Relationship of Dietary Fat and Serum Cholesterol Ester and Phospholipid Fatty Acids to Markers of Insulin Resistance in Men and Women With a Range of Glucose Tolerance

Jennifer C. Lovejoy, Catherine M. Champagne, Steven R. Smith, James P. DeLany, George A. Bray, Michael Lefevre, Yvonne M. Denkins, and Jennifer C. Rood

High-fat diets are associated with insulin resistance, however, this effect may vary depending on the type of fat consumed. The purpose of this study was to determine the relationship between intakes of specific dietary fatty acids (assessed by 3-day diet records and fatty acid composition of serum cholesterol esters [CEs] and phospholipids [PLs]) and glucose and insulin concentrations during an oral glucose tolerance test (OGTT). Nineteen men and 19 women completed the study. Nine subjects had type 2 diabetes or impaired glucose tolerance. Fasting insulin correlated with reported intakes of total fat (r = .50, P < .01), monounsaturated fat (r = .44, P < .01), and saturated fat (r = .49, P < .01), but not with *trans* fatty acid intake (r = .11, not significant [NS]). Fasting glucose also correlated with total (r = .39, P < .05) and monounsaturated fat intakes (r = .37, P < .05). In multivariate analysis, both total and saturated fat intake were strong single predictors of fasting insulin ($R^2 \sim .25$), and a model combining dietary and anthropometric measures accounted for 47% of the variance in fasting insulin. Significant relationships were observed between fasting insulin and the serum CE enrichments of myristic (C14:0), palmitoleic (C16:1), and dihomo-γ-linolenic (C20:3n-6) acids. In multivariate analysis, a model containing CE 14:0 and percent body fat explained 45% of the variance in fasting insulin, and C14:0 and age explained 30% of the variance in fasting glucose. PL C20:3n-6 explained 30% of the variance in fasting insulin, and a model including PL C18:1n-11 *cis,* C20:3n-6, age and body fat had an R^2 of .58. In conclusion, self-reported intake of saturated and monounsaturated fats, but not trans fatty acids, are associated with markers of insulin resistance. Furthermore, enhancement of dihomo-γ-linolenic and myristic acids in serum CE and PL, presumably markers for dietary intake, predicted insulin resistance.

HIGH INTAKES OF dietary fat have been associated with obesity and its comorbid conditions (reviewed in Bray and Popkin[1]). A number of studies have shown an association between high dietary fat intakes and insulin resistance in both animals[2-5] and humans.[6-9] Because insulin resistance has been associated with the development of both diabetes and heart disease, the association between dietary fat and insulin resistance may have great public health significance.

Certain classes of fatty acids may have a more deleterious effect on insulin action than others (reviewed in Lovejoy[10]). In animal models, Storlien et al[3,11] have shown that consumption of saturated and polyunsaturated fat induces severe insulin resistance, while monounsaturated fats and omega-3 fatty acids from fish oils are less detrimental. In humans, saturated fatty acid intake has been shown to be a significant independent predictor of fasting and postprandial insulin in middle-aged men,[12] as well as young men and women.[13]

The composition of lipids in serum or muscle (markers of dietary fatty acid intake) also correlates with insulin resistance. In a cross-sectional population study of over 4,000 healthy individuals, Folsom et al[14] found that fasting insulin (a marker of insulin resistance) was positively associated with the percentage of saturated fat in plasma phospholipids (PLs) and inversely associated with percentage of monounsaturated fat. Strong associations between increased palmitoleic acid (C16:1) and dihomo-γ-linolenic acid (C20:3n-6) in serum cholesterol esters (CEs) and subsequent risk of developing type 2 diabetes have also been reported.[15]

In the last decade, potential adverse health effects of *trans* fatty acids have come to light. *Trans* fatty acid intake has been associated with increased cardiovascular risk in some studies,[16,17] although not all.[18] Recently, a small study in obese individuals with type 2 diabetes showed that diets high in either saturated or *trans* fatty acids produced elevated insulin levels during an oral glucose tolerance test (OGTT) compared with diets high in (*cis*)oleic acid.[19] This is the only published study to our knowledge to address the question of *trans* fatty acids and insulin resistance in human populations, and it suggests that, at least in people with diabetes, *trans* fatty acid intake may have adverse effects on insulin action.

Taken as a whole, there is a strong suggestion that specific dietary fatty acids influence insulin resistance and increase the risk of type 2 diabetes mellitus. The purpose of the present study was to determine the relationship between self-reported dietary intake, serum CE and PL fatty acid composition, and insulin and glucose during an OGTT in human volunteers with a range of glucose tolerance. We hypothesized that increased intakes and plasma fatty acid enrichments of saturated and *trans* fatty acids would be associated with higher fasting insulin, glucose, insulin/glucose ratio during OGTT, and homeostasis model insulin resistance. The results support this hypothesis with regard to saturated, but not *trans*, fatty acids.

From the Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, LA.

Submitted February 9, 2000; accepted June 21, 2000.

Supported by US Department of Agriculture Grant No. 96034323-3031 (to G.A.B.).

