Relation between the intake of milk fat and the occurrence of conjugated linoleic acid in human adipose tissue[1–3]

Jin Jiang, Alicja Wolk, and Bengt Vessby

ABSTRACT

Background: Conjugated linoleic acid (CLA) is a group of naturally occurring fatty acids mainly present in fats from ruminants. CLA has been shown to be a potential anticarcinogen.

Objective: In this study, the relation between bovine milk fat intake and the occurrence of CLA in human adipose tissue was investigated.

Design: One hundred twenty-three men weighed and recorded the foods they consumed for 1 wk. Afterward, recall interviews were conducted by telephone monthly for 7 consecutive months to inquire about food consumption during the previous 24 h. The entire dietary recording procedure was repeated once. The fatty acid composition of adipose tissue and serum was analyzed.

Results: The average amount of one isomer of CLA—9-*cis*,11-*trans*-octadecadienoic acid (9*c*,11*t*-18:2)—as a percentage of total fatty acids was found to be 0.50% in adipose tissue and 0.25% in serum. The amount of 9*c*,11*t*-18:2 in adipose tissue was significantly correlated with milk fat intake ($r = 0.42$). The percentage of 9*c*,11*t*-18:2 in both adipose tissue and in serum was strongly correlated with myristoleic acid (14:1).

Conclusion: The amount of 9*c*,11*t*-18:2 in human adipose tissue was significantly related to milk fat intake. *Am J Clin Nutr* 1999;70:21–7.

KEY WORDS Conjugated linoleic acid; humans; adipose tissue; fatty acids; milk fat intake; men; Sweden; 9-*cis*,11-*trans*-octadecadienoic acid; 9*c*,11*t*-18:2

INTRODUCTION

Conjugated linoleic acid (CLA) is a generic name for a mixture of isomers of linoleic acid (18:2) with conjugated double bonds at positions 9 and 11 or 10 and 12, and each double bond may be in the *cis* or *trans* configuration (1). CLA has attracted considerable attention because of its potential beneficial health effects. It has been reported that CLA inhibits the initiation of mouse skin carcinogenesis as well as mouse forestomach and rat mammary tumorigenesis (1–4). Shultz et al (5) found that physiologic concentrations of CLA are cytostatic and cytotoxic to human malignant melanoma, colorectal, and breast cancer cells in vitro. CLA has also been found to be effective in preventing the catabolic effects of immune stimulation (6, 7). More recently, CLA was suggested to have hypocholesterolemic and antiatherogenic effects (8, 9). The mechanisms responsible for these unique physiologic effects are not known. Of the individual isomers of CLA, 9-*cis*,11-*trans*-octadecadienoic acid (9*c*,11*t*-18:2), has been implicated as the most important in terms of biological activity because, apparently, this isomer is the only one incorporated into the phospholipid fraction of tissues of animals fed a mixture of CLA isomers (3, 4).

CLA is a group of naturally occurring fatty acids, mainly present in foods from ruminant sources. CLA is produced from polyunsaturated fatty acids (PUFAs) by rumen bacteria during biohydrogenation (10). Dairy products are the major dietary sources of CLA, of which 9*c*,11*t*-18:2 is the major isomer, representing 80–90% of total CLA in bovine milk (11, 12).

CLA has also been identified in human serum and in bile and duodenal juices (13–15). The effect of diet on plasma and serum CLA concentrations has been studied by supplying human subjects with a high-CLA diet. In 2 such studies, it was found that such a dietary modification increased plasma and serum concentrations of CLA (16, 17). However, little is known about the amount of CLA in human adipose tissue and whether this reflects dietary CLA intake. The purpose of this study was to determine the amount of CLA in adipose tissue and serum of humans and the relation between CLA concentrations and milk fat intake.

SUBJECTS AND METHODS

Subjects

One hundred twenty-three men, randomly selected from Uppsala county in central Sweden, weighed and recorded the food they consumed for 1 wk. Afterward, recall interviews were conducted by telephone monthly for 7 consecutive months to inquire

[1] From the Department of Food Science, Swedish University of Agricultural Sciences, Uppsala; the Department of Medical Epidemiology, Karolinska Institute, Stockholm; and the Department of Geriatrics, University of Uppsala, Sweden.

[2] Supported by the Swedish Cancer Society and Arla, Stockholm. The conjugated linoleic acid standard was a gift from Sou Fei Chin (Food Research Institute, University of Wisconsin, Madison).

[3] Address reprint requests to J Jiang, Department of Food Science, Swedish University of Agricultural Sciences, PO Box 7051, S-750 07 Uppsala, Sweden. E-mail: Jin.Jiang@lmv.slu.se.

