Relation Between Plasma Phospholipid Saturated Fatty Acids and Hyperinsulinemia

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We determined whether plasma phospholipid fatty acid levels, an indicator of fatty acid composition in the diet, are associated with fasting serum insulin concentrations (a marker of insulin resistance). We examined cross-sectionally 4,304 middle-aged adults free of diabetes. Plasma fatty acid composition was quantified by gas chromatography. Fasting insulin was strongly and positively associated with the saturated fatty acid percentage in plasma phospholipids, moderately and inversely associated with the monounsaturated percentage, and not appreciably associated with the polyunsaturated percentage. Fasting insulin adjusted for age, smoking status, alcohol consumption, and sports participation, for example, was 29% higher in men and 33% higher in women per 1.9% greater level of saturated fatty acids (the interquartile range). After adjustment for body mass index (BMI) and other covariates, these estimates were 12% and 15% ($P < .01$ for the difference from zero). A 1.9% greater increment in saturated fatty acid level was also associated with a 2.4-fold higher odds of hyperinsulinemia (fasting insulin ≥ 143.5 pmol/L). These data are consistent with studies showing that fatty acid composition of cell membranes modulates insulin action, and support the hypothesis that increased habitual saturated fat intake or a related dietary pattern is a risk factor for hyperinsulinemia.

CONSIDERABLE evidence indicates that insulin resistance and its metabolic consequences play important roles in non–insulin-dependent diabetes mellitus and atherosclerotic cardiovascular disease. Insulin resistance is associated with obesity, disordered glucose metabolism, dyslipidemia, impaired fibrinolysis, and elevated blood pressure.[1] Hyperinsulinemia, a marker of insulin resistance, has been associated with increased atherosclerosis[2,3] and increased incidence of coronary heart disease.[4-6] However, this latter association has been disputed.[7]

It has been suggested for some time that excess total fat intake may lead to insulin resistance, either by direct effects on insulin action or indirectly by increasing obesity.[8] Recent evidence points further to a possible role of dietary fatty acid composition. Increasing the polyunsaturated fatty acid content of membranes of cultured cells increases the number and binding of insulin receptors and the rate of insulin-stimulated glucose transport.[9,10] Saturated fatty acids have an opposite effect.[9,11] A decreased concentration of polyunsaturated fatty acids in human skeletal muscle phospholipids is associated with decreased insulin sensitivity.[12] Even though short-term human feeding trials have not shown any consistent effect of dietary fat composition on insulin action,[13] habitual dietary saturated fat intake as measured by questionnaires is associated positively with blood insulin levels.[14-17]

The fatty acid composition of plasma phospholipids is a reasonably accurate, objective marker of dietary fat composition[18,19] and may reflect the generalized membrane response to a given pattern of fat intake. We therefore examined the association between plasma fatty acid composition and insulin levels cross-sectionally in a large sample of middle-aged adults participating in the Atherosclerosis Risk in Communities (ARIC) Study.[20] We hypothesized that insulin would be associated positively with saturated fatty acids and negatively with polyunsaturated fatty acids and the polyunsaturated to saturated (P/S) fatty acid ratio measured in plasma phospholipids.

SUBJECTS AND METHODS

The ARIC Study is a prospective investigation of atherosclerosis and cardiovascular disease being conducted in four US communities.[20] From 1987 through 1989, 15,800 participants, men and women, 45 to 64 years old, underwent a comprehensive clinical examination. Plasma phospholipid and cholesterol ester fatty acid composition was measured in nearly 4,000 participants in the Minneapolis ARIC field center.

Participants were asked to fast for 12 hours. Blood was drawn from seated participants, with less than 2 minutes' tourniquet time, into vacuum tubes containing EDTA or serum-separating gel. Plasma was separated and stored at −70°C. Insulin levels were measured in serum within a few weeks, and plasma fatty acids in EDTA-plasma approximately 2 years later. Moilanen and Nikkari[21] reported negligible deterioration of serum unsaturated fatty acids at −60°C over 1 year. Because of the chelating properties of EDTA, a low storage temperature (−70°C), and no evidence of drift in our frozen control plasma over the 2 years of analysis, we believe unsaturated fatty acid deterioration during storage was minimal.