Address reprint requests to Jennifer C. Lovejoy, PhD, Pennington Biomedical Research Center, 6400 Perkins Rd, Baton Rouge, LA 70808-4124.

0026-0495/01/5001-0008$10.00/0

doi:10.1053/meta.2001.19440

MATERIALS AND METHODS

Human Subjects

Volunteers were recruited from the Baton Rouge metropolitan area using flyers and newspaper advertisements. Men and women of any ethnic background who were over 18 years of age were included. Those taking medication (including birth control pills or hormone replacement therapy) with any chronic disease (other than type 2 diabetes), who reported a history of clotting problems or who had high levels of physical activity were excluded. All volunteers signed an informed consent form before participation in the study. Pennington Biomedical Research Center's Institutional Review Board approved the protocol, consent form, and all advertisements associated with the study.

Methods

A 2-hour OGTT was performed after an overnight fast. After collection of baseline blood samples, 75 g of glucose (Trutol, Baltimore, MD) was given orally, and additional blood was collected at 30, 60, and 120 minutes after glucose administration. Some volunteers (N = 12) had the OGTT performed as part of screening for another protocol, which only required blood collection at 0, 60, and 120 minutes. Insulin resistance was estimated by fasting insulin concentration and the ratio of the area under the glucose and insulin curves during the OGTT (a higher ratio of insulin to glucose area indicates relative insulin resistance).[20] Additionally, homeostasis model assessment (HOMA) insulin resistance (R) was calculated using the mathematical approximations described by Matthews et al.[21] Specifically, R = (fasting insulin $\times$ fasting glucose)/22.5.

All volunteers completed a 3-day diet record after a training session with a dietitian. The period during which the record was kept included 1 weekend day and 2 weekdays, and the record was collected within 1 to 2 weeks of metabolic testing. The diet record was analyzed using the Moore's Extended Nutrient (MENu) Database (copyright Pennington Biomedical Research Foundation, 1998). The *trans* fatty acid content of foods consumed was imputed using data from 3 main sources.[22-24] Some references contained information that matched closely with the products the subject consumed. For foods not identified by brand-name information, data from the American Society of Clinical Nutrition/American Institute of Nutrition (ASCN/AIN) Task Force on *Trans* Fatty Acids[22] were modified to reflect the average for disappearance and availability data. This yielded factors of 16.2% and 3.5% for percentage of *trans* from total fat for vegetable oil and animal/dairy foods, respectively.

Dual energy x-ray absorptiometry (DEXA; Hologic QDR2000, Waltham, MA) was performed to determine the body composition (lean and fat mass) of each subject using Hologic enhanced whole-body version 6.0 software.

Fatty Acid Composition in Serum

All volunteers provided a fasting serum sample for determination of serum CE and PL fatty acid content. Fatty acids in serum CEs and PLs are thought to reflect fat intake over the past 4 to 6 weeks.[25,26] Previous reports indicate that the CE fatty acids are most closely correlated with dietary fat intake, although there is generally a high correlation between the CE and PL fractions.[27]

Total plasma lipids were extracted with 2:1 chloroform/methanol. Preparative thin layer chromatography (TLC) was used to isolate CEs and PLs. The TLC plates were developed in a solution of hexane, ethyl ether, and acetic acid (79:20:1, vol:vol:vol), and bands were visualized with iodine. Methyl esters of the separated lipids were prepared by heating with a solution of BF_3-methanol, methanol, and benzene (34:30:36, vol:vol:vol) at 100°C for 45 minutes for CE and for 90 minutes for PLs. Saturated NaCl solution was then added to the reaction mixtures for the separation of organic layer from the aqueous phase by centrifuge. Fatty acid methyl esters were extracted in the organic layer. All samples were dried under nitrogen and stored in hexane under nitrogen at −20°C until analysis by gas chromatography.

Laboratory Analyses

Glucose was determined using the glucose oxidase method on a Beckman Synchron CX7 instrument (Beckman, Brea, CA). Insulin concentrations were determined using a microparticle enzyme immunoassay on an Abbott IMx analyzer (Abbott Laboratories, Abbott Park, IL). This assay has <1% cross-reactivity with proinsulin.

Statistical Analyses

All statistical analyses were performed using SAS-PC version 6.12 (SAS, Cary, NC). Descriptive data were obtained on all variables using the "univariate" procedure with an option allowing assessment of the normality of the distribution. All variables that were not normally distributed (including fasting insulin and glucose and the ratio of insulin/glucose area) were log transformed before analysis. Univariate correlation analysis between variables of interest was performed using Pearson product-moment correlations on log transformed data. Partial correlation values adjusted for total kcal are reported for all dietary fat intakes. Multiple regression analysis with R^2 selection was subsequently performed to determine optimal models for prediction of the metabolic parameters (log insulin, log glucose, log insulin/glucose ratio, and HOMA R). Three separate models were run: (1) a model including age, percent body fat, and dietary intakes of total fat, monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), saturated fat, and fiber; (2) a model with age, percent body fat, and serum CE fatty acid enrichments of C14:0, C16:1, and C20:3n-6, and C20:5n-3; and (3) a model with age, percent body fat, and serum PL fatty acid enrichments of C14:0, C18:1n-11 *cis*, C20:3n-6, and C20:5n-3. Total energy intake (kcal) was forced into the multiple regression model for all dietary fatty intakes to adjust absolute fat intake for total intake. Mean square error (MSE) was used in conjunction with R^2 to determine the optimal model for each variable.

