Conjugated linoleic acid in adipose tissue and risk of myocardial infarction¹⁻³

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ABSTRACT

Background: Despite the high saturated fat content of dairy products, no clear association between dairy product intake and risk of myocardial infarction (MI) has been observed. Dairy products are the main source of conjugated linoleic acid (CLA; 18:2n–7t), which is produced by the ruminal biohydrogenation of grasses eaten by cows. Pasture-grazing dairy cows have more CLA in their milk than do grain-fed cows. Some animal models have reported beneficial effects of CLA on atherosclerosis.

Objective: The objective was to determine the association between the 9c,11t-CLA isomer in adipose tissue and risk of MI.

Design: The studied population consisted of 1813 incident cases of a first nonfatal acute MI and 1813 population-based controls matched for age, sex, and area of residence. All subjects lived in Costa Rica—a country that uses traditional pasture-grazing for dairy cows. Conditional logistic regression was used to estimate multivariate odds ratios and 95% CIs.

Results: Adipose tissue 9c,11t-CLA was associated with a lower risk of MI in basic and multivariate models. Compared with the lowest quintile, odds ratios and 95% CIs were 0.80 (0.61, 1.04) for the second, 0.86 (0.64, 1.14) for the third, 0.62 (0.46, 0.84) for the fourth, and 0.51 (0.36, 0.71) for the fifth quintiles (p for trend <0.0001). Dairy intake was not associated with risk of MI, despite a strong risk associated with saturated fat intake.

Conclusion: 9c,11t-CLA, which is present in meaningful amounts in the milk of pasture-grazed cows, might offset the adverse effect of the saturated fat content of dairy products.

INTRODUCTION

Conjugated linoleic acid (CLA; 18:2n–7t) consists of a group of linoleic acid trans isomers that occur naturally in the diet. CLA is produced by biohydrogenation in ruminant animals such as cows, sheep, and goats (1) (Figure 1). Thus, CLA in the diet comes from dairy products and ruminant meats (2). Milk with a high content of CLA is produced when cows are fed pasture-based diets, as opposed to conserved forage or grain (3). Fresh grass contains a high concentration of soluble fiber and fermentable sugars, which create a more basic environment in the rumen that is favorable to the growth of the microbes responsible for CLA production (3). Experiments have shown that the CLA content of milk from cows grazing pastures was 5-fold higher than in cows fed conserved forage and grain (4).

As a trans fatty acid, CLA might play a role in heart disease. Some (5, 6), but not all (7), animal models suggest that the common 9c,11t isomer of CLA paradoxically could protect against atherosclerosis and that this protection is possibly mediated by its beneficial effects on lipoprotein metabolism (8). However, in humans, CLA supplements have not consistently affected plasma lipid risk factors (9). The CLA doses used in human intervention studies (range: 0.7–6.8 g/d) and the proportion of 9c,11t to 10c,12t isomers used in the supplements do not reflect natural sources of dietary CLA (9).

To our knowledge, no studies that evaluated the association between CLA from natural food sources at levels currently consumed and heart disease have been published. These studies are of great interest because of the lack of consistency in human studies and animal experiments that evaluate the effects of CLA on risk factors. Epidemiologic studies on the association between CLA intake assessed by questionnaires and heart disease are challenging because CLA and saturated fat share common food sources, mainly dairy products and beef. Therefore, biomarkers of dietary intake of CLA may be more likely to provide an accurate objective measure of long-term intake than are dietary questionnaires (10). Subcutaneous adipose tissue is considered the best choice for studies of long-term intake because of its slow turnover (11, 12).

The objective of this study was to test whether adipose tissue CLA is associated with risk of nonfatal myocardial infarction (MI). We measured 9c,11t-CLA in adipose tissue samples from 3626 individuals in Costa Rica with and without a history of nonfatal MI. In Costa Rica, the dairy industry uses mostly traditional pasture grazing for dairy cattle, and the content of CLA is higher than in milk produced in the United States from corn-fed cattle in feedlots (2, 13). Thus, Costa Rica is an especially favorable setting to study the relation between natural sources of CLA and heart disease.

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ADIPOSE TISSUE CLA AND MI RISK

FIGURE 1. Graphic representation of linoleic acid (cis) and the 9c,11t isomer of conjugated linoleic acid.