Serum insulin was assessed by radioimmunoassay (^{125}Insulin Kit; Cambridge Medical Diagnostics, Billerica, MA). Serum glucose was assessed by the hexokinase method. An intraindividual variability study was conducted by repeatedly measuring subjects over several weeks; the intraclass correlation coefficient for insulin was 0.81 and for glucose 0.84. The method coefficient of variation in the mid-normal range for insulin was 14.3% and for glucose 2.4%.

After thawing, 0.5 mL EDTA-plasma for fatty acid measurement was extracted under a nitrogen atmosphere with 0.5 mL methanol followed by 1.0 mL chloroform, and the lipid extract was filtered to remove protein. Phospholipid and cholesterol ester fractions were separated by thin-layer chromatography using a silica gel plate (Silica Gel H; Analtech, Newark, DE) and a two-stage mobile-phase development, using 80:20:1 (by volume) and 40:60:1 (by

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volume) mixtures of petroleum ether, diethyl ether, and glacial acetic acid, respectively. The plate was dried between development solvents, and the second mobile phase was allowed to migrate only half the plate length. After redrying, one lane was sprayed with dichlorofluorescein to visualize the phospholipid, cholesterol ester, triglyceride, and free fatty acid bands under UV light. Phospholipid and cholesterol ester bands were scraped into separate test tubes, and the lipids were converted to methyl esters of fatty acids by boron trifluoride catalysis.[22] The methyl esters were then separated and measured on a Hewlett Packard (Avondale, PA) 5890 gas chromatograph 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 a previously characterized synthetic mixture of fatty acids (GLC-68A; Nu-chek Prep, Elysian, MN). The relative amount of each fatty acid (percent of total fatty acid) was quantified by integrating the area under the peak and dividing the result by the total area for all fatty acids.

Groupings of saturated, monounsaturated, and polyunsaturated fatty acids were calculated by summing all the saturated, monounsaturated, or polyunsaturated acid peaks with 12- to 24-carbon atom chains. The P/S ratio was also calculated. Test-retest reliability coefficients, based on individuals sampled three times 2 weeks apart, ranged from 0.70 to 0.89 for individual major phospholipid fatty acids, except oleic acid ([18:1n-9] $r = .39$) and n-3 polyunsaturated fatty acids, for which reliability coefficients ranged from 0.31 to 0.58.[23]

Body mass index (BMI) was defined as weight in kilograms, divided by height in meters squared. Smoking status and usual alcohol consumption were determined by interview. A sports participation index was obtained through a leisure time questionnaire.[24]

Of 4,009 Minneapolis ARIC participants, 3,935 had fatty acid measurements. The number of nonwhite participants (n = 61), too small for meaningful analysis separately, was excluded, as were subjects taking lipid-lowering drugs (n = 56) or fish oil supplements (n = 109), because these might have altered fatty acid metabolism. We further excluded those who had not fasted (n = 112) or who had a history of or were treated for diabetes (n = 164), because these influence serum insulin concentrations. The final analysis included 3,403 participants (1,619 men and 1,784 women). The SAS package[25] was used for analysis. Arithmetic means, medians, quartile values, and Pearson correlation coefficients were calculated for descriptive purposes. To improve normality, fasting insulin subsequently was natural log-transformed. Age-adjusted geometric mean insulin levels were computed among quartiles of fatty acid composition and BMI using analysis of covariance by PROC GLM. Associations were further examined using multiple linear (least-squares) regression, using natural log–fasting insulin as the dependent variable, fatty acids as separate independent variables, and adjustment for other specified covariates. Participants were also categorized on whether they had fasting hyperinsulinemia ($\geq$143.5 pmol/L [$\geq$20 μU/mL]), and a logistic regression analysis was used to calculate odds ratios in relation to the major fatty acid categories. Because conclusions for fatty acid composition of phospholipids and cholesterol esters were virtually identical, we chose to present phospholipid results, mentioning only selected findings for cholesterol esters.

RESULTS

Among participants free of diabetes and cardiovascular disease, fasting mean serum insulin concentration was 68.5 pmol/L (median, 57.4); mean serum glucose was 5.6 mmol/L (Table 1). The fatty acid composition of phospholipids was approximately 41% saturates, 10% monounsaturates, and 43% polyunsaturates, and the remaining 6% related to various undescribed chromatographic peaks. The standard deviation of phospholipid fatty acid levels was narrow, reflecting relatively tight physiologic control.