RESULTS

Characteristics of the 19 male and 19 female subjects are shown in Table 1. The participants included had a wide range of age, degree of obesity, and intakes of total dietary fat. Five of the volunteers had type 2 diabetes by oral glucose tolerance criteria (2 of these had fasting glucose levels > 125 mg/dL), and 4 had impaired glucose tolerance by OGTT. The volunteers with abnormal glucose tolerance did not differ in any substantial way in age, body fat, or dietary fat intakes from those with normal glucose tolerance (data not shown).

Univariate correlation analysis results for dietary intakes are shown in Table 2. Log fasting insulin concentrations were significantly positively associated with age, body fat, and body mass index (BMI), as well as self-reported intakes of percent of calories from total fat, MUFA, and saturated fat (Fig 1). Fasting insulin did not correlate with PUFA or *trans* fatty acid intakes. Fasting glucose concentrations also correlated positively with total dietary fat intake, MUFA, and cholesterol, as well as with age and BMI. The ratio of the insulin/glucose area under the curve during OGTT correlated positively with percent body fat and inversely with dietary fiber intake. The analyses were also run on the data set after omitting individuals with abnormal

Table 1. Characteristics of the 19 Male and 19 Female Study Participants

Variable	Females	Males
Age (yr)	34.5 ± 3.2 (19.0-74.0)	34.9 ± 3.6 (19.0-71.0)
BMI (kg/m^2)	27.4 ± 1.8 (18.7-41.5)	28.0 ± 0.87 (20.9-34.2)
Percent body fat	40.7 ± 2.8 (23.3-56.8)	27.6 ± 1.7 (17.6-46.1)
Fasting insulin (μU/mL)	8.4 ± 1.0 (2.5-16.6)	10.9 ±2.1 (2.6-37.0)
Fasting glucose (mg/dL)	91.3 ± 1.9 (79-109)	103.4 ± 6.7 (74-210)
Dietary fat (g/d)	54.6 ± 5.8 (17.1-123.6)	80.1 ± 6.3 (24.4 + 142.5)
Dietary fat (% kcals)	31.5 ± 2.1 (16.5-48.6)	31.9 ± 1.6 (18.5-45.4)
Dietary monounsaturated fat (g/d)	21.1 ± 2.4 (7.6-47.3)	31.7 ± 2.6 (9.4-61.9)
Dietary polyunsaturated fat (g/d)	12.7 ± 1.6 (3.8-27.3)	14.4 ± 1.2 (5.6-25.7)
Dietary saturated fat (g/d)	16.6 ± 1.8 (4.1-39.1)	27.2 ± 2.6 (7.6-52.9)
Dietary *trans* fatty acids (g/d)	4.6 ± 0.6 (1.6-9.7)	6.0 ± 0.6 (2.5-11.9)
Dietary cholesterol (mg/d)	170.9 ± 18.3 (38.2-333)	320.3 ± 33.8 (77.9-754.1)

NOTE. Data are mean ± SE with range shown below.

glucose tolerance, and the correlation coefficients remained similar (data not shown).

A multiple regression analysis was performed to determine dietary and anthropometric predictors of metabolic parameters. Variables included in the model were age, percent body fat, and total fat, MUFA, PUFA saturated fat, and fiber intakes in grams. Total kcal intake was forced into the model to adjust all dietary variables for energy intake. Total fat intake was the strongest single predictor of fasting insulin concentration, with an R^2 of .26, although both saturated fat intake and percent body fat were nearly as strong as single predictors (R^2 = .25 and .23, respectively). The best combined model included age, saturated fat, and fiber and accounted for 54% of the variance in fasting insulin.

Table 2. Pearson Correlation Coefficients (*R* values) Between Anthropometric, Metabolic, and Dietary Variables in 38 Men and Women

	Log Insulin	Log Glucose	Log Insulin/ Glucose	HOMA Insulin Resistance (R)
Age (yr)	.45†	.40§	.10	.51†
Body fat (%)	.46†	.12	.49†	.27
BMI (kg/m^2)	.63‡	.33§	.37	.52‡
Dietary fat (g/d)*	.50†	.38§	.16	.43†
Dietary carbohydrate (g/d)	−.58‡	−.44†	−.16	−.52†
Dietary protein (g/d)	.21	.13	.12	.18
MUFA (g/d)	.44†	.37§	.13	.36§
PUFA (g/d)	.20	.06	.17	.09
Saturated fat (g/d)	.49†	.39§	.09	.50†
Trans fat (g/d)	.11	−.10	.28	−.02
Cholesterol (g/d)	.50†	.51†	.15	.48†
Fiber (g/d)	−.33	.16	−.37§	−.06

* Partial correlation coefficient values adjusted for total kcal intake are reported for all dietary intake variables.

† $P < .01$.

‡ $P < .001$.

§ $P < .05$.

