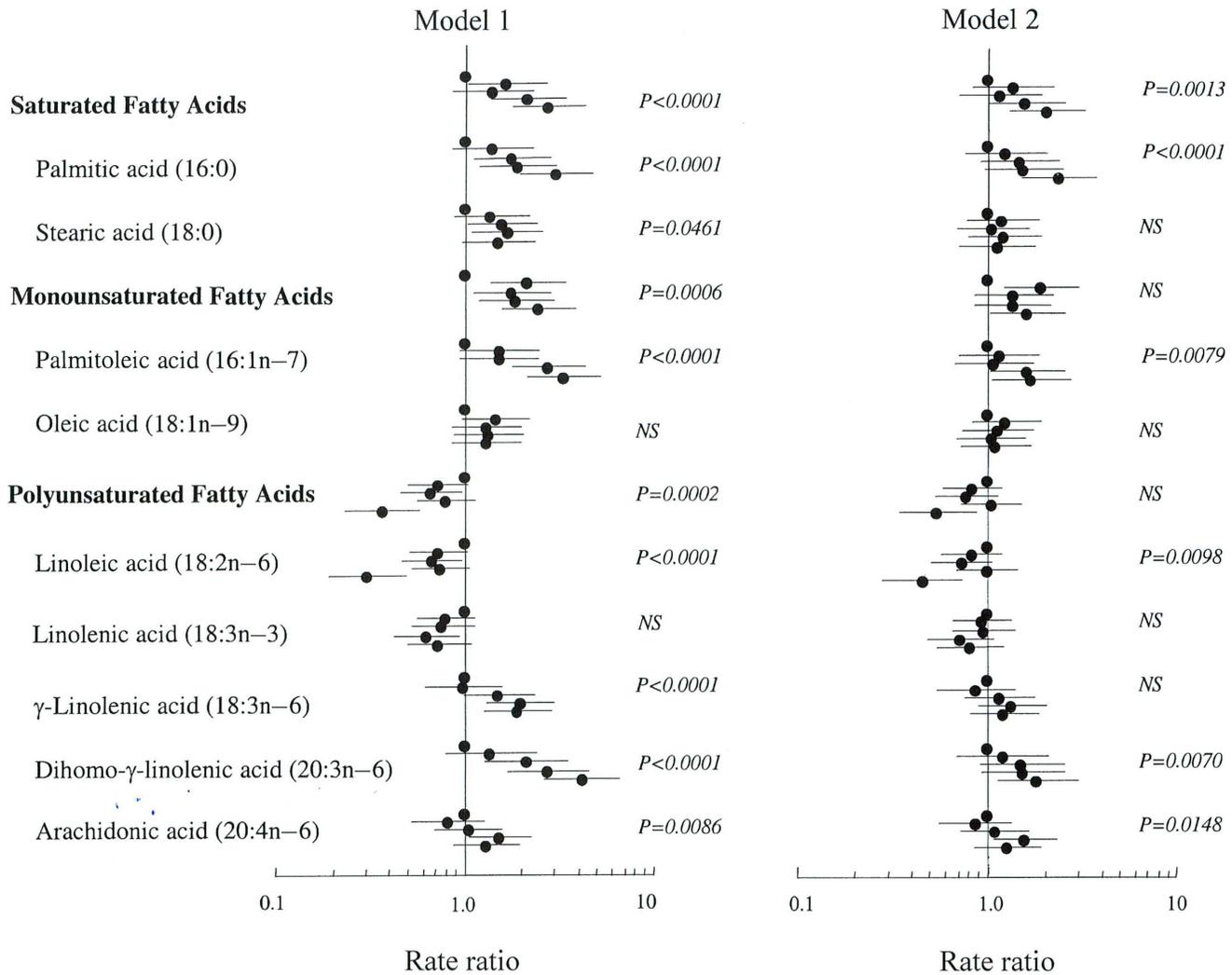


FIGURE 1. Rate ratios (and 95% CIs) for incident diabetes across quintiles of proportionate cholesterol ester fatty acid composition among participants in the Atherosclerosis Risk in Communities Study, Minneapolis Field Center, 1987–1998. Grouped fatty acids and each individual fatty acid constituent were examined separately in 2 multivariate models. Model 1 included adjustment for age (continuous) and sex. Model 2 included adjustment for the factors in model 1 plus body mass index (continuous), waist-to-hip ratio (continuous), cigarette-years of smoking (continuous), alcohol intake (continuous), sports index (continuous), education (\leq high school, college or vocational school, or graduate school), and parental history of diabetes (yes, no, or unknown). Rate ratios for incident diabetes in relation to the quintiles of fatty acid composition were estimated by using the lowest quintile of each fatty acid constituent or grouped fatty acids as the reference. *P* values represent tests for linear trends across quintiles.

