Specific plasma lipid classes and phospholipid fatty acids indicative of dairy food consumption associate with insulin sensitivity[1–3]

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ABSTRACT

Background: Reports have suggested that the consumption of dairy foods may reduce risk of type 2 diabetes on the basis of evidence of raised circulating ruminant fatty acids.

Objective: We determined whether certain phospholipid species and fatty acids that are associated with full-fat dairy consumption may also be linked to diminished insulin resistance.

Design: Four variables of insulin resistance and sensitivity were defined from oral-glucose-tolerance tests in 86 overweight and obese subjects with metabolic syndrome. Plasma phospholipids, sphingolipids, and fatty acids were determined by using a lipidomic analysis and gas chromatography–mass spectrometry to provide objective markers of dairy consumption. Food records provided information on dairy products. Associations were determined by using linear regression analyses adjusted for potential confounders age, sex, systolic blood pressure, waist:hip ratio, or body mass index (BMI) and corrected for multiple comparisons.

Results: Lysophosphatidylcholine, lyso-platelet-activating factor, and several phospholipid fatty acids correlated directly with the number of servings of full-fat dairy foods. Lysophosphatidylcholine and lyso-platelet-activating factor were also associated directly with insulin sensitivity when accounting for the waist:hip ratio (Matsuda index unadjusted, $P < 0.001$ for both; adjusted for multiple comparisons, $P < 0.02$ for both) and inversely with insulin resistance (fasting insulin unadjusted, $P < 0.001$ for both; adjusted, $P = 0.04$ and $P < 0.05$, respectively; homeostasis model assessment of insulin resistance adjusted, $P = 0.04$ for both; post–glucose insulin area under the plasma insulin curve during the 120 min of the test adjusted, $P < 0.01$ for both). The substitution of BMI for the waist:hip ratio attenuated associations modestly. Phospholipid fatty acid 17:0 also tended to be associated directly with insulin sensitivity and inversely with resistance.

Conclusion: Variables of insulin resistance were lower at higher concentrations of specific plasma phospholipids that were also indicators of full-fat dairy consumption. This trial was registered at clinicaltrials.gov as NCT00163943. *Am J Clin Nutr* 2014;99:46–53.

INTRODUCTION

Some evidence has suggested that a diet rich in saturated fat may affect insulin sensitivity. The evidence has been inconsistent but has favored the association of insulin resistance with saturated fat consumption. Cross-sectional (1), prospective (2), and intervention (3) studies have suggested an association of fat consumption with insulin resistance that was partially attributable to adiposity, whereas in other studies, the association was possibly independent of the nature of the fat (4). Mexican Americans were shown to become insulin resistance through the consumption of high-fat diets, which was an effect that was only partly dependent on adiposity (5).

Dairy foods have been shown to be possible exceptions to the saturated fat–insulin resistance hypothesis (6). In the prospective Atherosclerosis Risk in Communities study, newly diagnosed metabolic syndrome occurred less frequently with the consumption of dairy foods than by eating fat within fried foods (7). Studies in young adults (8) and in a large French prospective study have suggested that the consumption of dairy foods led to fewer cases of metabolic syndrome and impaired fasting glucose (9). Prospective studies have suggested that the consumption of dairy foods, especially low-fat dairy products, may also diminish risk of future type 2 diabetes (9–12). However, in at least one large prospective study, intakes of dairy foods were not associated with incident diabetes although intake of total fermented dairy products (ie, cheese, yogurt, and fermented milk) were associated with significantly fewer cases (13).

Specific fatty acids of ruminant origin may also predict diminished risk of incident diabetes. Raised concentrations of *trans* palmitoleic acid (16:1n−7 *trans*) derived from dairy fat were associated with lesser risk of developing type 2 diabetes. In the prospective Cardiovascular Health Study, Mozzafarrian et al (14) reported that plasma phospholipid *trans* palmitoleic acid concentrations were associated inversely with insulin resistance. In the Multi-Ethnic Study of Atherosclerosis (15), the concentration of *trans* palmitoleic acid, which correlated with the con-

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sumption of dairy fat, was associated with less incident diabetes and inversely with plasma fasting insulin (−9%), which suggested improved insulin sensitivity. Two other minor fatty acids that are also recognized as derived from ruminant milk are pentadecanoic acid (15:0) and heptadecanoic acid (17:0) and may also be associated with less type 2 diabetes (16). Because none of these fatty acids represents greater than ~ 0.5% of plasma total fatty acids, the findings may reflect the consumption of dairy foods rather than a metabolic effect.

