Cross-sectional study of conjugated linoleic acid in adipose tissue and risk of diabetes[1–3]

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ABSTRACT

Background: Some experimental studies on conjugated linoleic acid (CLA) and insulin regulation suggested that CLA could be associated with risk of diabetes, but epidemiologic studies are lacking.

Objective: The aim of the study was to test whether the amount of CLA in adipose tissue is associated with risk of diabetes.

Design: A cross-sectional design was used to test the study hypothesis in 232 adults with diabetes and 1512 adults without diabetes who lived in Costa Rica. The *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomers in adipose tissue and 48 other fatty acids were assessed by using gas chromatography. Prevalence ratios (PRs) and 95% CIs were estimated by using Poisson regression adjusted for potential confounders.

Results: The mean (±SD) percentage of total fatty acids of CLA for the *cis*-9, *trans*-11 isomer in adipose tissue was 0.57 ± 0.18% in adults without diabetes and 0.53 ± 0.17% in adults with diabetes ($P = 0.0078$). The *trans*-10, *cis*-12 CLA isomer was not detected in adipose tissue. The *cis*-9, *trans*-11 CLA isomer was associated with a lower risk of diabetes. In comparison with the first quintile, the PR (95% CI) for the fifth quintile was 0.48 (0.31, 0.76) (P-trend = 0.0005) in the basic and 0.46 (0.29, 0.72) (P-trend = 0.0002) in the multivariable model. Additional adjustment for other fatty acids in adipose tissue including *trans*-9 16:1, which is a fatty acid that was previously associated with diabetes, did not modify the results.

Conclusion: The observed inverse association between the *cis*-9, *trans*-11 CLA in adipose tissue and diabetes risk is consistent with the hypothesis that CLA may be involved in insulin regulation. *Am J Clin Nutr* 2012;96:175–81.

INTRODUCTION

Conjugated linoleic acid (CLA) represents a group of positional and geometric isomers of linoleic acid (1). CLA is produced in the rumen of ruminant animals by fermentative bacteria (2) or by synthesis via Δ 9-desaturase (3). CLA is in dairy products such as milk and cheese as well as ruminant meats such as beef and lamb (3). The most common CLA isomers in ruminant meats and milk are *cis*-9, *trans*-11 and *trans*-10, *cis*-12 (2). These isomers represent 80% and 10%, respectively, of all CLA isomers in the human diet (4).

Animal models suggested that CLA could have beneficial effects on diabetes (5, 6), but these effects could be isomer specific (7). Results from intervention studies in humans have yielded mixed results (8, 9), with adverse rather than beneficial effects on insulin resistance shown in some studies (10, 11). For example, a double-blinded, randomized trial in 25 obese men that measured insulin sensitivity directly by using a euglycemic clamp showed that CLA decreased insulin sensitivity by 15% (12). Another randomized, placebo-controlled trial in 32 subjects with type 2 diabetes showed that CLA increased fasting glucose and reduced postprandial insulin sensitivity (8). The interpretation of these studies is difficult because of the variety of studied subjects, lengths, doses, and types of isomers provided. For example, the typical dose of ~3 g CLA commonly used in intervention studies is 10 times higher than the average reported dietary intake (range: ~0.11–0.29 g/d) (13–15). Furthermore, it is possible that *cis*-9, *trans*-11 and *trans*-10, *cis*-12 have divergent health effects (16).

To our knowledge, there are no published observational studies that evaluated the association between CLA and diabetes because CLA is not generally present in nutrient databases, and most studies that assessed the fatty acid profile in human biospecimens have not included CLA. The goal of this study was to test whether CLA measured in adipose tissue is associated with risk of diabetes. Because the amount of CLA in humans is derived from ruminant fat, the amount of CLA in adipose tissue can provide an objective marker of CLA intake. A linear significant correlation has been reported between adipose tissue CLA, dietary CLA, and dairy intake, but no association has been detected for beef intake (13). Studies showed that the turnover of fatty acids in adipose tissue is >2 y; therefore, adipose tissue fatty acid concentrations represent a long-term integrated measure of exposure (17, 18). The association between CLA and plasma triglyceride and fasting glucose was also evaluated in control subjects.

SUBJECTS AND METHODS

Study population

The study population consisted of subjects who participated as control subjects in a population-based study of diet and heart

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disease in Costa Rica. In the 2119 control subjects in the heart-disease study, we selected 1744 subjects (82%) who had complete values for *cis*-9, *trans*-11 CLA in adipose tissue and information on diabetes and potential cofounders (19). A total of 232 subjects were classified as adults with diabetes according to the WHO diagnostic criteria of having a fasting whole blood glucose concentration ≥6.1 mmol/L and/or taking medication to control diabetic symptoms (20), and the other 1512 subjects were classified as persons without diabetes. The catchment area for this study consisted of 34 counties in the Central Valley of Costa Rica. Control subjects were randomly recruited from the free-living population to match cases of myocardial infarction by age (±5 y), sex, and area of residence. Control subjects were not recruited if they were unable to answer the questionnaire or if they had suffered a myocardial infarction in the past. Participation was 88% in control subjects. Participants signed a consent form approved by the institutional review boards at the Harvard School of Public Health and the University of Costa Rica.