Table 1. Mean ± SD and 25th, 50th, and 75th Percentile Values of Fasting Serum Insulin and Glucose, BMI, and Phospholipid Fatty Acids in 45- to 64-Year-Old Adults Free of Diabetes and Cardiovascular Disease (N = 3,403)

Variable	Mean ± SD	Percentile		
		25th	50th	75th
Insulin (pmol/L)	68.5 ± 47	36	57	86
Glucose (mmol/L)	5.56 ± 0.55	5.19	5.50	5.83
BMI (kg/m^2)	26.9 ± 4.5	23.7	26.3	29.4
Fatty acids (%)*				
SFA	40.6 ± 1.4	39.7	40.7	41.6
Palmitic (16:0)	25.4 ± 1.6	24.2	25.2	26.4
Stearic (18:0)	13.3 ± 1.2	12.5	13.3	14.1
MUFA	10.0 ± 1.3	9.2	9.8	10.6
Palmitoleic (16:1n-7)	0.64 ± 0.18	0.52	0.61	0.71
Oleic (18:1n-9)	8.6 ± 1.2	7.8	8.5	9.2
PUFA	42.8 ± 1.7	41.8	42.9	43.9
Linoleic (18:2n-6)	22.0 ± 2.7	20.4	22.0	23.7
Linolenic (18:3n-3)	0.14 ± 0.05	0.11	0.14	0.17
Di-homo-γ-linolenic (20:3n-6)	3.3 ± 0.8	2.8	3.3	3.8
Arachidonic (20:4n-6)	11.5 ± 2.0	10.1	11.4	12.7
Eicosapentaenoic (20:5n-3)	0.55 ± 0.28	0.39	0.51	0.65
Docosahexaenoic (22:6n-3)	2.8 ± 0.9	2.2	2.6	3.2
P/S	1.06 ± 0.07	1.01	1.06	1.10

Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

*Percent of total fatty acids.

Figure 1 depicts the sex-specific, age-adjusted geometric mean insulin level by quartiles of grouped saturated, monounsaturated, and polyunsaturated fatty acids, P/S ratio, and BMI. For both men and women, fasting insulin was associated positively with saturates and BMI and negatively with P/S ratio and monounsaturates. Geometric mean fasting insulin was more than 50% higher for quartile 4 than for quartile 1 of plasma saturated fatty acids.

Associations between insulin and fatty acids depicted in Fig 1 did not materially change with further adjustment for smoking status, alcohol intake, and sports participation score. For example, for an interquartile range increase in saturated fatty acids, fasting serum insulin was greater by a sizable 29% in men to 33% in women (Table 2). The association was greater for stearic acid (18:0) than for palmitic acid (16:0). A comparable interquartile range increment in monounsaturates, principally oleic acid (18:1), was accompanied by an 8% to 13% lower fasting insulin, even though the uncommon monounsaturate, 16:1n-7, was associated positively with fasting insulin. Polyunsaturates of the n-6 and n-3 series had inconsistent associations with fasting insulin, although the most common, linoleic (18:2n-6), was associated negatively with fasting insulin in

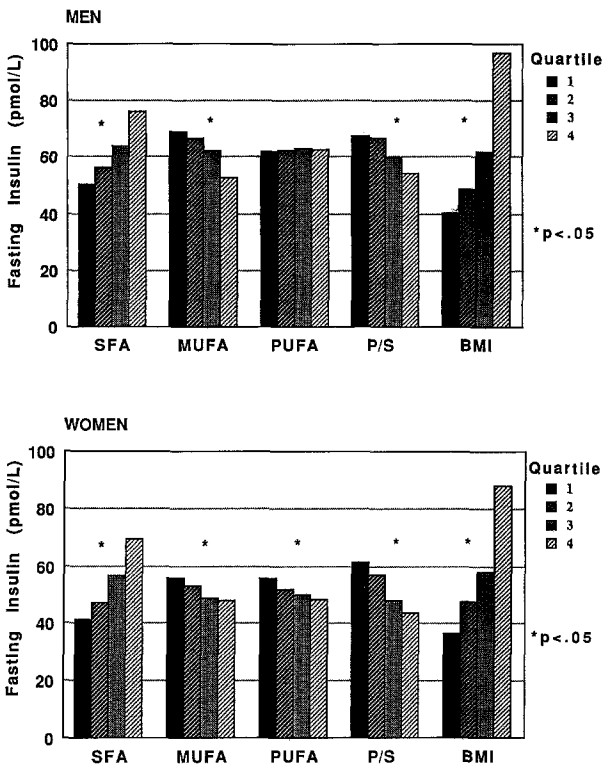

Fig 1. Age-adjusted geometric mean fasting insulin level according to quartiles of plasma phospholipid fatty acids and BMI in adults aged 45 to 64 years free of diabetes. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

women. The fatty acid, 20:3n-6, was strongly positively associated with fasting insulin.