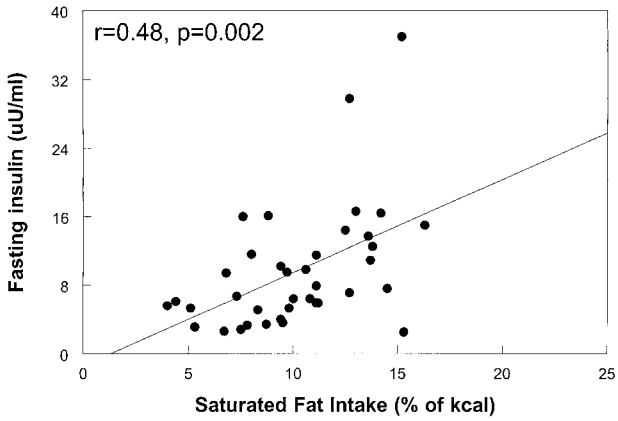

Fig 1. Association between saturated fat intake as a percent of energy and fasting insulin concentration in 38 volunteers. Exclusion of 2 individuals with fasting insulin greater than 30 μU/mL did not substantially alter the correlation (r = .43, P = .008).

Age was the strongest single predictor of the variance in fasting glucose (R^2 = .25), with dietary fat intakes and percent body fat accounting for 17% to 18% of the variance independently. The combined model with age, MUFA, and fiber accounted for 49% of the variance in fasting glucose. For the insulin/glucose ratio, fiber independently explained 22% of the variance, with percentage body fat, PUFA intake, and total fat intake all independently explaining roughly 11% of the variance in this parameter. The best combined model, consisting of fiber, MUFA, and body fat, explained only 26% of the variance in insulin/glucose ratio. Finally, for HOMA R, both age and saturated fat intake were strong independent predictors (R^2 = .27 and .25, respectively), and a combined model with age, percent body fat, total dietary fat, and saturated fat explained 52% of the variance in HOMA R.

Table 3 shows univariate correlations between fatty acid composition of serum CEs and metabolic parameters. Significant positive relationships were observed between log fasting insulin and the CE enrichments of myristic acid (C14:0), palmitoleic acid (C16:1), dihomo-γ-linolenic acid (C20:3n-6), and eicosapentanoic acid (EPA; C20:5n-3). Fasting glucose was also significantly associated with C14:0 and C20:3n-6 enrichment in CE fatty acids. Insulin resistance assessed by HOMA showed a similar pattern to fasting insulin, correlating significantly with C14:0, C16:1, C20:3n-6, and C20:5n-3 (Table 3). The ratio of the insulin/glucose area under the curve did not significantly correlate with any CE fatty acid. In general, similar correlations were seen between the metabolic parameters and PL fatty acid enrichments of C14:0 and C20:3n-6, how-

Table 3. Pearson Correlation Coefficients (*R* values) Between Serum Cholesterol Ester Fatty Acid Profiles and Metabolic Risk Factors in 38 Men and Women

	Log Insulin	Log Glucose	Log Insulin/ Glucose	HOMA Insulin Resistance (R)
C14:0	.46†	.45†	−.11	.55†
C16:0	.22	.11	−.16	.21
C16:1	.37‡	.23	.04	.34‡
C18:0	.06	−.10	−.17	−.02
C18:1n-9 *cis*	−.01	−.13	−.08	−.10
C18:1n-9 *trans**	−.11	.43	−.14	−.05
C18:2n-6 *trans*	−.19	−.17	−.26	−.15
C18:3n-6	.28	.06	.17	.14
C18:3n-3	.02	.22	−.17	.03
C20:3n-6	.43†	.32‡	−.01	.48†
C20:4n-6	−.04	−.13	.28	−.06
C20:5n-3	.42†	.31	.03	.45†

* Only 15 subjects had detectable C18:1 *trans* fatty acid in their serum cholesterol esters.

† $P < .01$.

‡ $P < .05$.

ever, fasting insulin and HOMA R were also correlated inversely with C18:1n-11 *cis* in the PL fraction ($r = -.33$ and $r = -.36$, respectively; $P < .05$), and were not significantly correlated with C16:1.

Multiple regression analyses were performed to look at CE and PL fatty acids as predictors of fasting insulin and glucose in a model that also included age and percent body fat (Table 4). The results of this analysis showed that both CE enrichment of C14:0 and percentage body fat were significant independent predictors of fasting insulin concentration, each independently explaining 24% of the variance, while C20:3ω6 accounted for 21% of the variance. A model with both C14:0 and body fat accounted for 44% of the variance in fasting insulin. CE C14:0 enrichment was also a significant predictor of fasting glucose, accounting for 28% of the variance in this parameter, and a model with C14:0 and age accounted for 30% of the variance in fasting glucose. For HOMA insulin resistance, C14:0 independently accounted for 37% of the variance, while a model including C14:0, age, and C20:5n-3 accounted for 48% of the variance.