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about food consumption during the previous 24 h. This procedure was repeated once; thus, two 1-wk dietary records were collected and 14 dietary interviews were completed for the subjects. The results of these 2 methods of dietary assessment—dietary records and 24-h recall interviews—were compared. All 123 men participated in the 24-h recall interviews; however, only 103 men completed the two 1-wk dietary records. Adipose tissue needle biopsy and blood samples were then collected and the following information was noted: age, height, and weight. The study was approved by the Ethical Committee of the Medical Faculty, Uppsala University, Sweden. All subjects signed an informed consent form before entering the study.

Dietary assessment

Before completing the self-administered dietary records, each participant obtained detailed instructions from a research dietitian about how to weigh and record all foods consumed. During telephone interviews, a standardized form for the 24-h dietary recall was used to collect the data. Dietary data from food records and telephone interviews were computerized by using the personal computer program MATS (18), which includes a listing of 1593 foods and dishes. Nutrients were calculated by using the Swedish Food Administration Food Database (19). This database provides information on the contents of 14 main fatty acids in different foods. Milk fat intake was estimated by summing the amounts of total fat from all dairy products and the milk fat from dishes containing dairy ingredients. Total energy and nutrient intakes for each subject were calculated as the mean daily intake derived from both the dietary records and 24-h recall interviews. The daily intake of CLA was estimated according to published data on the concentration of CLA in total fat in different foods (11, 20).

Blood sampling and adipose tissue biopsy

Blood samples were drawn from an antecubital vein under standardized conditions after a 12-h overnight fast. After the blood was coagulated for 2 h at room temperature, serum was separated by low-speed centrifugation (2000 × *g* for 10 min at 20 °C) and stored at 4 °C until analyzed for serum lipids, which was done within 24 h. Triacylglycerol and cholesterol concentrations were measured in serum enzymatically by using the IL Test Triacylglycerol Enzymatic-Colorimetric Method 181709-00 and IL Test Cholesterol Triander's method 181618-80, respectively, with a Monarch apparatus (Instrumentation Laboratory, Lexington, MA). Biopsies from subcutaneous adipose tissue were obtained by fine-needle biopsy of the upper-outer quadrant of the buttock as described by Beynen and Katan (21). The tissue samples were stored at −70 °C until analyzed.

Lipid extraction and analyses

Aspirated adipose tissue samples weighing 20–40 mg were homogenized and extracted with 15 mL chloroform:methanol (2:1, by vol) according to the method of Folch et al (22). For fatty acid analysis, 4 mL of the chloroform phase was dehydrated with anhydrous sodium sulfate and the solution was transferred into a screw-capped test tube. After the solvent was evaporated, the residue was dissolved in 1 mL chloroform. The total amounts of lipid were then quantified with a microbalance (Mettler MT/UMT; Mettler-Toledo AG, Grifensee, Switzerland).

The methyl esters of the extracted lipid were prepared by adding 2 mL 0.01 mol NaOH/L in dry methanol and heating in a continuously shaken glycerol bath for 15 min at 60 °C. Afterward, 3 mL BF_3 in methanol (20%) was added and the mixtures were heated for 15 min at 60 °C. The test tube was then removed from the glycerol bath and cooled under cold, running water. After 2 mL 20% NaCl and 1 mL hexane were added, the mixture was mixed vigorously and centrifuged at 2500 × *g* for 5 min at 20 °C. The hexane phase was collected and the fatty acid methyl esters were redissolved in 400 μL hexane for gas chromatographic quantification.

The methyl esters of CLA were analyzed with an HP5890 series II gas chromatograph (Hewlett-Packard Co, Rolling Meadows, IL) fitted with a flame ionization detector. The chromatogram was integrated by using computer software (JCL6000; Jones Chromatography Ltd, Mid Glam, United Kingdom). Samples were injected through the split injection port (split ratio, 40:1) onto a CP Sil 88 fused silica capillary column (50 m × 0.22 mm, 0.2-mm file thickness; Chrompack, Middelburg, Netherlands). The nitrogen carrier gas flow was 2 mL/min and the injector and detector temperatures were 250 °C. The oven temperature was programmed at 120 °C for 3 min, increased from 120 to 180 °C at a rate of 5 °C/min, and then held at 180 °C for 25 min. Individual fatty acids were identified by comparing relative retention times with individual fatty acid standards (Larodan Fine Chemical AB, Malmö, Sweden). Heneicosanoic acid methyl ester (Larodan Fine Chemical AB) was used as an internal standard for quantification. Analytic results for CLA and other fatty acids were expressed as percentages of total fatty acids. Lipid from the corresponding serum samples (n = 123) was extracted, methylated, and analyzed by gas chromatography by using the same methods used for adipose tissue analysis.

Data analysis

Spearman correlation was used to analyze the relations between dietary intake and the amounts of specific fatty acids in adipose tissue and serum. Significant relations between fatty acids were plotted by using linear regression analysis. The analyses were performed by using the SAS statistical package (23).