tive estimate of the type of fats proportionately consumed by an individual (11). In the ARIC Study, the proportionate composition of SFAs and PUFAs in plasma CE and PL correlated fairly well with the dietary pattern (as a percentage of total fat) as assessed by a semiquantitative food-frequency questionnaire (6). The highest correlations between dietary and plasma fatty acid composition were found for long-chain PUFAs, which are derived primarily from diet. Plasma MUFAs did not reflect dietary MUFAs ($r \leq 0.05$), as would be expected given their endogenous synthesis.

Our results are consistent with those of most epidemiologic studies examining the association between dietary fat composition and risk of type 2 diabetes. Earlier ecologic studies pointed to a Western lifestyle (high intake of total and animal fat, obesity, and low intake of carbohydrate and fish) as the culprit for increased diabetes rates (12–15). Findings from cross-sectional

studies using dietary questionnaires have generally been compatible with a deleterious effect of dietary saturated fat on fasting or postload serum glucose concentrations (16–20). Although 4 earlier cohort studies reported no association between dietary factors and diabetes incidence (21–24), these null findings could be explained by underascertainment of clinically undiagnosed diabetes, invalid diet assessment, or other methodologic issues. With the use of validated food-frequency questionnaires, the Nurses' Health Study (25) and the Iowa Women's Health Study (26) drew a consistent conclusion that vegetable fat intake is inversely associated with the incidence of type 2 diabetes. The Health Professionals Follow-up Study (27) reported that intakes of total and saturated fat are associated with a higher risk of type 2 diabetes, although the association was not independent of BMI. Among the cohort studies that have assessed incident