SUBJECTS AND METHODS

Participants were recruited from 34 counties that comprise the Central Valley of Costa Rica. Eligible case subjects were men and women who survived a first acute MI, as diagnosed by 2 independent cardiologists at any of the 6 recruiting hospitals, and who were permanent residents of the geographic area defined for the study. A permanent resident was defined as any subject sharing room and board in the area of residence ≥4 days/wk. Cases must have lived in the catchment area for ≥1 y. To achieve 100% ascertainment, fieldworkers visited the 6 hospitals daily. Criteria for MI matched those set by the World Health Organization and required typical symptoms plus either elevations in cardiac enzyme concentrations or diagnostic changes in the electrocardiogram. On average, patients remained in the hospital for 15 d in the electrocardiogram. On average, patients remained in the hospital 3 d. On average, patients remained in the hospital 5 d.

Subjects were ineligible if they died after recruitment but before the data collection had been completed, were aged ≥75 y on the day of their first MI, or were physically or mentally unable to answer the questionnaire. Enrollment was carried out while cases were in the hospital’s step-down unit. Of those originally recruited, 47 subjects died before the data collection was completed; therefore, they were not eligible for the study. Of those that met the eligibility criteria, 98% participated in the study.

Controls were randomly selected for each case and matched for age (± 5 y), sex, and area of residence by using the information available at the National Census and Statistics Bureau of Costa Rica. The specific residence of each eligible subject was located in a Census map by using map segments of 60 households each. The order by which eligible homes were visited was selected by a lottery system. Control subjects were ineligible if they had ever had an acute MI or if they were physically or mentally unable to answer the questionnaire. Because of the comprehensive social services provided in Costa Rica, all persons living in the catchment area had access to medical care without regard to income. Therefore, control subjects came from the source population that gave rise to the cases and were not likely to have cardiovascular disease that was not diagnosed because of poor access to medical care. On average, data were collected 30 ± 10 d after recruitment of the matched case. Of those that met the eligibility criteria, 88% participated in the study. Informed consent was given on documents approved by the Human Subjects Committee of the Harvard School of Public Health and the University of Costa Rica.

All study participants were visited in their homes for data collection. The subjects provided information on dietary intake, physical activity, and socioeconomic, demographic, and health characteristics during an interview (14, 15). The fatty acid composition of all foods commonly used in Costa Rica, including the content of 9c,11t-CLA, was determined and incorporated into the nutrient calculation for the adipose-diet correlation analyses (13). A subcutaneous adipose tissue biopsy sample was collected from the upper buttock with a 16-gauge needle and disposable syringe and analyzed by gas-liquid chromatography following procedures previously described (16). The between-run CV was 5.2% for 9c,11t-CLA.

All data were analyzed by using Statistical Analysis System software (version 9.1; SAS Institute Inc, Cary, NC). A total of 1813 case-control pairs were included in the analysis. The significance of differences in the distributions of categorical variables by case-control status was tested by using McNemar’s test; continuous variables were tested by using the paired t test if normally distributed or by the Wilcoxon’s signed-rank test if not normally distributed. Odds ratios (ORs) and 95% CIs for risk of MI between quintiles of median adipose tissue 9c,11t-CLA or dairy intake were estimated by using multiple conditional logistic regression models. Tests for trends were performed across quintiles by using the median value for each of the quintiles modeled as a continuous variable. Confounders included in the models were physical activity (quintiles), monthly household income (quintiles), smoking status (never, past, or <10, 10–20, or >20 cigarettes/d), alcohol intake (4 categories: 0 for non-drinkers <1 g/d and tertiles for those with ≥1 g/d), calorie-adjusted saturated fat intake (quintiles), adipose tissue x-linolenic acid (18:3n–3) (quintiles), history of diabetes (yes or no), hypertension (yes or no), history of hypercholesterolemia (yes or no), family history of heart disease (yes or no), and adipose tissue trans fatty acids (quintiles). The main sources of saturated fat in the Costa Rican diet are palm oil, beef, and dairy products (17). trans Fatty acids in adipose tissue include 16:1, 18:1, and 18:2t. Other potential confounders examined but not included in the final models were calcium, vitamin D, fiber, cholesterol, polyunsaturated fatty acid intake, and the adipose tissue fatty acids 18:2n–6, 18:3n–6, 15:0, 17:0, and 20:4n–6. In controls, a multiple linear regression model was used to test the trend for dietary CLA across adipose tissue CLA deciles adjusted for age, sex, and waist-to-hip ratio.