In the PL fraction analysis, C20:3n-6 enrichment, percent body fat, and age explained significant amounts of the variance in fasting insulin levels (30%, 29%, and 26%, respectively). A model containing C20:3n-6, C18:1n-11 *cis*, age, and body fat explained 58% of the variance in fasting insulin. Age and C20:3n-6 in PLs independently accounted for 18% and 16% of the variance in fasting glucose, and a model with C20:3n-6, C20:5n-3, and age explained 34% of the variance. For HOMA R, age and C20:3n-6 were significant independent predictors ($R^2 = .29$ and .26, respectively) and a model containing age, C18:1n-11 *cis*, C20:3n-6, and C20:5n-3 explained 57% of the variance.

DISCUSSION

The results of this study confirm that high total dietary fat intake is associated with adverse effects on markers of insulin resistance in humans with a range of glucose tolerance. In addition, these data show independent negative effects of dietary saturated and monounsaturated fatty acids, but not trans fatty acids, on insulin concentrations. Furthermore, greater enrichments of myristic acid (C14:0), palmitoleic acid (C16:1), dihomo-γ-linolenic acid (C20:3n-6), and EPA (C20:5n-3) in serum CEs and PLs were related to markers of insulin resistance.

Our results are consistent with the majority of studies in both animals and humans in showing that saturated fatty acid intake is associated with insulin resistance. Storlien et al[3] have systematically investigated the question of dietary fatty acids and insulin resistance in animal models and have shown that feeding saturated fat induces severe insulin resistance in rats. Hunnicutt et al[28] confirmed that long-term exposure to palmitic acid produces insulin resistance in isolated rat adipocytes with minimal effects on insulin binding, suggesting that the insulin resistance is due to a postreceptor mechanism. In humans, Parker et al[12] found that higher dietary saturated fat intakes were associated with hyperinsulinemia, and that saturated fatty acids were significant independent predictors of both fasting and postprandial insulin in middle-aged men.

The composition of lipids in membranes of various tissues (a marker of dietary fatty acid intake) has been previously reported to correlate with insulin action. In healthy men, the greater the percentage of long-chain (C20 to C22) polyunsaturated fatty acids in muscle membrane phospholipids, the greater the individual's insulin sensitivity while the more saturated the membrane lipid, the greater the insulin resistance.[29] A strong inverse relationship between palmitic acid (C16:0) content of muscle PLs and insulin sensitivity has been observed,[30] as has an association between increased palmitoleic acid (C16:1) in serum CEs and subsequent risk of developing type 2 diabetes.[15] Our observation that myristic and palmitoleic acids are associated with increased insulin concentrations is consistent with these epidemiologic findings, despite the fact that these fatty acids occur in relatively low amounts in serum lipids (<5%). We also observed an inverse relationship between PL C18:1n-11 *cis* and fasting insulin and HOMA R, although the more common MUFA, C18:1n-9 (oleic acid), did not correlate significantly with either variable ($r = -.20$ and $-.26$, respectively).

The observed positive association between dihomo-γ-linolenic acid (C20:3n-6) and fasting insulin is also consistent with previous reports. Vessby et al[15] observed that C20:3n-6 was a significant predictor of the longitudinal development of type 2 diabetes in a Swedish population and Das[31] found that the level of C20:3n-6 was significantly decreased in patients with type 2 diabetes compared with nondiabetic controls. Dihomo-γ-linolenic acid levels are likely to represent metabolism of linoleic acid, and diets low in linoleic acid have been shown to produce high C20:3n-6 levels in serum CEs.[32] Furthermore, as an intermediary metabolite in the arachidonic acid pathway, C20:3n-6 is directly converted to series 1 prostanoids, which are potent inhibitors of platelet aggregation and vasoconstriction. Although C20:3n-6 is generally less than 1.5% of fatty acids isolated from serum CEs and PLs (Fig 2), it is possible that insulin action may be influenced through its conversion to

Table 4. Multiple Regression Analysis to Identify Serum Fatty Acid Predictors of Fasting Insulin, Glucose, and HOMA Insulin Resistance (R)

Dependent Variable	No. in Model	Independent Variables	R^2	Mean Square Error
Cholesterol ester fatty acids				
Log insulin	1	% body fat	.24	.07
		C14:0	.24	.07
		C20:3n-6	.21	.07
	2	C14:0, % body fat	.44	.05
	3	C14:0, C16:1, % body fat	.46	.05
	4	C14:0, C16:1, C20:5n-3, % body fat	.48	.05
Log glucose	1	C14:0	.28	.004
		Age	.16	.005
		C20:3n-6	.12	.005
	2	C14:0, age	.30	.004
	3	C14:0, C20:5n-3, age	.38	.004
	4	C14:0, C20:3n-6, C20:5n-3, age	.41	.004
HOMA R	1	C14:0	.37	3.69
		Age	.25	4.44
		C20:3n-6	.25	4.45
	2	C14:0, age	.42	3.48
	3	C14:0, C20:5n-3, age	.48	3.29
	4	C14:0, C20:5n-3, age, % body fat	.50	3.29
Phospholipid Fatty Acids				
Log insulin	1	C20:3 ω6	.30	.07
		% body fat	.29	.07
		Age	.26	.08
	2	C20:3n-6, % body fat	.54	.05
	3	C18:1n-11 *cis,* C20:3n-6, % body fat	.57	.05
	4	C18:1n-11 *cis,* C20:3n-6, age, % body fat	.58	.05
Log glucose	1	Age	.18	.006
		C20:3n-6	.16	.006
		C20:5n-3	.04	.007
	2	C20:3n-6, age	.26	.006
	3	C20:3n-6, C20:5n-3, age	.34	.005
	4	C20:3n-6, C20:5n-3, age, % body fat	.35	.005
HOMA R	1	Age	.29	5.30
		C20:3n-6	.26	5.50
		C18:1n-11 cis	.13	6.51
	2	C20:3n-6, age	.42	4.48
	3	C20:3n-6, C20:5n-3, age	.52	3.90
	4	C18:1n-11 *cis,* C20:3n-6, C20:5n-3, age	.57	3.64