Of additional interest is the finding that several phospholipid classes (17–20) and ceramides (21) have been shown to affect glucose uptake and sensitivity of tissues to insulin.

We have investigated possible associations of specific phospholipid fatty acids as well as specific lipid classes with variables of insulin resistance and insulin sensitivity. Plasma phospholipids and sphingolipids were measured by using a lipidomic analysis and correlated with indexes of insulin resistance in 86 overweight subjects with metabolic syndrome in whom the consumption of dairy foods had also been assessed.

SUBJECTS AND METHODS

Subjects

A total of 86 middle-aged men and women, who were overweight [BMI (in kg/m^2) >27] or obese (mean BMI: 33.2) and shown on routine screening to have additional metabolic factors that were consistent with a diagnosis of metabolic syndrome, had been recruited by using an advertisement. Characteristics of the subjects are shown in **Table 1** and discussed in Results. Waist circumferences exceeded 102 cm in men and 88 cm in women, and each subject showed ≥2 additional criteria for metabolic syndrome (22). All subjects were nonsmokers. Exclusion criteria included frank type 2 diabetes and the presence of cardiovascular, hepatic, renal, or thyroid disease. The few subjects who had been receiving antihypertensive or lipid-lowering medication ceased treatment >4 wk before they were studied.

TABLE 1
Characteristics of 86 subjects[1]

	Values	Median (25–75% IQR)
Age (y)	55 ± 6[2]	56 (51–59)
M:F (%)	58:42	—
BMI (kg/m^2)	33.2 ± 4.1	32.2 (30.3–35.3)
Fasting insulin (mU/L)	18.7 ± 6.6	18.2 (14.8–22.2)
HOMA-IR	4.8 ± 1.8	4.6 (3.7–5.7)
$AUC_{0\text{-}120min}$ (mU/L)	10,586 ± 4251	10,859 (8127–13076)
Matsuda index of insulin sensitivity	2.0 ± 0.9	1.9 (1.5–2.3)
LDL cholesterol (mmol/L)	3.46 ± 0.97	3.52 (2.91–4.10)
HDL cholesterol (mmol/L)	1.22 ± 0.26	1.2 (1.0–1.4)
Cholesterol (mmol/L)	5.59 ± 0.96	5.55 (4.91–6.18)
Triglycerides (mmol/L)	1.9 ± 1.1	1.6 (1.2–2.2)
SBP (mm Hg)	134 ± 16	135 (125–144)
Waist circumference (cm)	108 ± 11	109 (100–116)
Fasting glucose (mmol/L)	5.8 ± 0.5	5.8 (5.5–6.1)

[1] $AUC_{0\text{-}120min}$, plasma insulin AUC between 0 and 120 min after an oral glucose load; SBP, systolic blood pressure.
[2] Mean ± SD (all such values).

TABLE 2
Macronutrient consumption from 4-d records in 82 subjects expressed as daily intakes[1]

Macronutrient	Values
Energy (kcal/d)	2136 ± 572
Total fat (%)	34.3 ± 4.8
Saturated fat (%)	14.6 ± 3.0
Polyunsaturated fat (%)	5.6 ± 1.4
Monounsaturated fat (%)	14.2 ± 2.4
Protein (%)	18.6 ± 3.1
Carbohydrate (%)	42.8 ± 5.8
Sugars (g/d)	98 ± 32
Starch (g/d)	124 ± 47
Alcohol (%)	3.6 ± 5.0

[1] All values are means ± SDs.

Approval for the study was given by the Human Research Ethics Committee of Southern Health that includes the Alfred Hospital in Melbourne where the clinical studies were carried out. Subjects provided voluntary signed consent.