Data collection

For the current study, we used already-collected data and laboratory results from the Costa Rica Heart Study (19). Data on demographic and social economic characteristics, smoking, health status, and physical activity, as well as anthropometric measurements and blood pressure, were collected at the homes of subjects. A 12–14-h fasting blood sample and an adipose tissue aspirate were collected in the morning. Adipose tissue samples were collected from the buttock with a 1.5 × 16-inch gauge needle and a 10-cc syringe by using the method of Beynen and Katan (21). The sample was left in the syringe, stored in a sealed plastic bag, and immediately immersed into ice and transported within 3 h to the field station. Samples were diluted in 1.5 cc hexane:isopropanol (3:2), sealed, and stored at –80°C until they were analyzed for fatty acid content at the Harvard School of Public Health. No changes in the fatty acid composition of adipose tissue samples taken from the buttock have been observed in samples stored for 1.5 y (21).

Fatty acids were extracted from adipose with hexane:isopropanol (3:2) and esterified as previously described by Lillington et al (22). The fatty acid methyl esters were separated on a 100 m Supelco 2330 column and Hewlett Packard 6980 gas chromatographer. The temperature program ranged from 90°C to 250°C. We identified, through qualitative analysis of the retention peak times of pure standards (NuCheck Prep), 50 fatty acids including *cis*-9, *trans*-11 CLA (23). Fatty acids were expressed as a percentage of the total fatty acid identified. The between-sample CV was 5.2% for *cis*-9, *trans*-11 CLA. The *trans*-10, *cis*-12 isomer of CLA was not detected in adipose tissue, and consequently, analyses included only the *cis*-9, *trans*-11 CLA isomer.

Plasma triglycerides were measured by using enzymatic reagents (Boehringer-Mannheim Diagnostics), and capillary whole blood glucose was measured by using an Accu-Check II Blood Glucose Monitor with Chemstrip bG Test Strips (Boehringer-Mannheim Diagnostics) immediately after sample collection.

All statistical analyses were performed with Statistical Analysis Systems software (version 9.1; SAS Institute Inc). Differences in distributions of descriptive characteristics between subjects with and without diabetes were tested by using the *t* test if normally distributed or Wilcoxon's signed-rank test if not normally distributed for continuous variables, and the chi-square test was used for categorical variables. Poisson regression with robust variance was used to estimate prevalence ratios (PRs) and 95% CIs of diabetes according to quintiles of fatty acids in adipose tissue (24). Tests for trends were performed by using quintiles as a continuous variable in linear regression models. Potential confounders of age (continuous), sex, area of residence, physical activity (measured in metabolic equivalent tasks), BMI, waist circumference, income, and current smoking status (yes or no) were determined by evaluating their distribution in subjects without diabetes. The following types of fatty acids were measured in adipose tissue and used as potential confounders categorized into quintiles in prevalence models: *trans*-9 16:1, total *trans* (18:1 *trans*, 18:2 *trans*), even-numbered saturated (14:0 + 16:0 + 18:0), odd-numbered saturated (15:0 + 17:0), n−3 polyunsaturated (18:3n−3, EPA, DHA, and docosapentaenoic acid), n−6 polyunsaturated (18:2n−6, 20:2n−6, and 20:4n−6), and monounsaturated (16:1n−7 and 18:1n−9).

PRs and 95% CIs between diabetes and quintiles of ruminant *trans*-9:16:1 fatty acid and SFAs (15:0 + 17:0) were also calculated because of their association with diabetes and ruminant fat in previous studies (25). These models were adjusted for all of the previously mentioned confounders. The associations between fasting glucose, plasma triglyceride concentrations, and CLA in adipose tissue were also examined by using Spearman's partial correlation coefficients adjusted for age, sex, BMI, physical activity, and smoking.

RESULTS

General characteristics of the study population are shown in **Table 1**. The prevalence of diabetes in the study population was 13%. Compared with adults without diabetes, adults with diabetes were less physically active and had a higher BMI and larger waist circumferences. Also, there were fewer smokers in adults with diabetes than in adults without diabetes. Adults with diabetes presented higher plasma triglyceride concentrations and lower LDL cholesterol. Adipose tissue amounts of *cis*-9, *trans*-11 CLA were significantly lower in adults with diabetes than in adults without diabetes. Our analyses included only the *cis*-9, *trans*-11 CLA isomer because the *trans*-10, *cis*-12 isomer was not detected in adipose tissue.