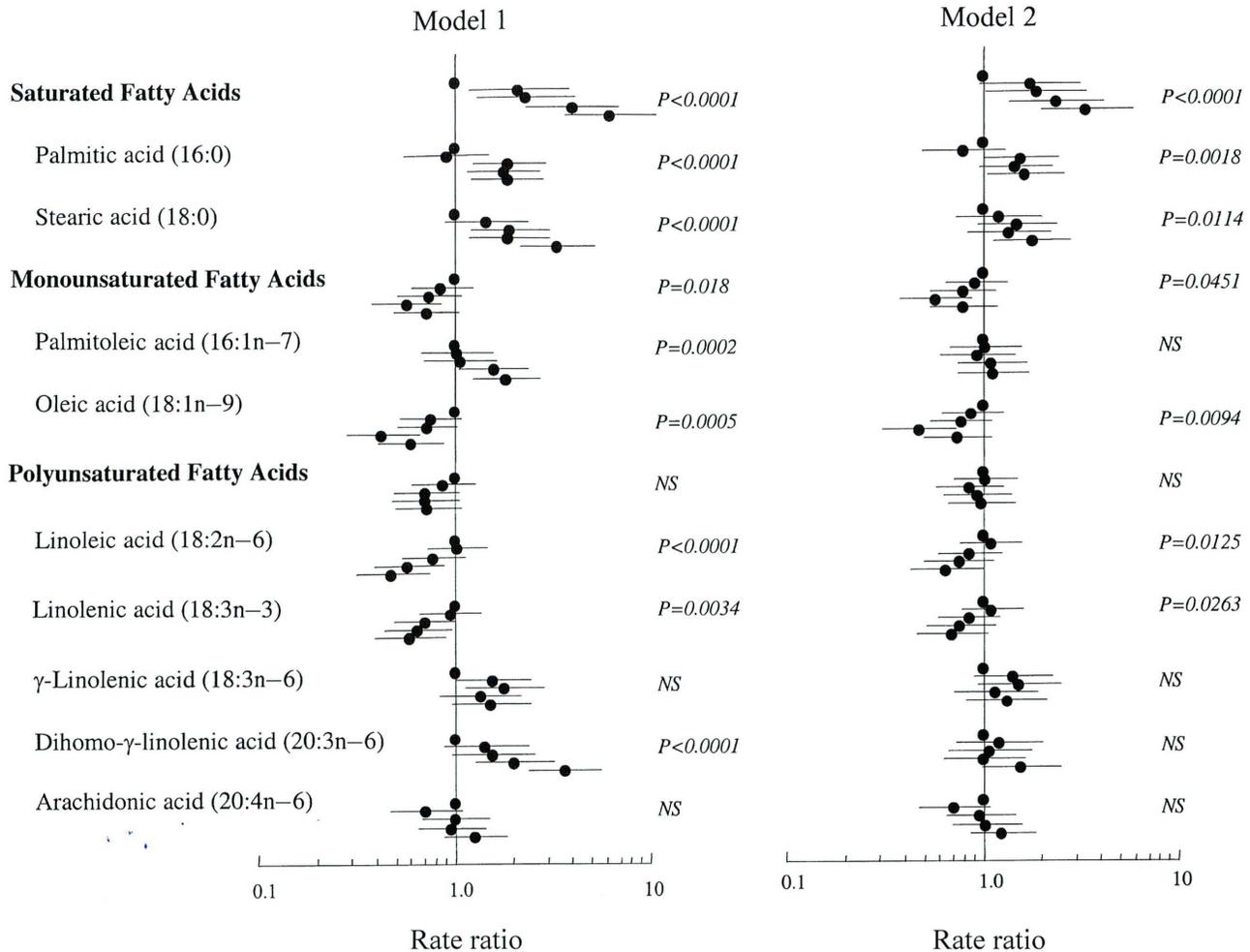


FIGURE 2. Rate ratios (and 95% CIs) for incident diabetes across quintiles of proportionate phospholipid fatty acid composition among participants in the Atherosclerosis Risk in Communities Study, Minneapolis Field Center, 1987–1998. Grouped fatty acids and each individual fatty acid constituent were examined separately in 2 multivariate models. Model 1 included adjustment for age (continuous) and sex. Model 2 included adjustment for the factors in model 1 plus body mass index (continuous), waist-to-hip ratio (continuous), cigarette-years of smoking (continuous), alcohol intake (continuous), sports index (continuous), education (\leq high school, college or vocational school, or graduate school), and parental history of diabetes (yes, no, or unknown). Rate ratios for incident diabetes in relation to the quintiles of fatty acid composition were estimated by using the lowest quintile of each fatty acid constituent or grouped fatty acids as the reference. *P* values represent tests for linear trends across quintiles.

diabetes by oral-glucose-tolerance-test screening, the Zutphen Elderly Study found that the incidence of glucose intolerance is significantly lower among subjects who usually eat fish than among those who do not (28). The Dutch and Finnish cohorts of the Seven Countries Study reported that intakes of SFAs and MUFAs 20 y before diagnosis were significantly higher in men with incident diabetes than in men with normal or impaired glucose tolerance (IGT) (29). Saturated fat was not a significant predictor of conversion of IGT to diabetes in the San Luis Valley Diabetes Study (30). However, in a study of Japanese Americans, intakes of animal fat and cholesterol were associated with an increased conversion of IGT to diabetes (31).

The results of several studies support a relation between biomarkers of fat intake and type 2 diabetes or its indicators (32–35). One study examined subjects with IGT, subjects with type 2 diabetes, and subjects with normal glucose tolerance (32). The proportions of 16:0, 16:1n-7, 18:3n-6, 20:3n-6, and arachidonic acid (20:4n-6) in serum CE were progressively higher from the

normal glucose tolerance group to the IGT group to the diabetes group. On the other hand, the proportion of 18:2n-6 was lower in diabetic subjects than in subjects with IGT or normal glucose tolerance. The other 2 cross-sectional studies (33, 34) also supported the hypothesis that serum fatty acid composition may modulate insulin action and that increased serum SFA concentrations are related to impaired glucose metabolism or diabetes. A 10-y prospective study (35) reported that subjects who developed diabetes had higher proportions of SFAs, 16:1n-7, 18:3n-6, and 20:3n-6 in serum CE at baseline than did nondiabetic subjects, but a lower proportion of 18:2n-6. These findings are largely consistent with ours.