Dietary assessment

The assessment of dairy food consumption was conducted from 4-d food records that included 1 weekend day and recorded individual dairy foods. Total dairy intake was calculated by using Australian Food Composition Tables (FoodWorks; Xyris Software). Data on dairy consumption were restricted to daily servings, which were defined as 250 mL milk, 200 g yogurt, 30 g butter, 40 g cheese, and 50 g ice cream. Subjects were instructed about portion sizes expressed in terms of cups, tubs, and slices, and appropriate measuring utensils were supplied. Nevertheless, accurate quantitative information was limited by individual perception of a serving size, and individual dairy products varied in their fat contents. One alternative was to more-precisely quantify plasma lipids that were likely representative of dairy fat and, therefore, could be better surrogates of dairy food consumption.

Major macronutrients calculated from 4-d food records expressed in daily amounts or percentages that were consumed at baseline 4 d before the measurement of insulin sensitivity are shown in **Table 2**. Details of daily dairy product consumption during this period are shown in **Table 3**.

Measurement of insulin sensitivity and resistance

The clinical study relevant to this investigation was a standard 75-g oral-glucose-tolerance test (liquid glucose Glucaid; Frontine) from which the following 4 key variables of insulin resistance and sensitivity were calculated: *1*) plasma insulin (subjects having fasted >12 h), *2*) the plasma insulin AUC between 0 and 120 min after an oral glucose load ($AUC_{0–120min}$) (plasma insulin concentrations were measured every 30 min for 2 h), *3*) the HOMA-IR (23), and *4*) the Matsuda index of insulin sensitivity [whole-body insulin sensitivity was calculated according to the equations published by Matsuda et al (23) and Matsuda and DeFronzo (24)]. Insulin sensitivity and resistance were calculated as

TABLE 3
Daily servings of dairy food consumption[1]

Dairy food	Mean ± SD	Median (25th–75th percentiles)
Milk		
HF (>2%)	0.87 ± 0.63	0.75 (0.30–1.25)
LF (≤2%)	0.99 ± 0.65	0.75 (0.50–1.50)
Yogurt		
HF	0.36 ± 0.26	0.25 (0.25–0.41)
LF	0.40 ± 0.32	0.25 (0.25–0.50)
Cheese		
HF	0.48 ± 0.42	0.38 (0.25–0.63)
LF	0.66 ± 0.58	0.44 (0.16–1.00)
Butter	0.23 ± 0.26	0.13 (0.07–0.25)
Cream		
HF	0.38 ± 0.27	0.25 (0.25–0.50)
LF	NA	NA
Dairy dessert		
HF	0.56 ± 0.42	0.50 (0.25–0.75)
LF	0.21 ± 0.07	0.25 (0.19–0.25)

[1] Derived from 4-d food records. Individuals who chose full-fat consumed mostly full-fat products; conversely, individuals who chose LF mostly consumed LF products. HF, high fat; LF, low fat; NA, not available.

$$10,000 \div \sqrt{(\text{FPG} \times \text{FPI})} \times (\text{mean PG} \times \text{mean PI}) \qquad (1)$$

where FPI is fasting plasma insulin (mU/L), FPG is fasting plasma glucose (mg/dL), and mean values are average concentrations of plasma glucose (PG) and plasma insulin (PI) at 0, 30, 60, 90, and 120 min. Subjects were stratified into insulin-sensitive and -resistance groups on the basis of Matsuda index cutoffs ≤2.1 or >2.1, respectively. The cutoff score for HOMA-IR was ≥2.5.

Measurements of plasma lipids and fatty acids

Blood samples were taken into tubes containing EDTA, and chilled plasma samples were separated and stored at −80°C. Plasma samples were analyzed for glucose, total cholesterol, HDL cholesterol, and triglyceride by using COBAS Integra 400 plus blood chemistry analyzer (Roche Diagnostics). Plasma insulin was measured by using a radioimmunoassay (Linco Research Inc).

Lipidomic analysis was performed by HPLC, electrospray ionization-tandem mass spectrometry by using an Agilent 1200 liquid-chromatography system combined with a Biosystems API 4000 Q/TRAP mass spectrometer with a turboionspray source (350°C) (AB SCIEX) and Analyst 1.5 data system (AB SCIEX) (25). We analyzed >300 individual lipid species including the major phospholipid and sphingolipid species. Individual species in each lipid class were summed for statistical evaluation. Median interassay CVs for lysophosphatidylcholine and lyso-platelet-activating factor were 8% and 10%, respectively.