The distribution of potential confounders by quintiles of *cis*-9, *trans*–11 CLA in adipose tissue is shown in **Table 2**. The median for the lowest quintile of *cis*-9, *trans*-11 CLA was 0.34% of total fatty acids, whereas the highest quintile was 0.83% of total fatty acids. Quintiles of *cis*-9, *trans*-11 CLA were positively correlated with age, female sex, and *trans* fatty acid in adipose tissue and inversely correlated with rural area of residence, physical activity, smoking, and SFAs, MUFAs, and n−3 and n−6 PUFAs in adipose tissue. A significant inverse trend was shown for fasting glucose and plasma triglyceride concentrations.

PRs and 95% CIs for the association between quintiles of *cis*-9, *trans*-11 CLA in adipose tissue and the prevalence of diabetes are shown in **Table 3**. Higher amounts of *cis*-9, *trans*-11 CLA in adipose tissue were significantly associated with a 52% lower prevalence of diabetes in the basic model [PR (95% CI) = 0.48 (0.31, 0.76) for fifth compared with first quintile]. Adjustment

TABLE 1
General characteristic of adults (n = 1744) with and without diabetes from the Central Valley of Costa Rica[1]

Variables	Without diabetes (n = 1512)	With diabetes (n = 232)
Age (y)	57 ± 11[2]	63 ± 9
Women (%)	24	33
Area of residence (percentage rural)	24	27
Monthly household income ($)	587 ± 432	549 ± 397
Current smokers (%)	22	15
BMI (kg/m^2)	26.2 ± 4.1	280.1 ± 4.5
Waist circumference (cm)	90.4 ± 9.9	95.2 ± 9.4
Physical activity (METs)	36 ± 15	33 ± 12
Blood glucose (mmol/L)	4.1 ± 0.7	7.9 ± 2.9
Total plasma triglycerides (mmol/L)	2.4 ± 1.4	3.0 ± 1.9
HDL cholesterol (mmol/L)	1.1 ± 0.2	1.1 ± 0.3
LDL cholesterol (mmol/L)	3.2 ± 1.0	2.9 ± 1.2
Adipose tissue CLA (percentage of total fatty acids)	0.57 ± 0.18	0.53 ± 0.17

[1] t Test and Wilcoxon's signed rank test for continuous variables and the chi-square test for categorical variables were used to determine the significance of differences. All variables, except area of residence, income, and HDL cholesterol, were significantly different between adults with and without diabetes ($P < 0.05$). CLA, conjugated linoleic acid; METs, metabolic equivalent tasks.
[2] Mean ± SD (all such values).

for physical activity, smoking status, or individual fatty acids in adipose tissue did not materially change the main result. We also tested whether other fatty acid markers of ruminant products *trans*-9 16:1 fatty acid and SFAs (15:0 + 17:0) were associated with diabetes risk. We showed no association for the *trans*-9 16:1 fatty acid and a suggestive, although not significant, association between SFAs (15:0 + 17:0) and higher diabetes risk.

A significant although weak inverse correlation was shown between concentrations of fasting glucose and adipose tissue *cis*-9, *trans*-11 CLA in adults without diabetes ($r = -0.072$, $P = 0.006$;

TABLE 2
General characteristics and potential confounders by quintiles of *cis*-9, *trans*-11 CLA in adipose tissue in adults without diabetes (n = 1512)[1]

	Quintiles of CLA in adipose tissue					
General characteristics	1	2	3	4	5	P-trend[2]
CLA (percentage of total adipose fatty acids)[3]	0.34	0.47	0.55	0.64	0.83	
Age (y)	55	56	57	58	59	<0.0001
Women (%)	12	18	27	32	37	<0.0001
Area of residence (percentage rural)	28	28	25	23	18	<0.0001
Monthly household income ($)	537	609	635	553	604	0.3
BMI (kg/m^2)	25.6	26.4	26.1	26.3	26.4	0.1
Waist circumference (cm)	89.8	91.5	90.1	90.2	90.4	0.9
Physical activity (METs)	39	37	36	34	33	<0.0001
Current smokers (%)	30	23	19	20	21	0.02
Blood glucose (mmol/L)	4.2	4.1	4.2	4.1	4.1	0.0004
Total triglycerides (mmol/L)	2.5	2.5	2.5	2.2	2.2	0.0002
HDL cholesterol (mmol/L)	1.0	1.0	1.1	1.0	1.1	0.07
LDL cholesterol (mmol/L)	3.1	3.2	3.1	3.4	3.2	0.4
Adipose tissue fatty acids (percentage of total fatty acids)						
trans Fatty acids	2.8	2.9	3.2	3.3	3.6	<0.0001
trans-9 16:1	0.13	0.14	0.15	0.14	0.15	<0.0001
18:1 *trans*	1.79	1.85	2.00	2.08	2.28	<0.0001
18:2 *trans*	1.00	1.05	1.15	1.20	1.37	<0.0001
SFAs (total)	27.3	25.9	25.2	24.6	23.5	<0.0001
Even chain (14:0 + 16:0 + 18:0)	27.3	25.9	25.2	24.5	23.5	<0.0001
Odd chain (15:0 + 17:0)	0.40	0.40	0.41	0.41	0.43	<0.0001
n−3 PUFAs	1.05	1.04	1.02	1.00	1.00	0.04
n−6 PUFAs	17.4	16.8	16.6	15.8	15.2	<0.0001
MUFAs	46.6	48.1	48.6	49.7	50.4	<0.0001