Although several mechanisms have been proposed to link a high-fat diet to the development of type 2 diabetes (4), potential mechanisms linking fatty acid composition with diabetes are less clear. An increase in the SFA content of cell membranes leads to decreased membrane fluidity, decreased insulin receptor affinity, and an increased number of low-affinity receptors (36). Feeding a

diet with a high ratio of PUFAs to SFAs increases the PUFA content of the major PLs of the adipocyte plasma membrane and improves the rates of insulin-stimulated glucose transport, oxidation, and lipogenesis (37). Other postulated mechanisms involving fat composition include changes in the activities of enzymes related to glucose metabolism (1) and alterations in lipoprotein synthesis, hence, a switch in the fuel available for energy consumption (38). Long-chain PUFAs inhibit the expression of the glucose-6-phosphate dehydrogenase gene, whereas MUFAs do not (39), and palmitate and myristate selectively mimic the effect of glucose and thereby augment insulin secretion under Ca^{2+} -free conditions (40).

A few limitations of the present study deserve comment. First, the measurement of tissue fatty acid composition does not perfectly represent the proportion of fatty acids in the diet (6) because of variability between individuals in the cellular utilization and endogenous synthesis of fatty acids. In our study, greater alcohol intake, more cigarette smoking, higher BMI, higher WHR, and less physical activity were related to a higher proportion of SFAs and MUFAs but a lower proportion of PUFAs in plasma. Although we controlled for these nondietary determinants of fatty acids in our analysis, the associations observed may still to some degree reflect the effect of varying metabolic responses to dietary fat. Second, in the present study, individual fatty acids were expressed as proportions of total fatty acids. Because endogenously synthesized fatty acids are included in the denominator, and the total fatty acid proportions must sum to 100 percent, proportional fatty acid measurements are inherently interdependent, both biologically and mathematically. That is, a high percentage of SFAs will automatically reflect a low percentage of unsaturated fatty acids. This makes it difficult to interpret the effect of single fatty acid constituents, independent of other fatty acids. Third, in the ARIC Study, the short-term and long-term reliability of plasma fatty acid composition was better for the major fatty acids (reliability coefficients > 0.65) and lower for fatty acids that compose < 1% of total fatty acids.

The ARIC Study used fasting glucose concentrations to define diabetes according to standard criteria (9). Not using an oral-glucose-tolerance test would tend to lead to underascertainment of both prevalent diabetes and incident diabetes. Yet, there is no evidence that the misclassification of diabetes would be differential by fatty acid composition and therefore lead to bias. On the contrary, the error in diabetes ascertainment is more likely to be independently and uniformly distributed within the range of plasma fatty acid composition; thus, the current findings would be attenuated estimates of the true association.

The associations observed between fatty acid biomarkers and diabetes need to be interpreted with caution. The prospective design is a strength of this study, verifying that plasma fatty acid composition predicts subsequent diabetes. However, because abnormalities in insulin sensitivity and metabolism may precede overt diabetes by many years, plasma fatty acid composition may merely reflect metabolic changes secondary to hyperinsulinemia (41, 42) or "pre-diabetes." In the supplemental multivariable models, we therefore adjusted the RRs for baseline serum glucose and insulin concentrations and for other diabetic risk factors, and the results yielded were similar (data not shown). Furthermore, when the analyses were stratified on the basis of baseline glucose concentrations, similar results were obtained for both glucose strata (< 110 and 110–125 mg/dL), which suggests that reverse causation is unlikely.

Confounding by obesity is another concern. Because obesity is the major risk factor for diabetes, we adjusted for baseline values

of BMI and WHR. In a supplemental analysis, we also adjusted for the change in weight during follow-up, because of a concern that a high SFA intake may lead to an increase in weight and in the proportion of SFAs in plasma but not be causally related to diabetes. However, literature linking dietary fatty acid composition to obesity is limited (43), and obesity is likely to be intermediate step between dietary fat intake and diabetes. Therefore, adjustment for BMI and WHR ratio could be an overadjustment. Finally, although we considered several known risk factors for diabetes to disentangle the independent association of fat composition with diabetes risk, we did not take into account other dietary factors that have been hypothesized to be related to diabetes development, such as the glycemic index, vitamin E, or cereal fiber.

In summary, our data showed that plasma PL and CE concentrations of SFAs are positively associated with the development of diabetes. Our findings are generally consistent with previous research on fatty acid composition and diabetes incidence and support the hypothesis that the dietary fat profile may play a role in the etiology of diabetes. 

We thank Linda Lewis for analyzing the plasma fatty acids.

LW was responsible for data analysis and manuscript preparation. ARF was responsible for data collection and manuscript preparation. Z-JZ was responsible for data analysis and critical editorial input. JSP was responsible for critical editorial input. JHE was responsible for funding and data collection. None of the authors had any financial or personal interest in any company or organization sponsoring the research.