series 1 prostaglandins, even at low levels. It is also possible that the effect is mediated through modulation of membrane fluidity, as discussed above.

Other mechanisms could also explain the impact of n-6 fatty acids on insulin action. Jucker et al[33] recently compared the effects of safflower oil (n-6) versus fish oil (n-3) feeding on metabolic fluxes in rat skeletal muscle. Using ^{13}C nuclear magnetic resonance imaging, these investigators showed that n-6-feeding increased intramuscular triglyceride content, produced insulin resistance, and decreased muscle glycolytic flux relative to n-3 fatty acid-feeding. This suggests that n-6 fatty acids may produce muscle insulin resistance by increasing fat oxidation and decreasing pyruvate dehydrogenase flux.

Given these findings, it is somewhat surprising that a positive association between serum EPA (C20:5n-3) and insulin levels was observed. Previous studies of EPA content of fatty acids in serum[15] or muscle[29] have found no relationship with insulin resistance or diabetes risk. Fish oil-feeding studies in individuals with diabetes have produced conflicting results, with some finding increases in insulin secretion after an EPA-rich meal[34] and others finding decreased insulin secretion and worsened glycemic control after 1 month of fish oil supplementation.[35] Studies in which fish oils rich in docosahexanoic acid (DHA; C22:6n-3) relative to EPA have been fed to volunteers, however, have shown beneficial effects on insulin sensitivity.[36] Further study is needed to determine the independent effects of EPA and DHA on insulin sensitivity in humans.

As mentioned previously, there has only been one study, to our knowledge, addressing *trans* fatty acid effects on insulin action in humans, although animal studies have shown that trans fatty acids increase insulin secretion.[37] In obese, type 2 diabetic patients, Christiansen et al[19] showed that a diet high in

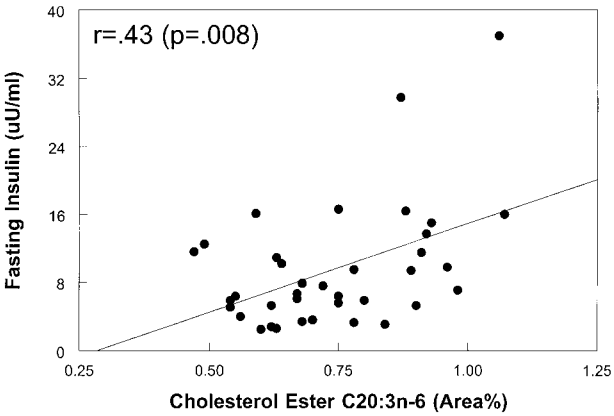

Fig 2. Association between serum cholesterol ester C20:3n-6 and fasting insulin concentration in 38 volunteers. Exclusion of 2 individuals with fasting insulin greater than 30 μU/mL did not substantially alter the correlation ($r = .34$, $P \pm .04$).

trans fatty acids produced hyperinsulinemia during an OGTT compared with a diet high in *cis* fatty acids. In the present study, we did not observe an association between either self-reported *trans* fatty acid intake or *trans* fatty acid enrichment in serum lipids and insulin concentrations during OGTT. It is possible that this discrepancy is due to methodologic differences between the present study and that of Christiansen et al.[19] For example, their study involved counseling participants in certain eating patterns, whereas the present study examined self-reported habitual intakes. In addition, Christiansen et al[19] used patients with diabetes, while our study included individuals with a range of glucose tolerance.

Several limitations of the present study deserve comment. First, as a descriptive study, the results indicate associations between fatty acid intakes and metabolic factors and cannot address causation. Studies are presently underway in our laboratory to test experimentally the effects of feeding specific fatty acids on insulin action. Furthermore, as in many studies examining dietary factors and health risks, we relied on self-reported intake data from free-living volunteers. It is well known that dietary self-report data tend to underestimate total intakes, and food records may not represent typical intakes. For this reason, we chose to also analyze fatty acid enrichments in serum CEs and PLs as biomarkers for dietary fat intake.