RESULTS

Characteristics of subjects

The average age of the subjects was 62 y (range: 46–72 y) and subjects had a mean (±SD) BMI (in kg/m^2) of 26.4 ± 3.2. Of the 123 subjects, 12 had a BMI >30 and 1 was grossly obese with a BMI of 41.3. Fifteen of the men had hypertriglyceridemia and 23 had hypercholesterolemia. None of the men had any acute illness in the 3 mo before the study. The data were analyzed for the whole group of men as well as for a subgroup who were normolipidemic with a BMI <30. Because the results were not significantly different between these 2 groups, we present the data for the whole group.

The average daily intake of selected nutrients is shown in **Table 1**. Mean intakes of total energy and nutrients were not significantly different between the 2 dietary recording methods. The average daily milk fat intake estimated in this study group was ≈25 g. The intakes of 10 major fatty acids were also estimated; palmitic acid (16:0), stearic acid (18:0), *cis*-oleic acid (*c*-18:1), and 18:2 accounted for >80% of the total fat intake. The estimated intake of CLA was ≈160 mg/d, which included CLA from both milk fat and the fat of ruminant meat products (11, 20).

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TABLE 1
Daily intake of energy and selected nutrients[1]

	Weighed dietary records (n = 103)	24-h Recall interviews (n = 123)
Total energy (kJ)	9.2 ± 1.6	2.1 ± 0.4
Protein (g)	80.9 ± 15.8	74.7 ± 15.1
Carbohydrate (g)	264.1 ± 69.3	247.7 ± 63.4
Fiber (g)	21.0 ± 6.9	18.8 ± 5.4
Cholesterol (g)	0.3 ± 0.1	0.3 ± 0.1
Total fat (g)[1]	81.7 ± 20.8	78.6 ± 20.9
SFA (g)	34.9 ± 10.6	33.5 ± 10.0
MUFA (g)	29.4 ± 7.6	28.4 ± 7.6
PUFA (g)	12.0 ± 3.0	11.6 ± 3.4
Milk fat (g)	25.2 ± 14.1	24.4 ± 11.5
Fatty acids (g)		
10:0	2.9 ± 1.4	2.8 ± 1.2
12:0	1.9 ± 0.7	1.9 ± 0.7
14:0	3.9 ± 1.5	3.7 ± 1.3
16:0	16.9 ± 5.0	16.2 ± 4.6
16:1	1.7 ± 0.4	1.6 ± 0.5
18:0	8.0 ± 2.3	7.7 ± 2.3
c-18:1	25.7 ± 6.6	24.9 ± 6.7
18:2	9.7 ± 2.7	9.4 ± 3.0
18:3	1.4 ± 0.4	1.3 ± 0.4
20:4	0.14 ± 0.06	0.13 ± 0.05
9*c*,11*t*-18:2[2]	0.16 ± 0.07	0.16 ± 0.06

[1] $\bar{x}$ ± SD. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
[2] 9-*cis*,11-*trans*-octadecadienoic acid [an isomer of conjugated linoleic acid (CLA)]; estimated according to published data on CLAs in different foods (11, 20).

Adipose tissue fatty acid composition

The mean percentage compositions of the major fatty acids as well as the sum of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and PUFAs in adipose tissue in the present study were compared with published data (24–26) (**Table 2**). The studies in the 1990s showed a decrease in the amount of SFAs and an increase in the amount of PUFAs in adipose tissue compared with data from 1964, eg, 18:2 increased from 7.2% in 1964 to 14.4% in 1991. The amount of 9*c*,11*t*-18:2 in adipose tissue in the present study ranged from 0.27% to 0.72% of total fatty acid methyl esters, with a mean of 0.50%. Significant correlations between the amounts of 9*c*,11*t*-18:2 and other fatty acids in adipose tissue are shown in **Figure 1**. Strong correlations were observed between 9*c*,11*t*-18:2 and myristoleic acid (14:1) and between 9*c*,11*t*-18:2 and palmitoleic acid (16:1). On the other hand, weak but significant correlations were found between 9*c*,11*t*-18:2 and the odd-chain fatty acid pentadecanoic acid (15:0) and between 9*c*,11*t*-18:2 and total *t*-oleic acid.

Relations between milk fat intake and adipose tissue fatty acid composition

Correlations between the amounts of long-chain fatty acids in adipose tissue and dietary milk fat intake were calculated by using data from both the dietary records and the 24-h dietary recall interviews (**Table 3**). Similar correlations were found with both methods. The amount of 9*c*,11*t*-18:2 in adipose tissue was significantly, positively correlated with the proportion of milk fat intake to total fat intake (r = 0.42, data from weighed dietary records), whereas a negative correlation was found for 18:2 (r = −0.46). The relation between the amount of 9*c*,11*t*-18:2 in adipose tissue and milk fat intake is shown in **Figure 2**. The correlation was similar to that between 9*c*,11*t*-18:2 in adipose tissue and the estimated total dietary CLA intake (r = 0.39).