Plasma phospholipid fatty acids were measured in extracted lipids that had been separated from neutral lipids and free fatty acids on aminopropyl columns. Fatty acids were hydrolyzed, methylesterified, and separated and quantified by using internal standard 13:0 on an SGE BPX70 (SGE Analytical Science) column and Agilent 7890 gas chromatograph coupled to a 5975C mass selective detector (Agilent Technologies). Interassay CVs differed for the various fatty acids with a range of 4.9% (15:0), 8.9% (17:0), and 10.5% (16:1n−7 *trans*).

Statistical analysis

A linear regression analysis was applied to test associations between concentrations of plasma lipids and phospholipid fatty acids and indexes of insulin sensitivity or insulin resistance. Linear regression analyses were adjusted for the following covariates that may also influence insulin sensitivity: age, sex, systolic blood pressure, waist:hip ratio, and BMI. Phospholipid and fatty acid concentrations were normalized to their respective IQRs to account for differences in the relative abundances of these lipids in plasma. β-coefficients obtained represented a change in respective measures of insulin sensitivity and resistance associated with an IQR increase in phospholipid species and classes or fatty acids. Associations between dairy product intakes and phospholipid species and classes or fatty acids were analyzed similarly. P values were corrected for multiple comparisons by using the Benjamini-Hochberg approach (26), and statistical significance was determined by a corrected 2-sided $P < 0.05$. The statistical package used was MATLAB R2012b (MathWorks).

RESULTS

There were 50 men and 36 women who were, on average, overweight or obese and clearly, on average, insulin resistant (Table 1). Fasting plasma insulin concentrations were elevated (mean: >18 mU/L), and HOMA-IR exceeded the normal value of 2.5 substantially (mean: >4.8), whereas the average Matsuda index was ≤2.1, which is the cutoff for insulin sensitivity. The data for the entire cohort have been analyzed as a single group (Table 1). Subjects were mildly dyslipidemic with both mean LDL cholesterol and triglycerides raised.

The macronutrient consumption in 82 subjects when the insulin metabolism studies were performed is shown in Table 2 and reveals a representative picture of the current Australian pattern of food consumption. The consumption of individual dairy foods expressed as daily servings is shown in Table 3. The studied cohort consumed approximately similar amounts of either full-fat or low-fat products but rarely both. Low-fat yogurt contains 1% fat, low-fat cheeses vary but generally contain <10% fat, and low-fat desserts vary but are generally <5% fat.

Association between dairy food consumption and lipid classes and fatty acids

Several plasma fatty acids are known to be derived predominantly from ruminant fat. We had satisfactory food records of the likely consumption of individual dairy foods at least in terms of the number of servings during 4 d as defined in the Australian Food Tables (Table 3). Next, we tested for associations between dairy consumption and lipid classes.

Of the 21 lipid classes expressed as interquartile changes in concentrations, several were directly associated with the number of dairy servings (**Table 4**). Significant positive associations were shown for lysophosphatidylcholine characterized by containing 15:0 and 17:0, which are 2 minor fatty acids of ruminant origin ($P < 0.001$ for both and $P < 0.01$ for both after correction for multiple comparisons). Two species of lyso-platelet-activating factor with 20:0 and 22:1 also showed a direct correlation with

TABLE 4
Linear regression of LPC and LPC(O) species against servings of full-fat dairy consumption, adjusted for age, sex, systolic blood pressure, and waist:hip ratio[1]

Predictor	β coefficient (95% CI)	P	Corrected P
LPC 15:0	0.95 (0.54, 1.35)	<0.001	<0.01
LPC 17:0	0.77 (0.39, 1.16)	<0.001	<0.01
LPC(O) 20:0	0.73 (0.19, 1.26)	<0.001	<0.01
LPC(O) 22:1	0.85 (0.28, 1.43)	<0.001	<0.01

[1] Corrected P values were corrected for multiple comparisons (all lipid species analyzed) by using the Benjamini-Hochberg approach. Only significant lipids are shown. LPC, lysophosphatidylcholine; LPC(O), lyso-platelet-activating factor.

the number of dairy servings ($P < 0.001$ for both and $P < 0.01$ after correction for multiple comparisons).