BMI was associated positively with fasting insulin ($r = .54$) such that for an interquartile increase in BMI, fasting insulin was greater by 54% in men and 38% in women. BMI and fasting glucose concentration were correlated positively with plasma saturated fatty acid percentage ($r = .29$ and $r = .21$, respectively), but were not correlated with polyunsaturates ($r = -.05$ and $r = .01$). With further adjustment for BMI and fasting glucose concentration (Table 3), associations between fatty acids and serum insulin were consistently attenuated by approximately 50% (Table 3 *v* Table 2). Many of the associations with individual fatty acids lost statistical significance with adjustment; however, statistically significant independent associations with insulin level persisted for higher levels of saturates (especially 18:0) and lower P/S ratios. For an interquartile range increase in saturated fatty acids, adjusted for BMI and fasting glucose, fasting serum insulin was greater by 12% in men and 15% in women.

Approximately 7.6% of the sample had elevated fasting insulin values of at least 143.5 pmol/L ($\geq$20 μU/mL). Logistic regression analysis demonstrated that saturated fatty acids were associated with hyperinsulinemia independently of monounsaturates and polyunsaturates, which were themselves not associated independently with hyperinsulinemia (Table 4). For an increment equal to the interquartile range of phospholipid saturated fatty acids, the odds ratio for hyperinsulinemia was elevated 3.7-fold. Even with further adjustment for BMI and fasting glucose, this

Table 2. Sex-Specific, Adjusted Percentage Difference (Mean ± SE) in Fasting Serum Insulin in Relation to Specified Phospholipid Fatty Acid Differences in 45- to 64-Year-Old Adults Free of Diabetes and Cardiovascular Disease

Fatty Acid		Insulin Difference (%)‡	
Type	Δ%†	Men (n = 1,160)	Women (n = 1,137)
SFA	1.9	+29 ± 2*	+33 ± 2*
16:0	2.2	+4 ± 2	+6 ± 2*
18:0	1.6	+33 ± 2*	+29 ± 2*
MUFA	1.4	−13 ± 2*	−8 ± 2*
16:1n-7	0.2	+7 ± 2*	+8 ± 2*
18:1n-9	1.4	−14 ± 2*	−10 ± 2*
PUFA	2.1	+1 ± 2	−6 ± 2*
18:2n-6	3.3	−2 ± 2	−9 ± 2*
18:3n-3	0.06	−3 ± 2	−5 ± 2*
20:3n-6	1.0	+33 ± 2*	+32 ± 2*
20:4n-6	2.6	−7 ± 2*	+2 ± 2
20:5n-3	0.3	+1 ± 2	+7 ± 2*
22:6n-3	1.0	+2 ± 2	−6 ± 2*
P/S	0.09	−14 ± 2*	−22 ± 2*

NOTE. See Table 1 for abbreviations.

*$P \leq .01$.

†Fatty acid increase in percentage points equal to the total sample's interquartile range (ie, from the 25th to 75th percentile).

‡Adjusted for age, smoking status, alcohol intake, and sports score using multiple linear regression. Each fatty acid was in a separate model.

Table 3. Sex-Specific, Adjusted Percentage Difference (mean ± SE) in Fasting Serum Insulin in Relation to Specified Phospholipid Fatty Acid Differences in 45- to 64-Year-Old Adults Free of Diabetes and Cardiovascular Disease, With Additional Adjustment for Fasting Glucose and BMI

Fatty Acid		Insulin Difference (%)‡	
Type	Δ%†	Men (n = 1,160)	Women (n = 1,137)
SFA	1.9	+12 ± 2*	+15 ± 2*
16:0	2.2	−1 ± 2	+2 ± 2
18:0	1.6	+18 ± 2*	+15 ± 2*
MUFA	1.4	−6 ± 1*	−3 ± 1
16:1n-7	0.2	+4 ± 2	+3 ± 1
18:1n-9	1.4	−6 ± 2*	−3 ± 2
PUFA	2.1	+1 ± 2	−3 ± 2
18:2n-6	3.3	+2 ± 2	−3 ± 2
18:3n-3	0.06	0 ± 2	0 ± 1
20:3n-6	1.0	+18 ± 2*	+15 ± 2*
20:4n-6	2.6	−8 ± 2*	−3 ± 2
20:5n-3	0.3	+1 ± 1	+2 ± 1
22:6n-3	1.0	+2 ± 1	−3 ± 1
P/S	0.09	−6 ± 2*	−11 ± 2*

*$P \leq .01$.