[1] All values are medians unless otherwise indicated. CLA, conjugated linoleic acid; METs, metabolic equivalent tasks.
[2] Tests for trends were performed by using quintiles as a continuous variable in linear regression models.
[3] All values are means.

TABLE 3
Risk of diabetes by quintiles of fatty acids in adipose tissue (n = 1744)

	Quintiles of adipose tissue					
	1	2	3	4	5	P-trend[1]
cis-9, *trans*-11 CLA[2]						
Percentage of total fatty acids[3]	0.34 (≤0.42)	0.47 (>0.42 to ≤0.51)	0.55 (>0.51 to ≤0.59)	0.64 (>0.59 to ≤0.70)	0.83 (>0.70 to ≤1.24)	—
Basic model[4]	1 (reference)[5]	0.93 (0.64, 1.34)	0.74 (0.50, 1.10)	0.67 (0.45, 1.01)	0.48 (0.31, 0.76)	0.0005
Model 1[6]	1 (reference)	0.86 (0.59, 1.25)	0.72 (0.48, 1.07)	0.62 (0.42, 0.94)	0.46 (0.29, 0.72)	0.0002
Model 2[7,8]	1 (reference)	0.85 (0.58, 1.25)	0.69 (0.46, 1.03)	0.59 (0.39, 0.90)	0.42 (0.26, 0.68)	0.0001
trans-9 16:1						
Percentage of total fatty acids[3]	0.071 (≤0.094)	0.11 (>0.094 to ≤0.13)	0.15 (>0.13 to ≤0.16)	0.17 (>0.16 to ≤0.19)	0.21 (>0.19 to ≤0.45)	—
Basic model[4]	1 (reference)	0.99 (0.63, 1.56)	1.10 (0.70, 1.71)	1.17 (0.77, 1.79)	1.24 (0.82, 1.88)	0.2
Model 1[6]	1 (reference)	0.94 (0.60, 1.48)	1.02 (0.65, 1.59)	1.09 (0.71, 1.67)	1.15 (0.76, 1.75)	0.4
Model 2[8,9]	1 (reference)	0.84 (0.53, 1.34)	0.88 (0.56, 1.39)	0.95 (0.61, 1.48)	1.05 (0.66, 1.66)	0.3
15:0 + 17:0						
Percentage of total fatty acids[3]	0.30 (≤0.33)	0.36 (>0.33 to ≤0.38)	0.40 (>0.38 to ≤0.42)	0.45 (>0.42 to ≤0.49)	0.54 (>0.49 to ≤1.23)	—
Basic model[4]	1 (reference)	0.78 (0.49, 1.24)	0.86 (0.55, 1.35)	1.24 (0.83, 1.85)	1.21 (0.81, 1.81)	0.07
Model 1[6]	1 (reference)	0.80 (0.51, 1.28)	0.88 (0.58, 1.39)	1.35 (0.90, 2.03)	1.33 (0.88, 2.01)	0.02
Model 2[8,10]	1 (reference)	0.82 (0.51, 1.31)	0.92 (0.58, 1.46)	1.30 (0.85, 2.01)	1.33 (0.84, 2.11)	0.06

[1] Tests for trends were performed by using quintiles as a continuous variable in linear regression models.
[2] CLA, conjugated linoleic acid.
[3] All values are medians; ranges in parentheses.
[4] Adjusted for age, sex, and area of residence.
[5] Prevalence; 95% CI in parentheses (all such values). Values were estimated by using Poisson regressions with robust variances.
[6] Adjusted as for basic model and for current smokers, BMI, and physical activity.
[7] Adjusted as for model 1 and for *trans*-9 16:1 and 15:0 + 17:0 fatty acids in adipose tissue.
[8] The addition of waist circumference, income, MUFAs, *trans* fatty acids, n−3 and n−6 PUFAs, and even-number SFAs did not modify results.
[9] Adjusted as for model 1 and for *cis*-9, *trans*-11 CLA and 15:0 + 17:0 fatty acids in adipose tissue.
[10] Adjusted as for model 1 and for *trans*-9 16:1 and *cis*-9, *trans*-11 fatty acids in adipose tissue.