REFERENCES

- Lichtenstein AH, Schwab US. Relationship of dietary fat to glucose metabolism. *Atherosclerosis* 2000;150:227–43.
- Fickova M, Hubert P, Cremel G, Leray C. Dietary (n–3) and (n–6) polyunsaturated fatty acids rapidly modify fatty acid composition and insulin effects in rat adipocytes. *J Nutr* 1998;128:512–9.
- Jucker BM, Cline GW, Barucci N, Shulman GI. Differential effects of safflower oil versus fish oil feeding on insulin-stimulated glycogen synthesis, glycolysis, and pyruvate dehydrogenase flux in skeletal muscle: a ^{13}C nuclear magnetic resonance study. *Diabetes* 1999;48:134–40.
- Feskens EJ, van Dam RM. Dietary fat and etiology of type 2 diabetes: an epidemiological perspective. *Nutr Metab Cardiovasc Dis* 1999;9:87–95.
- Vessby B. Dietary fat and insulin action in humans. *Br J Nutr* 2000;83(suppl):S91–6.
- Ma J, Folsom AR, Shahar E, Eckfeldt JH. Plasma fatty acid composition as an indicator of habitual dietary fat intake in middle-aged adults. *Am J Clin Nutr* 1995;62:564–71.
- The ARIC Investigators. The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. *Am J Epidemiol* 1989;129:687–702.
- Ma J, Folsom AR, Eckfeldt JH, Lewis L, Chambless LE. Short- and long-term repeatability of fatty acid composition of human plasma phospholipids and cholesterol esters. *Am J Clin Nutr* 1995;62:572–8.
- Anonymous. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 1997;20:1183–97.
- Baecke JA, Burema J, Frijters JE. A short questionnaire for the measurement of habitual physical activity in epidemiologic studies. *Am J Clin Nutr* 1982;36:936–42.
- Riboli E, Ronnholm H, Saracci R. Biological markers of diet. *Cancer Surv* 1987;6:685–718.
- O'Dea K. Obesity and diabetes in "the land of milk and honey." *Diabetes Metab Rev* 1992;8:373–88.
- West KM, Kalbfleisch JM. Influence of nutritional factors on prevalence of diabetes. *Diabetes* 1971;20:99–108.