RESULTS

The basic characteristics of the cases and controls are shown in Table 1. The distribution of potential confounders by quintiles of adipose tissue 9c,11t-CLA is shown in Table 2. Median 9c,11t-CLA values in adipose tissue ranged from 0.35% of fatty acids in the lowest quintile to 0.78% in the highest quintile. The median 9c,11t-CLA values correspond to the first and fifth adipose tissue CLA quintiles were 0.23 and 0.33 g/d, 0.27% and 0.37% of total fat, and 0.08% and 0.12% of energy, respectively. Higher adipose tissue 9c,11t-CLA values were associated with less smoking; lower physical activity, waist-to-hip ratios, and polyunsaturated fat, alcohol, and dietary fiber intakes; and lower amounts of linoleic (18:2n–6) and x-linolenic (18:3n–3) acid in adipose tissue. Higher adipose tissue 9c,11t-CLA values were associated with higher income, higher dairy product and saturated fat intakes, and higher amounts of trans fatty acids in adipose tissue.

ORs and 95% CIs for risk of MI across quintiles of 9c,11t-CLA in adipose tissue are shown in Table 3. Higher amounts of adipose tissue 9c,11t-CLA were associated with a 43% lower risk
of MI in the basic model (OR: 0.57; 95% CI: 0.45, 0.71). Adjustment for total energy by use of the residual method.

Further adjustment for saturated fat intake and trans fatty acids in adipose tissue strengthened the association (OR: 0.51; 95% CI: 0.36, 0.71). We also examined the association between dairy product intake and risk of MI in the basic model (OR: 0.57; 95% CI: 0.45, 0.71). Adjustment for saturated fat intake and trans fatty acids in adipose tissue attenuated the association (OR: 0.64; 95% CI: 0.48, 0.84). Further adjustment for saturated fat intake and trans fatty acids in adipose tissue strengthened the association (OR: 0.51; 95% CI: 0.36, 0.71). We also examined the association between dairy product intake and risk of MI (Table 4). No association between dairy intake and risk of MI was observed in the basic model that included physical activity, income, smoking, and history of hypertension and diabetes.

However, an inverse association between dairy intake and risk of MI was observed after adjustment for saturated fat intake and trans fatty acids in adipose tissue. To examine whether this inverse association could be attributed to 9c,11t-CLA, we further adjusted the model by deciles of dietary 9c,11t-CLA as a percentage of fatty acids shown in Figure 2. A significant positive relation was observed between dairy and adipose 9c,11t-CLA (r = 0.24; 95% CI: 0.19, 0.28). We also assessed the correlation between adipose tissue 9c,11t-CLA and intake of dairy products and beef. Median adipose tissue 9c,11t-CLA values as a percentage of total fatty acids by deciles of dairy product and beef intake as servings per day are shown in Figure 3. The intake of dairy products was significantly correlated with adipose tissue 9c,11t-CLA (r = 0.31; 95% CI: 0.26, 0.35). In contrast, no correlation was found between beef intake and 9c,11t-CLA in adipose tissue (r = −0.02; 95% CI: −0.07, 0.04).

Compared with dairy product intake, the variation in beef intake was small across adipose 9c,11t-CLA values. Beef intake ranged between 0 servings/d in the lowest decile to 1.2 servings/d in the highest decile of adipose tissue 9c,11t-CLA, whereas dairy product intake ranged from 0.2 in the lowest decile to 5.0 servings/d in the highest decile. The corresponding median 9c,11t-CLA value in adipose tissue was 0.56 for both deciles of beef intake. Thus, adipose tissue 9c,11t-CLA mostly reflected the intake of dairy products.

DISCUSSION

We examined whether adipose tissue 9c,11t-CLA, an isomer that represents ~80% of all CLA isomers in the diet, was associated with higher risk of MI. Our data showed that higher adipose tissue 9c,11t-CLA was associated with lower risk of MI.
Intake of dairy products was not associated with risk of MI. Subjects with higher adipose tissue c,11t-CLA values had higher c,11t-CLA intakes and higher intakes of dairy products. The results from this study appear counterintuitive because dairy fat also contains relatively high proportions of saturated fat with well-established pro-atherogenic characteristics. However, consistent with our study, most epidemiologic studies show that the intake of dairy products, per se, is either not associated with a higher risk of heart disease or is associated with a lower risk of heart disease (18). In a study in Greece, a one-portion increase in

### TABLE 2
General characteristics and potential confounders by quintile of adipose tissue conjugated linoleic acid (CLA; 9c,11t 18:2n–7) in population-based controls