n = 1512). In the same group, the concentration of plasma triglycerides was also inversely associated with higher adipose tissue *cis*-9, *trans*-11 CLA ($r = -0.083$, $P = 0.001$; $n = 1512$). These correlations were adjusted for age, sex, BMI, smoking, and physical activity. Our results were robust to outlier analysis.

DISCUSSION

We assessed amounts of *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA in adipose tissue in adults with and without diabetes. A significant inverse association was shown between the *cis*-9, *trans*-11 CLA isomer measured in adipose tissue and the prevalence of diabetes (a 42% lower prevalence of diabetes). The *trans*-10, *cis*-12 CLA isomer was not detected in adipose tissue in this population. This isomer was not detected in plasma or whole blood in the same studied population (26).

Short-term clinical trials on the role of CLA in insulin-related traits have yielded inconclusive results, and to our knowledge, there are no data from epidemiologic studies. Some studies showed detrimental effects of either *cis*-9, *trans*-11 CLA or *trans*-10, *cis*-12 CLA (12, 27) on insulin sensitivity. One study showed no effect of *cis*-9, *trans*-11 CLA on insulin sensitivity in healthy, middle-aged men after 6 wk (28). Furthermore, interventions that provided supplements that contained a 50%:50% combination of both CLA isomers (*trans*-10, *cis*-12 and *cis*-9, *trans*-11 CLA) have, in general, produced mixed results on insulin sensitivity (8, 29, 30). It is difficult to reconcile our findings with those of these intervention studies given the major differences in the way CLA intake was assessed, doses and types of isomers evaluated, and study designs. Our study was designed to evaluate an integrated long-term exposure (~2 y) to dietary CLA by measuring its amount in adipose tissue (17, 18). Intervention studies showed that *cis*-9, *trans*-11 CLA was not incorporated into adipose tissue after 3 mo (31, 32), even with CLA supplementation that was 12-fold higher (3.9 g/d) than the dietary intake previously reported for the Costa Rican population (0.29 g/d) (13). CLA did not affect insulin sensitivity in the study in which this measure was evaluated (31, 32). These data suggested that longer exposure periods may be needed to fully assess the potential effects of CLA. Epidemiologic studies that would evaluate dietary sources of CLA are also needed in other populations.

We showed that *trans*-9 16:1 fatty acid and SFAs (15:0 + 17:0) fatty acids in adipose tissue, which are 2 known biomarkers of dairy intake (23, 33), were not associated with the prevalence of diabetes. Mozaffarian et al (25) recently reported that the *trans*-9 16:1 measured in plasma phospholipids was both positively correlated with whole-fat dairy intake and inversely correlated with risk of diabetes in the prospective Cardiovascular Health Study. Whether the results of Mozaffarian et al (25) are confounded by *cis*-9, *trans*-11 CLA is uncertain because the study did not assess CLA. Nevertheless, our results and those of Mozaffarian et al (25) indicated that fatty acids shown in dairy products may have beneficial effects on risk of diabetes and supported previous studies that showed an inverse association between dairy intake and diabetes risk (34–36).

Available evidence suggested some potential mechanisms for the observed association between adipose tissue *cis*-9, *trans*-11 CLA and diabetes. A recent experimental study suggested that CLA could increase glucose-stimulated insulin secretion by activating the free fatty acid receptor 1, which is an emerging therapeutic target to treat type 2 diabetes (37). The same study also proposed a role for CLA as an activator of insulin production and activity. Other studies have shown that CLA is able to increase the expression of the *PPARG* gene, which is a key regulator of adipogenesis and insulin metabolism (38), in prediabetic rats (39) and human preadipocytes (40). However, other studies have shown no effect of *cis*-9, *trans*-11 CLA on *PPARG* gene expression in obese mice (41), 3T3-L1 murine preadipocytes (42), or human adipose tissue (43). Other potential mechanisms may involve antiinflammatory effects (5, 44) and changes in the phospholipid membrane of skeletal muscle and adipocytes (31, 42). We showed a significant inverse correlation between adipose *cis*-9, *trans*-11 CLA and whole blood fasting glucose and plasma triglycerides, which suggested that metabolic mechanisms that regulate glucose and triglyceride homeostasis may be, at least in part, related to the content of *cis*-9, *trans*-11 CLA in adipose tissue. Whether the observed association between CLA and a reduced risk of diabetes is mediated through changes in weight is unclear because intervention studies have failed to show a consistent effect of CLA supplementation on weight and BMI reduction (45, 46). We did not find an association between adipose tissue CLA and BMI, and the estimated PRs were not modified when they were adjusted for BMI.