Of the phospholipid fatty acids, significant direct associations were shown between several fatty acids and full-fat diary food consumption (**Table 5**). Concentrations of 15:0, 16:1n-7 *trans*, 18:1n−7 (vaccenic acid), and 17:0 (trend only), all predominantly of ruminant origin, were significantly directly correlated with the number of full-fat dairy servings.

Plasma lipid classes and indexes of insulin sensitivity and resistance

Because adiposity influences insulin sensitivity, we adjusted for the following 2 indexes of adiposity: the waist:hip ratio and BMI. Correlations with one or the other variable adjusted are shown in **Tables 6** and **7**.

Possible associations of 21 lipid classes with the index of insulin sensitivity (Matsuda index) and 3 indexes of insulin resistance (ie, fasting plasma insulin, HOMA-IR, and the $AUC_{0–120min}$) were tested. Of the many phospholipid species that are quantifiable by lipidomic analysis, several species showed significant associations with insulin sensitivity and resistance but became nonsignificantly associated after multiple comparisons (data not shown). However, 2 phospholipid classes showed significant associations with insulin sensitivity and resistance (Table 6). After adjustment for the potential confounders age, sex, waist:hip ratio, or BMI and systolic blood pressure and for multiple comparisons both lysophosphatidylcholine and lyso-platelet-activating factor were directly associated with the Matsuda index ($P < 0.01$ after adjustment for the waist:hip ratio; $P < 0.02$ after adjustment for BMI). Lysophosphatidylcholine and lyso-platelet-activating factor were inversely associated with HOMA-IR ($P = 0.04$ for both, corrected for multiple comparisons and adjusted for waist:hip ratio but not BMI), fasting plasma insulin ($P = 0.04$ and <0.05, respectively, corrected for multiple comparisons and adjusted for the waist:hip ratio but not BMI), and $AUC_{0–120min}$ ($P < 0.01$ for both, corrected for multiple comparisons and adjusted for waist:hip ratio; adjustment for BMI led to lesser significance at $P = 0.06$ and $P < 0.05$, respectively).

None of the other lipids approach significance after correction for multiple comparisons including sphingomyelin, ceramide and its precursor dihydroceramide, G_{M3} ganglioside, as well as nonlysophospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylserine) and phospholipid plasmalogens (alkenylphosphatidylcholine and alkenylphosphatidylethanolamine).

Plasma phospholipid fatty acids and indexes of insulin resistance and sensitivity

Associations of 2 plasma phospholipid fatty acids expressed as percentages of the total measured fatty acids in this lipid with variables of insulin sensitivity and resistance are shown in Table 7. Fatty acids, with the exception of 17:0, were shown not to be significantly associated with indexes of insulin sensitivity and resistance. Data on 16:1n−7 *trans* are shown because of a special interest in *trans* palmitoleic acid. The minor fatty acid 17:0 tended to be significantly associated directly with the Matsuda index and inversely associated with fasting insulin and HOMA-IR more strongly when adjusted for the waist:hip ratio than for BMI.

Of fatty acids that did not suggest any association were 16:1n−7 *trans* (*trans* palmitoleic acid) and 18:1n−7 (vaccenic acid) both of which are ruminant-derived fatty acids. Associations of fatty acids with $AUC_{0–120min}$ are not shown because they were not significant.

Because data had shown that full-fat dairy consumption was associated with several phospholipid fatty acids and species that, in turn, showed negative associations with insulin resistance, it was important to determine whether either dairy fat (calculated as servings eaten; Table 3) or total fat consumption (Table 2) was related to insulin resistance. The analysis of data showed no such associations with any of the indexes of insulin resistance or sensitivity (adjusted as in the previous analyses for age, sex, waist:hip ratio, or BMI and systolic blood pressure and corrected for multiple comparisons). None of the regressions of either dairy fat or total fat intake on any variable of insulin resistance or sensitivity approached significance.

We also investigated the possibility that insulin resistance and sensitivity may also be related to fermented dairy products alone, namely cheese and yogurt. However, no significant relations were shown.