†Fatty acid increase in percentage points equal to the total sample's interquartile range (ie, from the 25th to 75th percentile).

‡Adjusted for age, smoking status, alcohol intake, sports score, fasting glucose, and BMI using multiple linear regression. Each fatty acid was in a separate model.

Table 4. Odds Ratios and 95% Confidence Intervals for Fasting Hyperinsulinemia in Relation to Specified Phospholipid Fatty Acid Differences in 45- to 64-Year-Old Adults Free of Diabetes and Cardiovascular Disease

Fatty Acid Group	Δ%	Odds Ratio	
		Model 1†	Model 2‡
Saturated	1.9	3.7 [2.7, 4.9]*	2.4 [1.7, 3.3]*
Monounsaturated	1.4	0.8 [0.6, 1.2]	0.9 [0.6, 1.4]
Polyunsaturated	2.1	1.2 [0.8, 1.8]	1.2 [0.7, 1.9]

NOTE. 95% confidence intervals are in brackets.

*$P < .0001$.

†Odds ratio for hyperinsulinemia (≥143.5 pmol/L) per increase in each fatty acid group's interquartile range. Fatty acids were adjusted for each other and for age, sex, smoking status, alcohol intake, and sports score.

‡Also adjusted for BMI and glucose concentration.

odds ratio was 2.4. In a similar model using the cholesterol ester data (not shown), the odds ratio for hyperinsulinemia associated with an interquartile increment of saturated fatty acids was 1.9 ($P < .0001$).

DISCUSSION

This large cross-sectional study of middle-aged adults free of diabetes found that fasting serum insulin was associated positively with the percentage of saturated fatty acids in fasting plasma phospholipids. The detected association was strong: even after adjustment for BMI and other variables, an increment in saturated fatty acids equal to the interquartile range of phospholipid saturated fatty acids was associated with approximately a 14% higher fasting insulin concentration and 2.4-fold higher odds ratio of hyperinsulinemia. In contrast, insulin concentration had a weak negative association with monounsaturated fatty acids and no consistent association with polyunsaturated fatty acids. However, there was some variability among specific monounsaturated and polyunsaturated fatty acids. For example, palmitoleic acid (16:1n-7), which is mostly consumed with saturated fatty acids or synthesized from palmitic acid (16:0), was associated positively with insulin concentration, whereas oleic acid (18:1n-9) was associated negatively with insulin.

We used fasting serum insulin in the ARIC Study because sophisticated measures of insulin resistance or secretion are not feasible in a large epidemiologic study. In nondiabetics, fasting insulin has a relatively high correlation with insulin action measured using the hyperinsulinemic, euglycemic clamp technique or the intravenous glucose tolerance test with minimal modeling.[26] Fasting insulin also has been reported to be an independent risk factor for coronary heart disease,[4-6] although the etiologic role of insulin remains controversial.[7]

Fatty acid composition was expressed as a percentage of total fatty acid mass in plasma phospholipids. Levels reflect the fatty acid composition of the recent (weeks to months) diet,[18,19] not total fat intake or absolute intake of specific fatty acids. The correlation between phospholipid fatty acid percentages and dietary fatty acid intake tends to be stronger for long-chain polyunsaturated fatty acids derived solely from diet than for saturated and monounsaturated fatty acids, which may be synthesized endogenously. In the ARIC Study, for example, correlations (r) of fatty acids measured in phospholipids with those measured by a food frequency questionnaire were .25 for polyunsaturates, .22 for linoleic acid, .15 for saturates, and .05 for monounsaturates.[19]

It must be acknowledged that the fatty acid percentage not only reflects fatty acid composition of the diet, but likely a certain dietary pattern. For example, people who eat more saturated fat may also have a higher total fat intake and a lower carbohydrate and dietary fiber intake. It could be that the association we attributed to saturated fatty acid is a result of competing effects of these nutrients on serum insulin.