CLA in serum

The content of 9*c*,11*t*-18:2 in serum samples ranged from 0.12% to 0.50% of total fatty acids. The mean value was 0.25%, which was about half the value found in adipose tissue. The rela-

TABLE 2
Fatty acid composition of adipose tissue triacylglycerol in men[1]

Fatty acid	Heffernan (24), 1964, England (n = 8)	Ito et al (25), 1991, USA (n = 76)	Tjønneland et al (26), 1993, Denmark (n = 23)	Present study, 1998, Sweden (n = 123)
	% of total fatty acids			
SFA	33.4	28.1	27.2	30.6
MUFA	59.6	52.3	58.5	55.9
PUFA	7.2	16.4	11.2	13.3
12:0	0.9 ± 0.1	0.4 ± 0.2	0.2 ± 0.1	0.5 ± 0.2
14:0	4.2 ± 0.3	2.7 ± 0.5	2.4 ± 0.7	3.8 ± 0.7
14:1	1.1 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0.5 ± 0.2
15:0	0.6 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
16:0	23.3 ± 3.3	20.1 ± 1.9	20.8 ± 1.3	21.9 ± 1.9
16:1	10.1 ± 1.7	5.9 ± 1.6	8.4 ± 2.4	6.7 ± 1.5
17:0	ND	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
18:0	4.4 ± 0.8	3.7 ± 0.1	3.3 ± 1.2	3.9 ± 0.8
t-18:1[2]	ND	2.3 ± 0.3	ND	2.2 ± 0.5
c-18:1[3]	48.4 ± 3.3	40.3 ± 1.9	49.7 ± 2.0	46.5 ± 1.9
18:2	7.2 ± 1.5	14.4 ± 2.3	10.1 ± 2.9	10.8 ± 1.9
18:3	ND	0.6 ± 0.2	0.8 ± 0.3	1.7 ± 0.2
9*c*,11*t*-18:2[4]	ND	ND	ND	0.5 ± 0.1
20:4	ND	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1

[1] SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
[2] Total *trans*-18:1, including *trans* isomers at positions 8–11.
[3] 9-*cis*-18:1.
[4] 9-*cis*,11-*trans*-octadecadienoic acid, an isomer of conjugated linoleic acid.

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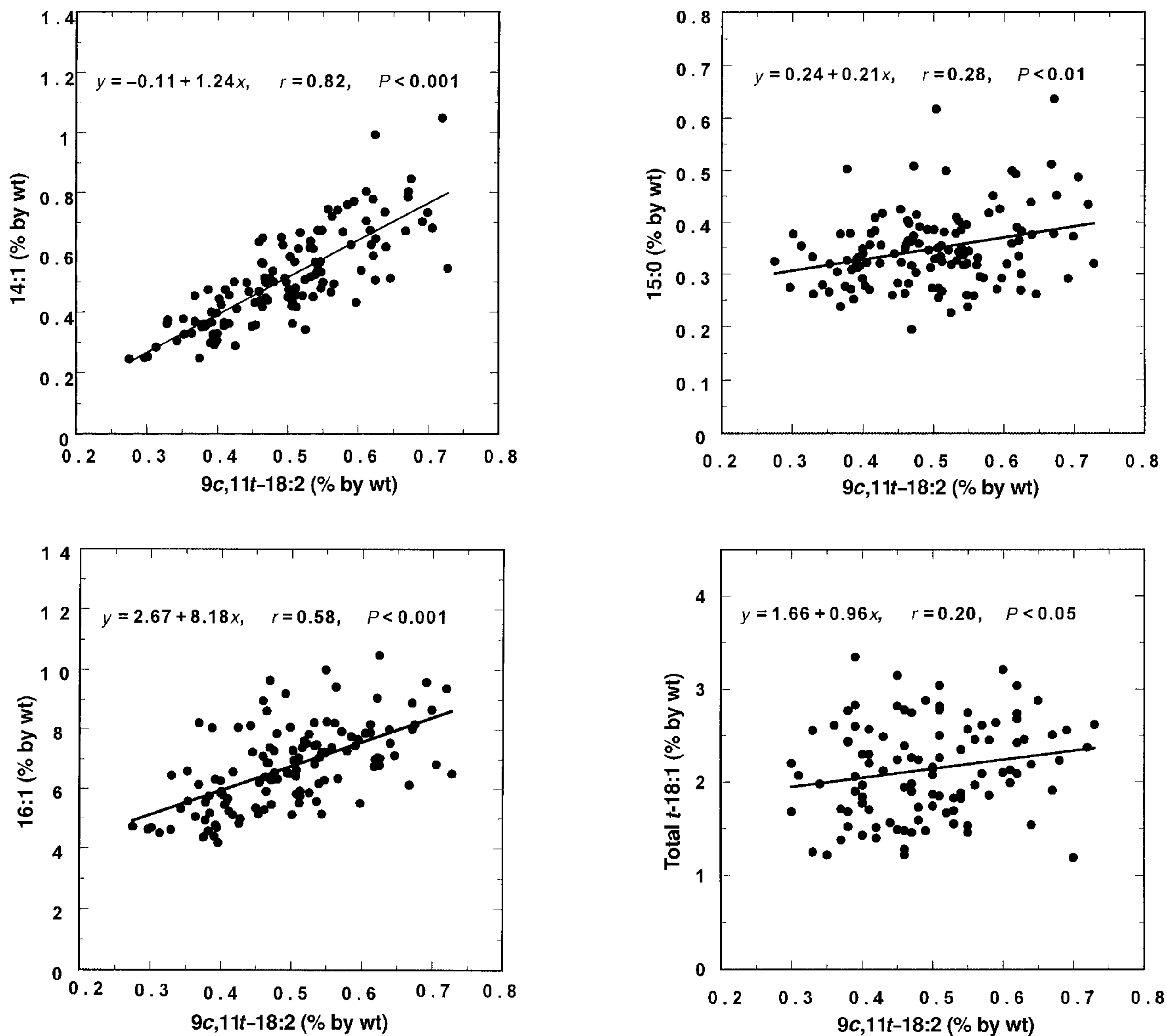