DISCUSSION

We have shown that several circulating phospholipid classes and probably also the phospholipid fatty acid 17:0 are directly associated with insulin sensitivity and inversely with insulin resistance. Correlations with insulin resistance appeared robust

TABLE 5
Linear regressions of plasma phospholipid fatty acids against servings of full-fat dairy foods[1]

Predictor	β coefficient (95% CI)	P	Corrected P
14:0	1.37 (−0.60, 3.35)	0.18	0.27
15:0	2.48 (1.04, 3.92)	<0.001	<0.01
16:1n−7 *trans*[2]	2.01 (0.45, 3.57)	<0.01	<0.05
17:0	1.67 (−0.04, 3.39)	0.06	0.10
18:1n−7[3]	2.11 (0.41, 3.81)	<0.02	<0.05

[1] Of 8 fatty acids with a potential link to dairy consumption, only 5 fatty acids are shown for clarity. Corrected P values were corrected for multiple comparisons by using the Benjamini-Hochberg approach.
[2] *trans* Palmitoleic acid.
[3] Vaccenic acid.

TABLE 6
Linear regressions of phospholipid classes on indexes of insulin sensitivity and insulin resistance adjusted for age, sex, systolic blood pressure, and either waist:hip ratio or BMI[1]

	Phospholipid class			
	LPC[2] Waist:hip ratio	LPC[3] BMI	LPC(O)[2] Waist:hip ratio	LPC(O)[3] BMI
Matsuda index				
β coefficient (95% CI)	0.39 (0.18, 0.60)	0.37 (0.14, 0.59)	0.44 (0.20, 0.68)	0.42 (0.16, 0.67)
P	<0.001	<0.001	<0.001	<0.001
Corrected *P*	<0.01	<0.02	<0.01	<0.02
Fasting insulin				
β coefficient (95% CI)	−3.02 (−4.84, −1.20)	−2.82 (−4.77, −0.88)	−3.03 (−5.03, −1.02)	−2.79 (−4.87, −0.71)
P	<0.001	<0.01	<0.001	<0.01
Corrected *P*	0.04	0.12	<0.05	0.12
$AUC_{0–120min}$				
β coefficient (95% CI)	−1758 (−2776, −739)	−1622 (−2723, −520)	−2153 (−3320, −987)	−2001 (−3222, −779)
P	<0.001	<0.01	<0.001	<0.01
Corrected *P*	<0.01	0.06	<0.01	<0.05
HOMA-IR				
β coefficient (95% CI)	−0.78 (−1.27, −0.30)	−0.70 (−1.21, −0.19)	−0.81 (−1.34, −0.28)	−0.72 (−1.27, −0.17)
P	<0.001	<0.01	<0.001	<0.01
Corrected *P*	0.04	0.14	0.04	0.14

[1] Corrected *P* values were corrected for multiple comparisons by using the Benjamini-Hochberg approach. $AUC_{0-120min}$, plasma insulin AUC between 0 and 120 min after an oral glucose load; LPC, lysophosphatidylcholine; LPC(O), lyso-platelet-activating factor.
[2] Adjusted for covariates age, sex, systolic blood pressure, and waist:hip ratio.
[3] Adjusted for covariates age, sex, systolic blood pressure, and BMI.

because more subjects were insulin resistant by HOMA-IR and the Matsuda index than sensitive because they were an overweight group with metabolic dysfunction. Of additional interest was the finding that lipids that were associated directly with insulin sensitivity were also correlated directly and significantly with full-fat dairy consumption. Thus, it was not surprising that some of the lipid classes are enriched with fatty acids of ruminant derivation.

The fatty acid 17:0, which is the only dairy-linked fatty acid to suggest a significant association with insulin sensitivity, is

TABLE 7
Linear regressions of plasma phospholipid fatty acids on indexes of insulin sensitivity and insulin resistance adjusted for age, systolic blood pressure, and either waist:hip ratio or BMI[1]

	Fatty acids			
	17:0[2] Waist:hip ratio	17:0[3] BMI	16:1n−7 *trans*[4] Waist:hip ratio	16:1n−7 *trans*[5] BMI
Matsuda index				
β coefficient (95% CI)	0.30 (0.02, 0.58)	0.23 (−0.05, 0.52)	0.02 (−0.22, 0.26)	0.02 (−0.22, 0.26)
P	0.04	NS	NS	NS
Corrected *P*	NS	NS	NS	NS
Fasting insulin				
β coefficient (95% CI)	−2.41 (−4.63, −0.18)	−2.19 (−4.42, 0.04)	−0.76 (−2.65, 1.13)	−0.80 (−2.67, 1.07)
P	0.04	0.06	NS	NS
Corrected *P*	NS	NS	NS	NS
HOMA-IR				
β coefficient (95% CI)	−0.70 (−1.28, −0.12)	−0.68 (−1.21, −0.04)	−0.17 (−0.67, 0.33)	−0.19 (−0.68, 0.31)
P	0.02	0.04	NS	NS
Corrected *P*	NS	NS	NS	NS