Another limitation of our study was the lack of any direct measure of whole body insulin sensitivity such as euglycemic clamp, minimal model method, or insulin suppression test. Because our basic goal was to screen a heterogeneous mix of individuals to determine whether dietary trans fatty acids related to metabolic risk, we chose to use surrogate markers for insulin resistance including fasting insulin, the ratio of insulin to glucose area during an OGTT, and the HOMA method. While these measures have been reported to correlate well with direct measures of whole-body insulin sensitivity,[20,38] they are clearly indirect markers. Nevertheless, the results of our study are in general agreement with studies in which direct measures of insulin sensitivity were used, such as the Insulin Resistance Atherosclerosis Study (IRAS), which reported significant associations between total fat intake and intakes of specific fatty acids and insulin sensitivity.[39]

The use of surrogate measures of insulin sensitivity in the present study may, however, explain the differences in the magnitude of correlations between the dietary measures and the different markers of insulin action. While both fasting insulin and HOMA insulin sensitivity were well correlated with several dietary fatty acid measures, the insulin/glucose ratio was generally not correlated with dietary intakes and correlated with different serum fatty acids than did the other measures. This was unexpected and may indicate that the insulin/glucose ratio during OGTT is a poorer marker of insulin sensitivity in this diverse population or, at least, that it is reflecting a different aspect of carbohydrate metabolism and insulin action.

Finally, we need to comment on statistical power and the distinction between statistically and physiologically relevant results. In general, the present study had statistical power to detect correlation values in the range of .30 or greater. This corresponds to an R^2 of greater than or equal to .09. It is likely that anything that accounts for less than 9% of the variance in a given variable is not strongly biologically or clinically relevant. It is also worth noting that while total body fatness and/or age accounted for significant portions of the variance in fasting insulin and other metabolic markers, the dietary intakes and relevant serum fatty acid components were strongly and independently predictive in many cases (Table 4). Nevertheless, the regression models that provided the best explanatory power almost always included body fatness (or age), as well as the dietary markers. Thus, as expected, body fatness is clearly an important marker for metabolic risk. Neither do our data discount the significant role that genetic factors play in determining risk, although this was not assessed in the present study, because at most, only ~50% of the variance in any metabolic parameter was explained by dietary and anthropometric factors. The results may, however, imply that high dietary fat intake, which is strongly predictive of obesity, mediates some of the risk factors associated with obesity.

In summary, this study showed significant negative effects of total dietary fat, saturated, and monounsaturated fat intakes on fasting glucose and markers of insulin resistance. Saturated fatty acids, in particular, were associated with adverse metabolic profiles in these volunteers with a range of glucose tolerance. Additionally, the data indicate that dihomo-γ-linolenic, myristic, and palmitoleic acids in serum CE and PL are associated with markers of insulin resistance. Further studies are needed to address the mechanisms by which fat intake alters insulin action, and to confirm experimentally that feeding specific fatty acids alters insulin sensitivity both acutely and chronically in humans.

ACKNOWLEDGMENT

The authors would like to thank the research volunteers who participated in these studies. The following individuals are thanked for their technical contributions to the collection of the data: Mark Klemperer, Paige McCown, Chris Ricord, and Susan Mancuso.