FIGURE 1. Relations between one isomer of conjugated linoleic acid—9-*cis*,11-*trans*-octadecadienoic acid (9*c*,11*t*-18:2)—and 14:1, 16:1, 15:0, and total *t*-18:1 in adipose tissue in men (n = 103).

TABLE 3
Spearman correlation coefficients between the composition of 18-carbon fatty acids in adipose tissue and estimated dietary milk fat intake (milk fat intake/total fat intake) in men

Fatty acids	Weighed dietary records (n = 103)	24-h Recall interviews (n = 123)
18:0	0.11	0.17
t-18:1[1]	−0.02	−0.08
c-18:1[2]	−0.19	−0.23[3]
18:2	−0.46[4]	−0.45[4]
18:3	−0.22[3]	−0.15
CLA[5]	0.42[4]	0.35[4]

[1] Total *trans*-18:1, including isomers at positions 8–11.
[2] 9-*cis*-18:1.
[3] $P < 0.05$.
[4] $P < 0.001$.
[5] 9-*cis*,11-*trans*-octadecadienoic acid, an isomer of conjugated linoleic acid.

tion between 9*c*,11*t*-18:2 in serum and in adipose tissue is shown in **Figure 3.** No significant correlation was found between serum CLA and adipose tissue CLA. The amounts of 9*c*,11*t*-18:2 and 14:1 in serum were also positively correlated (**Figure 4**).

DISCUSSION

The occurrence of CLA in human blood and human milk has been reported in the literature (12, 14, 15, 17) and 9*c*,11*t*-18:12 has been identified as the major conjugated diene in human serum (15). The amount of 9*c*,11*t*-18:2 in human serum and milk was shown to be 0.30–0.54% and 0.31–1.25% of total fatty acids, respectively (12). The amount of 9*c*,11*t*-18:2 was interpreted initially as an indicator of free radical attack on PUFAs (27), but other studies indicated that the concentration of 9*c*,11*t*-18:2 in human serum and milk is influenced by the diet, mainly by diets containing dairy and ruminant meat fats (12, 17).

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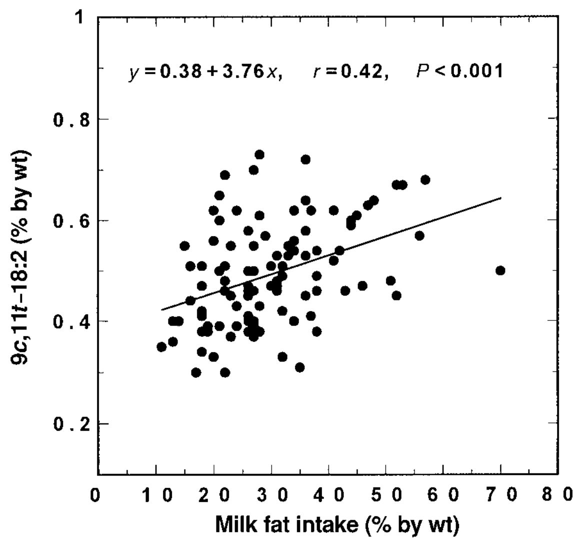

FIGURE 2. Relations between dietary milk fat intake and one isomer of conjugated linoleic acid—9-*cis*,11-*trans*-octadecadienoic acid (9*c*,11*t*-18:2)—in adipose tissue in men (n = 103). Intake was measured by using weighed dietary records for 2 wk.

Jack et al (28) also found that certain pathogenic bacteria can generate 9*c*,11*t*-18:2 in vitro. Consequently, the use of 9*c*,11*t*-18:2 as a marker of free radical activity in humans has been questioned.