<table>
<thead>
<tr>
<th>Quintile of adipose CLA</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>P for trend^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median adipose CLA (% of total fatty acids)</td>
<td>0.35</td>
<td>0.45</td>
<td>0.53</td>
<td>0.62</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>CLA intake (g/d)</td>
<td>0.23</td>
<td>0.26</td>
<td>0.29</td>
<td>0.30</td>
<td>0.33</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>(% of total fat)</td>
<td>0.27</td>
<td>0.30</td>
<td>0.33</td>
<td>0.34</td>
<td>0.37</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>(% of energy)</td>
<td>0.08</td>
<td>0.10</td>
<td>0.11</td>
<td>0.11</td>
<td>0.12</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.96</td>
<td>0.96</td>
<td>0.95</td>
<td>0.95</td>
<td>0.93</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Physical activity (METs)</td>
<td>1.66</td>
<td>1.59</td>
<td>1.58</td>
<td>1.50</td>
<td>1.42</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Monthly household income (US$)</td>
<td>520</td>
<td>608</td>
<td>620</td>
<td>563</td>
<td>583</td>
<td>0.03</td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>24</td>
<td>24</td>
<td>17</td>
<td>18</td>
<td>20</td>
<td>0.49</td>
</tr>
<tr>
<td>Alcohol (% drinkers)</td>
<td>50</td>
<td>46</td>
<td>40</td>
<td>35</td>
<td>36</td>
<td>0.0006</td>
</tr>
<tr>
<td>History of diabetes (%)</td>
<td>17</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>13</td>
<td>0.007</td>
</tr>
<tr>
<td>History of hypertension (%)</td>
<td>29</td>
<td>31</td>
<td>29</td>
<td>29</td>
<td>31</td>
<td>0.28</td>
</tr>
</tbody>
</table>

### TABLE 3
Odds ratios and 95% CIs for risk of myocardial infarction by quintile of adipose tissue conjugated linoleic acid (CLA; 9c,11t 18:2n–7)

<table>
<thead>
<tr>
<th>Quintile of adipose CLA</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median adipose tissue CLA (% of total fatty acids)</td>
<td>0.35</td>
<td>0.45</td>
<td>0.53</td>
<td>0.62</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>Model 1^2</td>
<td>1.00 (ref)</td>
<td>0.78 (0.64, 0.96)</td>
<td>0.75 (0.60, 0.93)</td>
<td>0.65 (0.52, 0.81)</td>
<td>0.57 (0.45, 0.71)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Model 2^3</td>
<td>1.00 (ref)</td>
<td>0.84 (0.66, 1.08)</td>
<td>0.94 (0.72, 1.22)</td>
<td>0.73 (0.56, 0.95)</td>
<td>0.66 (0.51, 0.88)</td>
<td>0.002</td>
</tr>
<tr>
<td>Model 3^4</td>
<td>1.00 (ref)</td>
<td>0.85 (0.66, 1.09)</td>
<td>0.93 (0.71, 1.22)</td>
<td>0.72 (0.55, 0.94)</td>
<td>0.64 (0.48, 0.84)</td>
<td>0.0007</td>
</tr>
<tr>
<td>Model 4^5</td>
<td>1.00 (ref)</td>
<td>0.80 (0.61, 1.04)</td>
<td>0.86 (0.64, 1.14)</td>
<td>0.62 (0.46, 0.84)</td>
<td>0.51 (0.36, 0.71)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

1 All values are medians. n = 1813. METs, metabolic equivalent tasks.
2 General linear regression models were used for tests for trends across quintiles by using the median value for each of the quintiles modeled as a continuous variable.
3 Smokers of ≥10 cigarettes/d.
4 Adjusted for total energy by use of the residual method.
5 Sum of 18:1n−7t (vaccenic acid), 18:1n−9t (elaidic acid), and 18:1n−12t (petroselaidic acid).
6 Sum of 18:2n−6t (linoelaidic acid), 18:2n−6c, and 18:2n−6c.
dairy product consumption was associated with 12% lower risk of MI (19). Studies conducted using biomarkers of dairy intake have yielded conflicting results. In Norway, a higher content of 15:0 in adipose tissue was associated with a lower risk of MI (20). In contrast, a higher content of 15:0 in plasma was associated with a higher risk of MI in the United States (21). Our findings on conjugated linoleic acid (CLA) by deciles of beef (19) and saturated fat intake in dairy products (23) are consistent with these findings. Intake of dairy products in this study (238 g/d) is lower than estimated intakes in a study conducted in the United States (247 g/d) (24). However, the reported average 9c,11t-CLA intake in Costa Rica (0.29 g/d) was higher than estimated intakes in studies conducted in the United States (0.11 g/d) (25) but similar to adipose tissue values observed in Sweden (26). These differences could be explained by a higher content of 9c,11t-CLA in Costa Rica. For example, the content of CLA measured in milk fat in Costa Rica is higher (15.8 mg CLA/g fat) (13) than the contents reported for the United States (4.5 mg CLA/g fat) (2).