The major limitation of the current study was its cross-sectional design, and therefore, no causal relationships or temporality could be established. Because adults with and without diabetes were derived from the control group from the original myocardial infarction study, it remains to be assessed whether our studies can be generalized to other populations. Also, residual confounders for different factors could not be completely ruled out, and the use of self-reported diabetes and fasting glucose in whole blood to identify diabetic cases may have produced some degree of unidentified diabetic subjects. However, we used the cutoff of a fasting whole blood glucose concentration of 6.1 mmol/L for a diagnosis of diabetes as proposed by the WHO for whole blood in epidemiologic studies (20), and the use of capillary whole blood glucose is a reliable and cost-effective tool for use in epidemiologic studies (47). To our knowledge, this is the first observational study to report an association between adipose tissue *cis*-9, *trans*-11 CLA and the prevalence of diabetes; thus, our findings need to be replicated in independent studies.

A major strength of the current study was the availability of unique measurements of fatty acids in adipose tissue that reflected long-term intakes. One study showed that adipose tissue fatty acids had higher correlations with their corresponding dietary fatty acids than with whole blood or plasma fatty acids (26). Furthermore, the availability of measurements of other fatty acids in adipose tissue allowed us to control for potential confounding because of those different fatty acids. For example, in our population, the content of *cis*-9, *trans*-11 CLA was inversely correlated with even-numbered saturated fat and positively correlated with *trans* fat and odd-numbered saturated fat in adipose tissue. The failure to adjust for other fatty acids could lead to potential confounding by their effects.

In conclusion, higher amounts of the *cis*-9, *trans*-11 CLA isomer in adipose tissue were inversely correlated to the prevalence of diabetes, fasting plasma triglycerides, and fasting blood glucose concentrations. This study supports the hypothesis that CLA may play a role in the development of diabetes. Future

studies are needed to confirm our findings as well as to elucidate potential biologic mechanisms that mediate the observed associations.

The authors' responsibilities were as follows—HC: designed the study; EAR-N and NC-W: conducted the research and analyzed data; NC-W: wrote the manuscript; HC and EAR-N: designed and supervised the statistical analysis, contributed to interpretation of the results, and edited the manuscript; and all authors: read and approved the final manuscript. None of the authors had a conflict of interest.