The effect of the consumption of dairy products on plasma CLA concentrations in humans was studied by supplementing the diet of men with cheddar cheese, which resulted in a 19–27% increase in the plasma CLA concentration (16). This finding indicated that dietary modification can increase the CLA concentration in human plasma. It is known that dietary intake is also an important factor that can influence the fatty acid composition of human adipose tissue (29, 30). In the present study, the correlation between the fatty acid profile of adipose tissue and dietary milk fat intake determined by 2 different dietary assessment methods showed similar results, ie, the amount of 9*c*,11*t*-18:2 in adipose tissue was significantly correlated with milk fat intake.

In addition to the dietary factor, the endogenous formation of 9*c*,11*t*-18:2 was shown in rats by means of desaturation of 11-octadecanoic acid by liver microsomes (31) and by conversion of free 18:2 to CLA by the intestinal flora (32). Similar processes may occur in humans; eg, it was shown that dietary *trans* fatty acids increase CLA concentrations in human serum, which suggests that CLA in human tissues is partly derived from the diet, but that part of it may be formed via the conversion of dietary *trans* fatty acids (33). An elevated concentration of CLA in the cervix was observed as a result of bacterial colonization and activity (34). However, a recent study of the possible isomerization of 18:2 to CLA by human intestinal microflora reported that the supplementation of safflower oil with triacylglycerol-esterified 18:2 did not increase plasma concentrations of esterified CLA in total lipids (35).

The incorporation of dietary CLA into various tissues was studied and was found to be tissue dependent, eg, adipose tissue and lung tissue contain the most and brain tissue the least (36). In the present study, it was found that the average amount of CLA in human adipose tissue was twice as high as in serum, and there was no significant correlation between the amount of CLA in adipose tissue and in serum. These results warrant further studies of the incorporation of dietary CLA into human adipose tissue and the turnover rate, endogenous formation, and metabolism of CLA in humans.

Regarding the relations between different fatty acids in adipose tissue, no positive correlation was found between 9*c*,11*t*-18:2 and 18:2, which agrees with the results reported for human plasma (16). Jiang et al (37) showed previously a strong correlation between 9*c*,11*t*-18:2 and *trans*-vaccenic acid (11*t*-18:1) in bovine milk (r = 0.78). However, a weaker correlation (r = 0.20) was observed between 9*c*,11*t*-18:2 and total *t*-18:1 in human adipose tissue in this study. The result that 9*c*,11*t*-18:2 was significantly correlated with 15:0 might substantiate the observed correlation

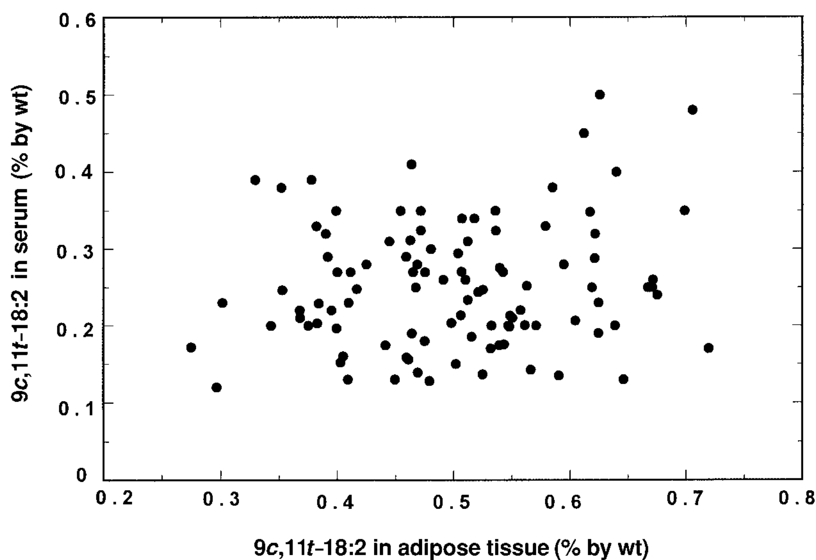

FIGURE 3. Relations between one isomer of conjugated linoleic acid—9-*cis*,11-*trans*-octadecadienoic acid (9*c*,11*t*-18:2)—in adipose tissue and serum in men (n = 103).