14. Kawate R, Yamakido M, Nishimoto Y, Bennett PH, Hamman RF, Knowler WC. Diabetes mellitus and its vascular complications in Japanese migrants on the island of Hawaii. *Diabetes Care* 1979;2:161-70.
15. Kromann N, Green A. Epidemiological studies in the Upernavik District, Greenland. Incidence of some chronic diseases 1950-1974. *Acta Med Scand* 1980;208:401-6.
16. Feskens EJ, Kromhout D. Habitual dietary intake and glucose tolerance in euglycaemic men: The Zutphen Study. *Int J Epidemiol* 1990;19:953-9.
17. Trevisan M, Krogh V, Freudenheim J, et al. Consumption of olive oil, butter, and vegetable oils and coronary heart disease risk factors. The Research Group ATS-RF2 of the Italian National Research Council. *JAMA* 1990;263:688-92.
18. Tsunehara CH, Leonetti DL, Fujimoto WY. Diet of second-generation Japanese-American men with and without non-insulin-dependent diabetes. *Am J Clin Nutr* 1990;52:731-8.
19. Marshall JA, Hamman RF, Baxter J. High-fat, low-carbohydrate diet and the etiology of non-insulin-dependent diabetes mellitus: the San Luis Valley Diabetes Study. *Am J Epidemiol* 1991;134:590-603.
20. Mooy JM, Grootenhuys PA, de Vries H, et al. Prevalence and determinants of glucose intolerance in a Dutch Caucasian population. The Hoorn Study. *Diabetes Care* 1995;18:1270-3.
21. Bennett PH, Knowler WC, Baird HR, Butler WJ, Pettitt DJ, Raid JM. Diet and development of noninsulin-dependent diabetes mellitus: an epidemiological perspective. In: Pozza G, Micossi P, Catapano AL, Paoletti R, eds. *Diet, diabetes, and atherosclerosis*. New York: Raven Press, 1984:109-19.
22. Medalie JH, Papier C, Herman JB, et al. Diabetes mellitus among 10,000 adult men. I. Five-year incidence and associated variables. *Isr J Med Sci* 1974;10:681-97.
23. Feskens EJ, Kromhout D. Cardiovascular risk factors and the 25-year incidence of diabetes mellitus. The Zutphen Study. *Am J Epidemiol* 1989;130:1101-8.
24. Lundgren H, Bengtsson C, Blohme G, et al. Dietary habits and incidence of noninsulin-dependent diabetes mellitus in a population of women in Gothenburg, Sweden. *Am J Clin Nutr* 1989;49:708-12.
25. Salmeron J, Hu FB, Manson JE, et al. Dietary fat intake and risk of type 2 diabetes in women. *Am J Clin Nutr* 2001;73:1019-26.
26. Meyer KA, Kushi LH, Jacobs DR Jr, Folsom AR. Dietary fat and incidence of type 2 diabetes in older Iowa women. *Diabetes Care* 2001;24:1528-35.
27. van Dam RM, Stampfer M, Willett WC, Hu FB, Rimm EB. Dietary fat and meat intake in relation to risk of type 2 diabetes in men. *Diabetes Care* 2002;25:417-24.
28. Feskens EJ, Bowles CH, Kromhout D. Inverse association between fish intake and risk of glucose intolerance in normoglycemic elderly men and women. *Diabetes Care* 1991;14:935-41.
29. Feskens EJ, Virtanen SM, Rasanen L, et al. Dietary factors determining diabetes and impaired glucose tolerance. A 20-year follow-up of the Finnish and Dutch cohorts of the Seven Countries Study. *Diabetes Care* 1995;18:1104-12.
30. Marshall JA, Hoag S, Shetterly S, Hamman RF. Dietary fat predicts conversion from impaired glucose tolerance to NIDDM. *Diabetes Care* 1994;17:50-6.
31. Leonetti DL, Tsunehara CH, Wahl PW, Fujimoto WY. Baseline dietary intake and physical activity of Japanese American men in relation to glucose tolerance at 5-year follow-up. *Am J Hum Biol* 1996;8:55-67.
32. Salomaa V, Ahola I, Tuomilehto J, et al. Fatty acid composition of serum cholesterol esters in different degrees of glucose intolerance: a population based study. *Metabolism* 1990;39:1285-91.
33. Vessby B, Tengblad S, Lithell H. Insulin sensitivity is related to the fatty acid composition of serum lipids and skeletal muscle phospholipids in 70-year-old men. *Diabetologia* 1994;37:1044-50.
34. Folsom AR, Ma J, McGovern PG, Eckfeldt JH. Relation between plasma phospholipid saturated fatty acids and hyperinsulinemia. *Metabolism* 1996;45:223-8.
35. Vessby B, Aro A, Skarfors E, Berglund L, Salminen I, Lithell H. The risk to develop NIDDM is related to the fatty acid composition of the serum cholesterol esters. *Diabetes* 1994;43:1353-7.
36. Ginsberg BH, Brown TJ, Simon I, Spector AA. Effect of the membrane lipid environment on the properties of insulin receptors. *Diabetes* 1981;30:773-80.
37. Field CJ, Ryan EA, Thomson AB, Clandinin MT. Diet fat composition alters membrane phospholipid composition, insulin binding, and glucose metabolism in adipocytes from control and diabetic animals. *J Biol Chem* 1990;265:11143-50.
38. Storlien LH, Kraegen EW, Chisholm DJ, Ford GL, Bruce DG, Pascoe WS. Fish oil prevents insulin resistance induced by high-fat feeding in rats. *Science* 1987;237:885-8.
39. Stabile LP, Klautky SA, Minor SM, Salati LM. Polyunsaturated fatty acids inhibit the expression of the glucose-6-phosphate dehydrogenase gene in primary rat hepatocytes by a nuclear posttranscriptional mechanism. *J Lipid Res* 1998;39:1951-63.
40. Komatsu M, Sharp GW. Palmitate and myristate selectively mimic the effect of glucose in augmenting insulin release in the absence of extracellular Ca^{2+} . *Diabetes* 1998;47:352-7.
41. Eck MG, Wynn JO, Carter WJ, Faas FH. Fatty acid desaturation in experimental diabetes mellitus. *Diabetes* 1979;28:479-85.
42. Horrobin DF. Fatty acid metabolism in health and disease: the role of delta-6-desaturase. *Am J Clin Nutr* 1993;(suppl):732S-7S.
43. Storlien LH, Hulbert AJ, Else PL. Polyunsaturated fatty acids, membrane function and metabolic diseases such as diabetes and obesity. *Curr Opin Clin Nutr Metab Care* 1998;1:559-63.