[1] 17:0 showed significant associations with dairy food consumption; 16:1n−7 *trans* was a fatty acid of interest. Corrected *P* values were corrected for multiple comparisons including fatty acids listed in Table 5 by using the Benjamini-Hochberg approach.
[2] Heptadecanoic acid adjusted for covariates age, systolic blood pressure, and waist:hip ratio.
[3] Adjusted for covariates age, systolic blood pressure, and BMI.
[4] *trans* Palmitoleic acid adjusted for covariates age, systolic blood pressure, and waist:hip ratio.
[5] Adjusted for covariates age, systolic blood pressure, and BMI.

a minor fatty acid (<1% of total fatty acids) and, therefore, is likely to be simply a marker of dairy fat consumption. Our finding was consistent with recent reports of other minor fatty acids of ruminant origin, in particular *trans* palmitoleic acid being associated with less insulin resistance and fewer cases of incident type 2 diabetes (14, 15). We could not confirm those findings, but our sample of 86 overweight individuals was far fewer than in the large prospective studies, but in general, the current group was more homogeneous. In contrast to the reported link between insulin sensitivity and the *trans* form of palmitoleic acid, the *cis* form of 16:1 has been shown to be associated with insulin resistance in one study (27) although this association was not confirmed by Fabrini et al (28). The fatty acid 15:0 is also considered a marker of dairy consumption as shown in the current study, and some (16) but not all (29) studies have reported a possible link of this fatty acid to diabetes.

As expected, the phospholipid fatty acid 17:0 that tended to be correlated with insulin sensitivity was also associated with full-fat dairy consumption. Nevertheless, we failed to show that total dairy fat consumption calculated as the number of servings was associated with insulin sensitivity. The latter finding was not necessarily at odds with results from some other reports (7, 9) and suggested that the chemically derived lipid data may have been a more robust and objective indicator of dairy consumption than food records, which may be distorted through unintentional bias and imperfect recollection.

Associations between concentrations in plasma of lysophosphatidylcholine and lyso-platelet-activating factor with less insulin resistance and directly with full-fat dairy consumption were more substantial. These lipids are membrane lipids from which 1 fatty acid molecule has been cleaved by phospholipases leaving one acyl chain generally of 16, 18, or 20 carbons; the species with 15:0 derives from dairy fat. Lysophosphatidylcholine is also generated during the lecithin:cholesterol acyltransferase reaction with the formation of cholesteryl esters. Although phospholipids are minor constituents of milk total lipids, they are major constituents of the milk-fat globule, which is susceptible to phopholipase hydrolysis with the release of lysophosphatidylocholine; this appears a possible explanation for the relation between dairy fat consumption and the lysophosphatidylcholine concentration in plasma. We have shown (unpublished observations) that the lysophosphatidylcholine concentration in milk fat is <1% of the phosphatidylcholine concentration and, thus, would be an unlikely significant direct source of plasma lysophosphatidylcholine.

Our finding raises the issue of whether there may be a metabolic aspect to the link to insulin sensitivity. That plasma lysophosphatidylcholine is associated with improved glucose clearance has been reported together with cellular mechanisms that support those observations. Yea et al (17) showed that lysophosphatidylcholine stimulated the glucose transporter *GLUT4* in 3T3-L1 adipocytes enhancing glucose uptake . In addition, the administration of lysophosphatidylcholine lowered blood glucose in normal and insulin-deficient mice (17). In another study, mice fed a high-fat diet showed reduced concentrations of plasma lysophosphatidylcholine (19), and several species of this phospholipid that were enriched with SFAs had declined in the livers. However, contrary reports suggested that lysophosphatidylcholine may adversely affect insulin sensitivity. Han et al (30) showed that L6 myotubules incubated with palmitic acid developed insulin resistance through insulin-receptor substrate *1 Ser307* phosphorylation. Because the effect was reversible by inhibiting the incorporation of palmitate into lysophosphatidylcholine, it appeared that lysophosphatidylcholine may act as an intermediate linking SFAs to insulin resistance.