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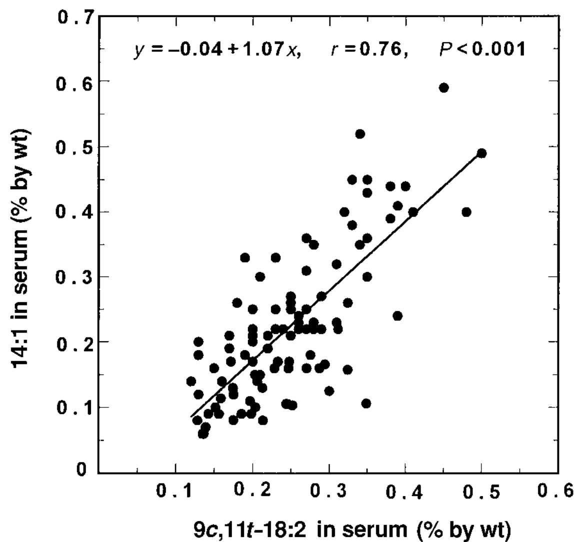
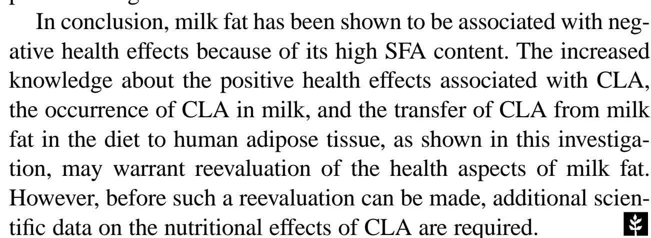


FIGURE 4. Relations between one isomer of conjugated linoleic acid—9-*cis*,11-*trans*-octadecadienoic acid (9*c*,11*t*-18:2)—and 14:1 in serum in men ($n = 103$).

between 9*c*,11*t*-18:2 and milk fat intake because 15:0 has been shown to be a good biological marker of milk fat intake in human adipose tissue (38). Of interest are the strong correlations between 9*c*,11*t*-18:2 and 14:1 and of 9*c*,11*t*-18:2 and 16:1 in adipose tissue. The reason for these relations is not understood and thus requires further investigation. The data published in 1964 (24) showed that the amounts of 14:1 and 16:1 in adipose tissue in men were twice as high in the 1960s as in the more recent investigations. This may indicate a higher concentration of CLA in human adipose tissue in the 1960s, as judged from the strong correlations between CLA and 14:1 and 16:1 found in the present study. This observation is consistent with the reduced consumption of total animal fat (eg, butter, full-fat milk, and fat from ruminant meats) in recent decades, although the reason for these differences may be due in part to divergent fat intakes in different countries.


In conclusion, milk fat has been shown to be associated with negative health effects because of its high SFA content. The increased knowledge about the positive health effects associated with CLA, the occurrence of CLA in milk, and the transfer of CLA from milk fat in the diet to human adipose tissue, as shown in this investigation, may warrant reevaluation of the health aspects of milk fat. However, before such a reevaluation can be made, additional scientific data on the nutritional effects of CLA are required.

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REFERENCES

1. Ha YL, Grimm NK, Pariza MW. Anticarcinogens from fried ground beef: heat-altered derivatives of linoleic acid. Carcinogenesis 1987;8:1881–7.
2. Pariza MW, Hargraves WA. A beef-derived mutagenesis modulator inhibits initiation of mouse epidermal tumours by 7,12 dimethylbenz(*a*)anthracene. Carcinogenesis 1985;6:591–3.
3. Ha YL, Storkson J, Pariza MW. Inhibition of benzo(a)pyrene-induced mouse forestomach neoplasias by conjugated dienoic derivatives of linoleic acid. Cancer Res 1990;50:1097–101.
4. Ip C, Chin SF, Scimeca JA, Pariza MW. Mammary cancer prevention by a conjugated dienoic derivative of linoleic acid. Cancer Res 1991;51:6118–24.
5. Shultz TD, Chew BP, Seaman WR. Differential stimulatory and inhibitory responses of human MCF-7 breast cancer cells to linoleic acid and conjugated linoleic acid in culture. Anticancer Res 1992;12:2143–6.
6. Cook ME, Miller CC, Park Y, Pariza M. Immune modulation by altered nutrient metabolism: nutritional control of immune-induced growth depression. Poultry Sci 1993;72:1301–5.
7. Miller CC, Park Y, Pariza MW, Cook ME. Feeding conjugated linoleic acid to animals partially overcomes catabolic responses due to endotoxin injection. Biochem Biophys Res Commun 1994;198:1107–12.
8. Lee KN, Kritchevsky D, Pariza MW. Conjugated linoleic acid and atherosclerosis in rabbits. Atherosclerosis 1994;108:19–25.
9. Nicolosi RJ, Laitinen L. Dietary conjugated linoleic acid reduces aortic fatty streak formation greater than linoleic acid in hypercholesterolemic hamsters. FASEB J 1996;10:2751 (abstr).
10. Kepler CR, Hirons KP, McNiell JJ, Tove SB. Intermediates and products of the biohydrogenation of linoleic acid by *Butyrivibrio fibrisolvens*. J Biol Chem 1966;241:1350–4.
11. Chin SF, Liu W, Storkson JM, Ha YL, Pariza MW. Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognised class of anticarcinogens. J Food Comp Anal 1992;5:185–97.
12. Fogerty AC, Ford GL, Svoronos D. Octadeca-9,11-dienoic acid in foodstuffs and in the lipids of human blood and breast milk. Nutr Rep Int 1988;38:937–44.
13. Cawood P, Wickens DG, Iversen SA, Braganza JM, Dormandy TL. The nature of diene conjugation in human serum, bile and duodenal juice. FEBS Lett 1983;162:239–43.
14. Iversen SA, Cawood PM, Dormandy TL. A method for the measurement of linoleic acid, 18:2(9,11), in serum phospholipid, and possible origins. Ann Clin Biochem 1985;22:137–40.
15. Iversen SA, Cawood PM, Madigan J, Lawson AM, Dormandy TL. Identification of a diene conjugated component of human lipid as octadeca-9,11-dienoic acid. FEBS Lett 1984;171:320–4.
16. Huang YC, Luedecke LO, Shultz TD. Effect of cheddar cheese consumption on the plasma conjugated linoleic acid concentration in men. Nutr Res 1994;14:373–86.
17. Britton M, Fong C, Wickens D, Yudkin J. Diet as a source of phospholipid esterified 9,11-octadecadienoic acid in humans. Clin Sci 1992;83:97–101.
18. Rudans Lättdata. MATS program, version 2.2. Västerås, Sweden: Rudans Lättdata, 1992.
19. Bergström L. Food database, PC version 1992. Uppsala, Sweden: Swedish Food Administration, 1992. (Report no. 14.)
20. Jiang J, Björck L, Fondén R. Conjugated linoleic acid in Swedish dairy products with special reference to manufacturing of hard cheeses. Int Dairy J 1997;7:863–7.
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. Folch J, Lees M, Stanley GHS. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 1957;226:497–509.
23. SAS Institute, Inc. SAS system for Windows, release 6.11. Cary, NC: SAS Institute Inc, 1995.
24. Heffernan AGA. Fatty acid composition of adipose tissue in normal and abnormal subjects. Am J Clin Nutr 1964;15:5–10.
25. Ito Y, Hudgins LC, Hirsch J, Shike M. Adipose-tissue fatty acid composition in recipients of long-term total parenteral nutrition (TPN). Am J Clin Nutr 1991;53:1487–92.
26. Tjønneland A, Overvad K, Thorling E, Ewertz M. Adipose tissue fatty acids as biomarkers of dietary exposure in Danish men and women. Am J Clin Nutr 1993;57:629–33.
27. Dormandy TL, Wickens DG. The experimental and clinical pathway of diene conjugation. Chem Phys Lipids 1987;45:353–64.