REFERENCES

1. Mensink RP. Metabolic and health effects of isomeric fatty acids. Curr Opin Lipidol 2005;16:27–30.
2. Evans M, Brown J, McIntosh M. Isomer-specific effects of conjugated linoleic acid (CLA) on adiposity and lipid metabolism. J Nutr Biochem 2002;13:508.
3. Griinari JM, Corl BA, Lacy SH, Chouinard PY, Nurmela KV, Bauman DE. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Δ^9-desaturase. J Nutr 2000;130:2285–91.
4. Bhattacharya A, Banu J, Rahman M, Causey J, Fernandes G. Biological effects of conjugated linoleic acids in health and disease. J Nutr Biochem 2006;17:789–810.
5. Moloney F, Toomey S, Noone E, Nugent A, Allan B, Loscher CE, Roche HM. Antidiabetic effects of cis-9, trans-11-conjugated linoleic acid may be mediated via anti-inflammatory effects in white adipose tissue. Diabetes 2007;56:574–82.
6. Noto A, Zahradka P, Yurkova N, Xie X, Truong H, Nitschmann E, Ogborn MR, Taylor CG. Dietary conjugated linoleic acid decreases adipocyte size and favorably modifies adipokine status and insulin sensitivity in obese, insulin-resistant rats. Metabolism 2007;56:1601–11.
7. Halade GV, Rahman MM, Fernandes G. Differential effects of conjugated linoleic acid isomers in insulin-resistant female C57Bl/6J mice. J Nutr Biochem 2010;21:332–7.
8. Moloney F, Yeow TP, Mullen A, Nolan JJ, Roche HM. Conjugated linoleic acid supplementation, insulin sensitivity, and lipoprotein metabolism in patients with type 2 diabetes mellitus. Am J Clin Nutr 2004;80:887–95.
9. Joseph SV, Jacques H, Plourde M, Mitchell PL, McLeod RS, Jones PJ. Conjugated linoleic acid supplementation for 8 weeks does not affect body composition, lipid profile, or safety biomarkers in overweight, hyperlipidemic men. J Nutr 2011;141:1286–91.
10. Risérus U. Trans fatty acids and insulin resistance. Atheroscler Suppl 2006;7:37–9.
11. Taylor CG, Zahradka P. Dietary conjugated linoleic acid and insulin sensitivity and resistance in rodent models. Am J Clin Nutr 2004;79:1164S–8S.
12. Risérus U, Vessby B, Arnlov J, Basu S. Effects of cis-9,trans-11 conjugated linoleic acid supplementation on insulin sensitivity, lipid peroxidation, and proinflammatory markers in obese men. Am J Clin Nutr 2004;80:279–83.
13. Smit LA, Baylin A, Campos H. Conjugated linoleic acid in adipose tissue and risk of myocardial infarction. Am J Clin Nutr 2010;92:34–40.
14. Salas-Salvadó J, Marquez-Sandoval F, Bullo M. Conjugated linoleic acid intake in humans: a systematic review focusing on its effect on body composition, glucose, and lipid metabolism. Crit Rev Food Sci Nutr 2006;46:479–88.
15. Mushtaq S, Heather Mangiapane E, Hunter KA. Estimation of cis-9, trans-11 conjugated linoleic acid content in UK foods and assessment of dietary intake in a cohort of healthy adults. Br J Nutr 2010;103:1366–74.
16. Reynolds CM, Roche HM. Conjugated linoleic acid and inflammatory cell signalling. Prostaglandins Leukot Essent Fatty Acids 2010;82:199–204.
17. Katan MB, Deslypere JP, van Birgelen AP, Penders M, Zegwaard M. Kinetics of the incorporation of dietary fatty acids into serum cholesteryl esters, erythrocyte membranes, and adipose tissue: an 18-month controlled study. J Lipid Res 1997;38:2012–22.
18. Dayton S, Hashimoto S, Dixon W, Pearce ML. Composition of lipids in human serum and adipose tissue during prolonged feeding of a diet high in unsaturated fat. J Lipid Res 1966;7:103–11.
19. Kabagambe EK, Baylin A, Ruiz-Narvarez E, Siles X, Campos H. Decreased consumption of dried mature beans is positively associated with urbanization and nonfatal acute myocardial infarction. J Nutr 2005;135:1770–5.
20. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998;15:539–53.
21. Beynen AC, Katan MB. Rapid sampling and long-term storage of subcutaneous adipose-tissue biopsies for determination of fatty acid composition. Am J Clin Nutr 1985;42:317–22.
22. Lillington JM, Trafford DJ, Makin HL. A rapid and simple method for the esterification of fatty acids and steroid carboxylic acids prior to gas-liquid chromatography. Clin Chim Acta 1981;111:91–8.
23. Baylin A, Kabagambe EK, Siles X, Campos H. Adipose tissue biomarkers of fatty acid intake. Am J Clin Nutr 2002;76:750–7.
24. Spiegelman D, Hertzmark E. Easy SAS calculations for risk or prevalence ratios and differences. Am J Epidemiol 2005;162:199–200.
25. Mozaffarian D, Cao H, King IB, Lemaitre RN, Song X, Siscovick DS, Hotamisligil GS. Trans-palmitoleic acid, metabolic risk factors, and new-onset diabetes in U.S. adults: a cohort study. Ann Intern Med 2010;153:790–9.
26. Baylin A, Kim MK, Donovan-Palmer A, Siles X, Dougherty L, Tocco P, Campos H. Fasting whole blood as a biomarker of essential fatty acid intake in epidemiologic studies: comparison with adipose tissue and plasma. Am J Epidemiol 2005;162:373–81.
27. Risérus U, Vessby B, Arner P, Zethelius B. Supplementation with trans10cis12-conjugated linoleic acid induces hyperproinsulinaemia in obese men: close association with impaired insulin sensitivity. Diabetologia 2004;47:1016–9.
28. Tricon S, Burdge GC, Jones EL, Russell JJ, El-Khazen S, Moretti E, Hall WL, Gerry AB, Leake DS, Grimble RF, et al. Effects of dairy products naturally enriched with cis-9,trans-11 conjugated linoleic acid on the blood lipid profile in healthy middle-aged men. Am J Clin Nutr 2006;83:744–53.
29. Ahrén B, Mari A, Fyfe CL, Tsofliou F, Sneddon AA, Wahle KW, Winzell MS, Pacini G, Williams LM. Effects of conjugated linoleic acid plus n−3 polyunsaturated fatty acids on insulin secretion and estimated insulin sensitivity in men. Eur J Clin Nutr 2009;63:778–86.
30. Raff M, Tholstrup T, Basu S, Nonboe P, Sorensen MT, Straarup EM. A diet rich in conjugated linoleic acid and butter increases lipid peroxidation but does not affect atherosclerotic, inflammatory, or diabetic risk markers in healthy young men. J Nutr 2008;138:509–14.
31. Goedecke JH, Rae DE, Smuts CM, Lambert EV, O'Shea M. Conjugated linoleic acid isomers, t10c12 and c9t11, are differentially incorporated into adipose tissue and skeletal muscle in humans. Lipids 2009;44:983–8.
32. Benito P, Nelson GJ, Kelley DS, Bartolini G, Schmidt PC, Simon V. The effect of conjugated linoleic acid on plasma lipoproteins and tissue fatty acid composition in humans. Lipids 2001;36:229–36.
33. Sun Q, Ma J, Campos H, Hu FB. Plasma and erythrocyte biomarkers of dairy fat intake and risk of ischemic heart disease. Am J Clin Nutr 2007;86:929–37.
34. Elwood PC, Pickering JE, Givens DI, Gallacher JE. The consumption of milk and dairy foods and the incidence of vascular disease and diabetes: an overview of the evidence. Lipids 2010;45:925–39.
35. Malik VS, Sun Q, van Dam RM, Rimm EB, Willett WC, Rosner B, Hu FB. Adolescent dairy product consumption and risk of type 2 diabetes in middle-aged women. Am J Clin Nutr 2011;94:854–61.
36. Tong X, Dong JY, Wu ZW, Li W, Qin LQ. Dairy consumption and risk of type 2 diabetes mellitus: a meta-analysis of cohort studies. Eur J Clin Nutr 2011;65:1027–31.
37. Schmidt J, Liebscher K, Merten N, Grundmann M, Mielenz M, Sauerwein H, Christiansen E, Due-Hansen ME, Ulven T, Ullrich S, et al. Conjugated linoleic acids mediate insulin release through islet G protein-coupled receptor FFA1/GPR40. J Biol Chem 2011;286:11890–4.
38. Tontonoz P, Spiegelman BM. Fat and beyond: the diverse biology of PPARgamma. Annu Rev Biochem 2008;77:289–312.
39. Houseknecht KL, Vanden Heuvel JP, Moya-Camarena SY, Portocarrero CP, Peck LW, Nickel KP, Belury MA. Dietary conjugated linoleic acid normalizes impaired glucose tolerance in the Zucker diabetic fatty fa/fa rat. Biochem Biophys Res Commun 1998;244:678–82.
40. Brown JM, Boysen MS, Jensen SS, Morrison RF, Storkson J, Lea-Currie R, Pariza M, Mandrup S, McIntosh MK. Isomer-specific regulation of metabolism and PPARgamma signaling by CLA in human preadipocytes. J Lipid Res 2003;44:1287–300.