To our knowledge, only two previous human studies have directly examined the relation between plasma fatty acid content and insulin. Pelikánová et al[27] reported that fasting and postglucose insulin levels, as well as insulin action measured by euglycemic clamp studies, were strongly and inversely correlated with the n-6 to saturated fatty acid ratio of phospholipids in 11 nondiabetics. Fasting insulin had a correlation of .38 with saturated fatty acids.[27] Vessby et al[28] recently reported that insulin sensitivity, measured by the euglycemic, hyperinsulinemic clamp technique, was negatively correlated with the proportion of palmitic ($r = -.31$), palmitoleic ($r = -.25$), and di-homo-γ-linoleic ($r = -.33$) acids and positively correlated with linoleic acid ($r = .28$) in serum cholesterol esters. These findings are generally consistent with our results. Also generally consistent with our results, Salomaa et al[29] found that the cholesterol ester proportions of palmitic acid, palmitoleic acid, and di-homo-γ-linoleic acid increased and the proportion of linoleic acid decreased, successively, from a normal glucose tolerance group to an impaired glucose tolerance group to a non–insulin-dependent diabetes mellitus group.

Clinical and epidemiologic studies using dietary questionnaires have generally shown that insulin concentrations are associated positively with habitual saturated fat intake and/or negatively with polyunsaturated fat intake,[14-17] although in some cases total fat intake appeared to be more important than fat composition.[16,30,31] These observations contrast with those in fat-feeding trials in which fat alteration did not greatly influence insulin action.[13] It is possible that long-term dietary saturated fat intake outweighs any detectable short-term effect assessed by feeding trials.

Rather than dietary fatty acid composition altering insulin action, it could be that (1) a common factor influences both or (2) insulin resistance or insulin concentration alters fatty acid composition of phospholipids. Pelikánová et al[32] showed that 5 hours of insulin infusion did not alter fatty acid composition of phospholipids or cholesterol esters despite alterations in triglyceride and free fatty acid composition, but perhaps 5 hours of infusion is too short. In rats, insulin is known to activate δ-6 and δ-9 desaturating enzymes.[33,34] Our data showing positive relations of fasting insulin with palmitoleic acid (16:1n-7) and di-homo-γ-linolenic acid (20:3n-6) and a negative relation with linoleic acid (18:2n-6) support the possibility of insulin-induced

fatty acid desaturation influencing plasma fatty acid composition. Palmitoleic acid levels are probably determined more by fatty acid desaturation than by diet, and linoleic acid is converted to di-homo-γ-linolenic acid following δ-6 desaturation and chain elongation before formation of arachidonic acid.

Other studies corroborate a role of saturated fatty acids or the P/S ratio in the pathogenesis of insulin resistance. Increasing the ratio of polyunsaturated to saturated fatty acids increases the fluidity of cell membranes, the number and binding of insulin receptors, and the rate of insulin-stimulated glucose transport.[9-12] Borkman et al,[12] for example, reported that fasting serum insulin concentration was correlated negatively ($r = -.61$, $P < .001$) with the average degree of fatty acid unsaturation of phospholipids in rectus abdominus muscle. In the same study, insulin sensitivity measured by euglycemic clamp studies was associated positively ($r = .62$, $P < .05$) with the average degree of fatty acid unsaturation of vastus lateralis. Because skeletal muscle is a major site of insulin action, the investigators suggested that a decreased concentration of phospholipid polyunsaturates (and hence increased saturates) may modulate the action of insulin. Vessby et al[28] recently offered supportive evidence by showing that the proportion of palmitic acid in phospholipids of the vastus lateralis was inversely correlated ($r = -.48$) with peripheral insulin sensitivity. Furthermore, men who developed diabetes over 10 years were found to have increased serum levels of saturated fatty acids and palmitoleic, γ-linolenic (18:3n-6), and di-homo-γ-linolenic acids, and reduced levels of linoleic acid.[35]

Cross-sectional findings should not be taken as causal. Yet our data are consistent with the hypothesis that habitually high intake of saturated fatty acids or related dietary patterns may increase fasting insulin levels. Further research is needed to clarify the discrepancy between short-term fat-feeding trials, which show no material effect of total or saturated fat, and observational studies of habitual dietary fat composition such as this one.

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