A positive relation was found between dietary and adipose tissue 9c,11t-CLA. Consistent with the findings of a previous study (26), adipose tissue 9c,11t-CLA was positively associated with dairy product intake. In contrast, no relation was found between adipose tissue 9c,11t-CLA and beef intake. This observation could be explained by the small range of beef intake (0 serving/d in the lowest decile to 1.2 servings/d in the highest decile) compared with dairy product intake (0.2 serving/d in the lowest to 5.0 servings/d in the highest decile). Also, 9c,11t-CLA from dairy products contributed 59% of total dietary CLA, whereas beef contributed 19%. These findings suggest that 9c,11t-CLA from dairy products played a role in the inverse association between adipose tissue 9c,11t-CLA and risk of MI.

Large between-country differences exist in the consumption of dairy products worldwide, with some countries consuming as low as 27 g/d (23). The intake of dairy products in this study (238 g/d) was somewhat lower than that reported for adults in the United States (247 g/d) (24). However, the reported average 9c,11t-CLA intake in Costa Rica (0.29 g/d) was higher than estimated intakes in a study conducted in the United States (0.11 g/d) (25) but similar to adipose tissue values observed in Sweden (26). These differences could be explained by a higher content of 9c,11t-CLA in Costa Rica. For example, the content of CLA measured in milk fat in Costa Rica is higher (15.8 mg CLA/g fat) (13) than the contents reported for the United States (4.5 mg CLA/g fat) (2).
Several mechanisms can be proposed to explain our findings, but the results from animal models and intervention trials are far from clear. Experimental research in animal models has found that CLA can protect against atherosclerosis and that this effect could be mediated by its effect on plasma lipids (5, 6, 8). These findings have not been consistent because null and positive effects have been also detected (7). CLA intake causes weight reduction in animal models (27)—an effect that has been attributed to an increase in energy expenditure and energy lost in excreta (28). In support of this hypothesis, we found an inverse association between adipose tissue 9c,11t-CLA and the waist-to-hip ratio, but this trend was not significant after adjustment for confounders. Studies of the effects of CLA on weight reduction in humans are ambiguous (9, 29), and the unadjusted associations in this study are relatively small. It is also possible that CLA could have beneficial antiinflammatory effects. Feeding obese mice a 9c,11t-CLA–enriched diet reduced macrophage infiltration in adipose tissue and down-regulated several inflammatory markers, including tumor necrosis factor-α, and nuclear transcription factor κB expression, DNA binding, and transcriptional activity (30). Other studies have shown peroxisome proliferator–activated receptor-α activation by CLA (31). Thus, human research studies of the pathways by which 9c,11t-CLA at doses commonly found in the diet could affect heart disease in humans are needed.

The main limitation of this study was the use of a retrospective case-control design in which causal effects cannot be studied. Residual confounding by other factors was also a possibility. For example, confounding by other nutrients in dairy products, such as calcium, potassium, and vitamins D and B, were evaluated but not included in the final models because they did not modify the results. These nutrients have been shown to be associated with beneficial outcomes (32–35), but they were measured with a food-frequency questionnaire that has more measurement error than CLA in adipose. Also, the observed results may not be totally determined by dietary CLA, because adipose tissue fatty acids also reflect metabolism. For example, trans-vaccenic acid can be converted to CLA in humans (36). The lack of a clear mechanism that can explain these results is also a concern and deserves further research.

The CLA content of milk and of other dairy products ranges from 0.34% to 1.07% of total fat and is influenced by the diet of cows (3). Range-fed animals or those grazing solely on pastures have a higher content of CLA in their milk than do those raised on grains and forage (4). However, it is uncertain at this point whether an increase in CLA in dairy products will have beneficial effects that would counterbalance the adverse effects of saturated fat. Some studies have also found adverse effects of specific CLA isomers when very high doses and different isomer combinations are used (9). Thus, promotion of products containing CLA as beneficial is misguided at this point.

In conclusion, this study showed that higher adipose tissue 9c,11t-CLA is associated with a reduced risk of nonfatal acute MI in Costa Rica. Dairy product intake was not associated with risk of MI. It is possible that the potentially beneficial effect of CLA on MI offsets the adverse effects of saturated fat. The higher proportion of 9c,11t-CLA among traditional pasture grazing dairy cows could improve the adverse effects of saturated fat in dairy products.

The authors’ responsibilities were as follows—LAS: drafted the manuscript; HC: conceptualized and designed the study, acquired the data, and obtained funding; and LAS, AB, and HC: conducted the statistical analyses, interpreted the results, provided critical revision of the manuscript for important intellectual content, approved the submitted manuscript, participated sufficiently in the work to take public responsibility for the entire manuscript, had full access to all of the data in the study, and took responsibility for the integrity of the data and the accuracy of the data analysis. None of the authors reported conflicts of interest.

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