Studies in small groups of obese and of type 2 diabetic subjects have shown lower plasma lysophosphatidylcholine concentrations that were inversely related to plasma insulin concentrations than those of healthy lean subjects (19), and similar findings have been shown in a study of glucose-intolerant patients (18). Short-term 28-d caloric overfeeding in healthy humans resulted in insulin resistance with lower concentrations of lysophosphatidylcholine (31), consistent with similar findings in fat-fed mice and our study that showed an inverse association between lysophosphatidylcholine and insulin resistance.

In the current study, plasma total lyso-platelet-activating factor was also shown to be directly associated with insulin sensitivity and full-fat dairy intake (for the species with 20:0 and 22:1). Lyso-platelet-activating factor is known to oppose the inflammatory and thrombogenic activities of the parent compound platelet-activating factor. Welch et al (32) have shown that the activation by platelet-activating factor of neutrophils and platelets is balanced by the opposing effect of lyso-platelet-activating factor. Kudolo et al (33) reported that circulating platelet-activating-factor hydrolase, which results in the formation of lyso-platelet-activating factor and, hence, is a surrogate for platelet-activating factor itself was higher in a small group of obese and of type 2 diabetic subjects than in lean healthy subjects. In addition, platelet-activating-factor hydrolase was correlated directly with the plasma insulin concentration, which suggested that lyso-platelet-activating factor may oppose this effect of platelet-activating factor on insulin resistance. In a larger study that included 50 patients with type 2 diabetes and a similar number of healthy subjects, plasma platelet-activating-factor hydrolase was also shown to be significantly elevated in patients with diabetes (34). We could not show associations between insulin-sensitivity status and sphingolipids, including sphingomyelin, ceramide, and glycosphingolipids.

Limitations of this study included the absence of an association between dairy food consumption and insulin sensitivity on the basis of dietary records, which we attributed to the variance in such recordings that may be difficult to quantify in this free-living population. An additional limitation was related to CVs in the measurement of lipid classes by lipidomics, which limited the power of the analyses, and the large number of lipid species and classes that required correction for multiple comparisons.

A possible limitation was the apparent discordant result between some of the findings when we adjusted for adiposity with the waist:hip ratio or BMI. Although β coefficients were similar in most analyses, the variance around some of the coefficients was greater with BMI adjustment. That visceral adiposity is a driver of insulin resistance is generally recognized, but whether it is better predicted by the waist:hip ratio or BMI has been mildly contentious. In the Health Professionals Follow-Up Study, both waist circumference and BMI predicted risk of diabetes although the former appeared slightly superior (35). A detailed investigation of subjects in the Diabetes Prevention Program, in which visceral adiposity was quantified by using computed tomography, also showed that both the waist:hip ratio and BMI predicted diabetes (36).

In conclusion, in 86 overweight and obese men and women in whom variables of insulin sensitivity and resistance had been defined from oral-glucose-tolerance tests, several plasma phospholipid classes, lysophosphatidylcholine, and lyso-platelet-activating factor as well as the phospholipid fatty acid 17:0 were shown to be associated with insulin sensitivity and inversely with insulin resistance. These lipids were also correlated with a higher intake of full-fat dairy foods, which defined them as indicators of full-fat dairy consumption in the current population. This result has led to our conclusion that the consumption of full-fat dairy foods is more likely to be associated with favorable rather than adverse effects, whereas this finding could not be shown from food-record data. To our knowledge, the current study included the largest population of insulin resistant nondiabetic individuals in whom plasma lysophosphatidylcholine and lyso-platelet-activating-factor concentrations have been shown to be inversely related to insulin resistance. Nevertheless, the conclusions are limited to this group of overweight and obese individuals with metabolic syndrome.

The authors' responsibilities were as follows—PJN, PJM, and NS: designed the research project, analyzed data, and wrote the article; NS, MTG, NAM, DPDS, DLT, and CKB: developed the methodology and conducted experiments; and NAM and GW: carried out statistical analyses. None of the authors had a conflict of interest.

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