REFERENCES

1. Bray GA, Popkin BM: Dietary fat intake does affect obesity! Am J Clin Nutr 68:149-1150, 1998
2. Storlien LH, James DE, Burleigh KM, et al: Fat feeding causes widespread in vivo insulin resistance, decreased energy expenditure and obesity in rats. Am J Physiol 251:E567-583, 1986
3. Storlien LH, Pan DA, Kriketos AD, et al: High fat diet-induced insulin resistance. Lessons from animal studies. Ann N Y Acad Sci 683:82-90, 1993
4. Kraegen EW, Clark PW, Jenkins AB, et al: Development of muscle insulin resistance after liver insulin resistance in high-fat fed rats. Diabetes 40:1397-1403, 1991
5. Yakubu F, Lin D, Peters JC, et al: Insulin action in rats is influenced by amount and composition of dietary fat. Obesity Res 1:481-488, 1993
6. Chen M, Bergman RN, Porte D Jr: Insulin resistance and beta-cell dysfunction of aging: The importance of dietary carbohydrate. J Clin Endocrinol Metab 67:951-957, 1988
7. Fukagawa NK, Anderson JW, Hageman G, et al: High-carbohydrate, high-fiber diets increase peripheral insulin sensitivity in healthy young and old adults. Am J Clin Nutr 52:524-528, 1990
8. Lovejoy J, DiGirolamo M: Habitual dietary intake and insulin sensitivity in lean and obese adults. Am J Clin Nutr 55:1174-1179, 1992
9. Lovejoy JC, Windhauser MM, Rood JC, et al: Effect of a controlled high-fat versus low-fat diet on insulin sensitivity and leptin levels in African-American and Caucasian women. Metabolism 47: 1520-1524, 1998
10. Lovejoy JC: Dietary fatty acids and insulin resistance. Curr Atherosclerosis Rep 1:215-220, 1999
11. Storlien LH, Jenkins AB, Chisholm DJ, et al: Influence of dietary fat composition on the development of insulin resistance in rats: Relationship to muscle triglyceride and ω-3 fatty acids in muscle phospholipids. Diabetes 40:280-289, 1991
12. Parker DR, Weiss ST, Troisi R, et al: Relationship of dietary saturated fatty acids and body habitus to serum insulin concentrations: The Normative Aging Study. Am J Clin Nutr 58:129-136, 1993
13. Ludwig DS, Pereira MA, Kroenke CH, et al: Dietary fiber, weight gain, and cardiovascular disease risk factors in young adults. JAMA 282:1539-1546, 1999
14. Folsom AR, Ma J, McGovern PG, et al: Relation between plasma phospholipid saturated fatty acids and hyperinsulinemia. Metabolism 45:223-228, 1996
15. Vessby B, Aro A, Skarfors E, et al: The risk to develop NIDDM is related to the fatty acid composition of serum cholesterol esters. Diabetes 43:1353-1357, 1994
16. Ascherio A, Hennekens CH, Buring JE, et al: Trans-fatty acids intake and risk of myocardial infarction. Circulation 89:94-101, 1994
17. Willett WC, Stampfer MJ, Manson JE, et al: Intake of trans fatty acids and risk of coronary heart disease among women. Lancet 341: 581-585, 1993
18. Aro A, Kardinall AF, Salminen I, et al: Adipose tissue isomeric trans fatty acids and risk of myocardial infarction in nine countries: The EURAMIC study. Lancet 345:273-278, 1995
19. Christiansen E, Schnider S, Palmvig B, et al: Intake of a diet high in trans monounsaturated fatty acids or saturated fatty acids: Effects on postprandial insulinemia and glycemia in obese patients with NIDDM. Diabetes Care 20:881-886, 1997
20. Reaven G: The role of insulin resistance in human disease. Diabetes 37:1595-1607, 1988
21. Matthews DR, Hosker JP, Rudenski AS, et al: Homeostasis model assessment: Insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28: 412-419, 1985
22. ASCN/AIN Task Force on Trans Fatty Acids: Special Task Force Report: Position paper on *trans* fatty acids. Am J Clin Nutr 63:663-670, 1996.
23. United States Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory (USDA/ARS/NDL): Provisional Table of *Trans* Fatty Acid Content of Foods. Riverdale, MD, USDA/ ARS/NDL, 1995
24. Wootan M, Liebman B, Rosofsky W: *Trans:* The phantom fat. Nutr Action Healthletter 23:10-13, 1996
25. Nikkari T, Luukkainen P, Pietinen P, et al: Fatty acid composition of serum lipid fractions in relation to gender and quality of dietary fat. Ann Med 27:491-498, 1995
26. Ma J, Folsom AR, Shahar E, et al: Plasma fatty acid composition as an indicator of habitual dietary fat intake in middle-aged adults. Am J Clin Nutr 62:564-571, 1995
27. Nikkari T. Serum fatty acids and coronary heart disease in Finnish populations. Prog Lipid Res 25:437-450, 1986
28. Hunnicutt JW, Hardy RW, Williford J, et al: Saturated fatty acid-induced insulin resistance in rat adipocytes. Diabetes 43:540-545, 1994
29. Borkman M, Storlien LH, Pan DA, et al: The relation between insulin sensitivity and the fatty-acid composition of skeletal-muscle phospholipids. N Engl J Med 328:238-244, 1993
30. 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 37:1044-1050, 1994
31. Das UN: Essential fatty acid metabolism in patients with hypertension, diabetes mellitus and coronary heart disease. Prostaglandins Leukot Essent Fatty Acids 52:387-391, 1995
32. Lasserre M, Mendy F, Spielmann D, et al: Effects of different dietary intake of essential fatty acids on C20:3 omega 6 and C20:4 omega 6 serum levels in human adults. Lipids 20:227-233, 1985
33. Jucker BM, Cline GW, Barucci M, et al: Differential effects of safflower oil versus fish oil feeding on insulin-stimulated glycogen synthesis, glycolysis, and pyruvate dehydrogenase flux in skeletal muscle. Diabetes 48:134-140, 1999
34. Lardinois CK, Starich GH, Mazzaferri EL, et al: Polyunsaturated fatty acids augment insulin secretion. J Am Coll Nutr 6:507-515, 1987
35. Glauber H, Wallace P, Griver K, et al: Adverse metabolic effect of omega-3 fatty acids in non-insulin dependent diabetes mellitus. Ann Intern Med 108:663-668, 1988
36. Popp-Snijders C, Schouten JA, Heine RJ, et al: Dietary supplementation of omega-3 fatty polyunsaturated fatty acids improves insulin sensitivity in non-insulin dependent diabetes. Diabetes Res 4:141-147, 1987
37. Alstrup KK, Gregersen S, Jensen HM, et al: Differential effects of *cis* and *trans* fatty acids on insulin release from isolated mouse islets. Metabolism 48:22-29, 1999
38. Anderson RL, Hamman RF, Savage PJ, et al: Exploration of simple insulin sensitivity measures derived from frequently-sampled intravenous glucose tolerance (FSIGT) tests. Am J Epidemiol 142:724-732, 1995
39. Mayer-Davis EJ, Monaco JH, Hoen HM, et al: Dietary fat and insulin sensitivity in a triethnic population: the role of obesity. The Insulin Resistance Atherosclerosis Study (IRAS). Am J Clin Nutr 65:79-87, 1997