41. Wargent E, Sennitt MV, Stocker C, Mayes AE, Brown L, O'Dowd J, Wang S, Einerhand AW, Mohede I, Arch JR, et al. Prolonged treatment of genetically obese mice with conjugated linoleic acid improves glucose tolerance and lowers plasma insulin concentration: possible involvement of PPAR activation. Lipids Health Dis 2005;4:3.
42. Evans M, Park Y, Pariza M, Curtis L, Kuebler B, McIntosh M Trans-10, cis-12 conjugated linoleic acid reduces triglyceride content while differentially affecting peroxisome proliferator activated receptor gamma2 and aP2 expression in 3T3-L1 preadipocytes. Lipids 2001;36:1223–32.
43. Herrmann J, Rubin D, Hasler R, Helwig U, Pfeuffer M, Auinger A, Laue C, Winkler P, Schreiber S, Bell D, et al. Isomer-specific effects of CLA on gene expression in human adipose tissue depending on PPARgamma2 P12A polymorphism: a double blind, randomized, controlled cross-over study. Lipids Health Dis 2009;8:35.
44. Turpeinen AM, Ylonen N, von Willebrand E, Basu S, Aro A. Immunological and metabolic effects of cis-9, trans-11-conjugated linoleic acid in subjects with birch pollen allergy. Br J Nutr 2008; 100:112–9.
45. Blankson H, Stakkestad JA, Fagertun H, Thom E, Wadstein J, Gudmundsen O. Conjugated linoleic acid reduces body fat mass in overweight and obese humans. J Nutr 2000;130:2943–8.
46. Whigham LD, Watras AC, Schoeller DA. Efficacy of conjugated linoleic acid for reducing fat mass: a meta-analysis in humans. Am J Clin Nutr 2007;85:1203–11.
47. Priya M, Mohan Anjana R, Pradeepa R, Jayashri R, Deepa M, Bhansali A, Mohan V. Comparison of capillary whole blood versus venous plasma glucose estimations in screening for diabetes mellitus in epidemiological studies in developing countries. Diabetes Technol Ther 2011;13:586–91.

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