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28. Jack CIA, Ridgway E, Jackson MJ, Hind CRK. Serum octadeca-9,11-dienoic acid—an assay of free radical activity or a result of bacterial production? Clin Chim Acta 1994;224:139–46.
29. Field CJ, Clandinin MT. Modulation of adipose tissue fat composition by diet: a review. Nutr Res 1984;4:743–55.
30. van Staveren WA, Deurenberg P, Katan MB, Burema J, de Groot LCGM, Hoffmans MDAF. Validity of the fatty acid composition of subcutaneous fat tissue microbiopsies as an estimate of long-term average fatty acid composition of the diet of separate individuals. Am J Epidemiol 1986;123:455–63.
31. Holman RT, Mafouz MM. *Cis* and *trans* octadecadienoic acids as precursors of polyunsaturated acids. Prog Lipid Res 1981;20:151–6.
32. Chin SF, Storkson JM, Liu W, Albright KJ, Pariza MW. Conjugated linoleic acid (9,11- and 10,12-octadecadienoic acid) is produced in conventional but not germ-free rats fed linoleic acid. J Nutr 1994;124:694–701.
33. Salminen I, Mutanen M, Jauhiainen M, Aro A. Dietary *trans* fatty acids increase conjugated linoleic acid levels in human serum. Nutr Biochem 1998;9:93–8.
34. Fairbank J, Hollingworth A, Griffin J, et al. Octadeca-9,11-dienoic acid in cervical intraepithelial neoplasia: a colposcopic study. Clin Chim Acta 1989;186:53–8.
35. Herbel BK, McGuire MK, McGuire MA, Shultz TD. Safflower oil consumption does not increase plasma conjugated linoleic acid concentrations in humans. Am J Clin Nutr 1998;67:332–7.
36. Sugano M, Tsujita A, Yamasaki M, Yamada K, Ikeda I, Kritchevsky D. Lymphatic recovery, tissue distribution, and metabolic effects of conjugated linoleic acid in rats. J Nutr Biochem 1997;8:38–43.
37. Jiang J, Bjoerck L, Fondén R, Emanuelson M. Occurrence of conjugated *cis*-9,*trans*-11-octadecadienoic acid in bovine milk: effects of feed and dietary regimen. J Dairy Sci 1996;79:438–45.
38. Wolk A, Vessby B, Ljung H, Barrefors P. Evaluation of a biological marker of dairy fat intake. Am J Clin Nutr 1998;68:291–5.

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