High 18:2 *Trans*-Fatty Acids in Adipose Tissue Are Associated with Increased Risk of Nonfatal Acute Myocardial Infarction in Costa Rican Adults[1,2]

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ABSTRACT *Trans*-fatty acid intake is associated with coronary heart disease (CHD), but the atherogenic potential of individual *trans*-fatty acids (FA) from partially hydrogenated oils (18:1 and 18:2) or meat and dairy products (16:1 and 18:1) is unclear. Incident cases (n = 482) of a first nonfatal myocardial infarction (MI) were matched with population controls (n = 482) for age, gender and area of residence, all living in Costa Rica. *Trans*-FA in adipose tissue samples were assessed by gas chromatography. Odds ratios (OR) and 95% confidence intervals were calculated from conditional logistic regression models. Total adipose tissue *trans*-fat was positively associated with risk of MI. After adjusting for established risk factors and other confounders, the OR by quintiles of total *trans*-fat were 1.00, 1.34, 2.05, 2.22 and 2.94 (P-test for trend < 0.01). This association was attributed mainly to 18:2 *trans*-FA that were abundant in both adipose tissue and in partially hydrogenated soybean oil, margarines and baked products used by this population; OR = 1.00, 0.96, 2.09, 3.51 and 5.05 (P-test for trend < 0.001). Adipose tissue 16:1 *trans*-FA were also associated with MI; OR = 1.00, 1.57, 1.39, 1.34 and 2.58 (P-test for trend < 0.05). An association with 18:1 *trans*-FA was not detected. High 18:2 *trans*-FA in adipose tissue are associated with increased risk of MI. Because the use of hydrogenated oils is increasing worldwide, consumers should be aware of the harmful effects of products containing partially hydrogenated oils. J. Nutr. 133: 1186–1191, 2003.

KEY WORDS: • *fatty acids* • *myocardial infarction* • *diet*

The link between *trans*-fatty acid intake and coronary heart disease (CHD)[4] has been a controversial topic (1–4). Although *trans*-fatty acids (FA) are found in many foods, they are more prevalent in ruminant fat (meats and dairy products) and partially hydrogenated vegetable oils. The food source (animal or vegetable) determines the type and content of specific *trans*-isomers in adipose tissue (5). Most hydrogenated products have substantially higher amounts of *trans*-FA than meats and dairy products (1). Partially hydrogenated oils are the major source of dietary *trans*-FA in the United States (6) and Europe, whereas in Mediterranean countries, animal products provide >50% of *trans*-FA (7). In the last two decades, the intake of *trans*-FA in Europe has decreased, mainly as a result of the availability of margarines with lower *trans*-FA concentrations (7,8). Although softer margarines are available in the United States, the current intake of *trans*-FA is similar to that observed in the 1960s (2% of energy). This is due mainly to high use of hydrogenated oils in baked products and fast foods (9).

All five prospective studies performed with dietary data from food-frequency questionnaires (FFQ) and other dietary assessment methods showed a positive association between total *trans*-fat intake and myocardial infarction (MI) (8,10–14). A comprehensive analysis of four of these studies showed that a 2% increase in energy intake from *trans*-FA was associated with a 25% [95% confidence interval (CI) 11%–40%] increase in the risk of CHD (8). Surprisingly, case-control studies using biomarkers of *trans*-fatty acid intake (15–19) have not been consistent; several studies showed no association (16–18,20) and some studies suggested that *trans*-FA vary in their potential for atherogenicity and that the type of *trans*-fat consumed may explain some of the differences across populations (15,19).

Cardiovascular disease (CVD) is the main cause of death in several developing countries (21), many of which are gradually replacing traditional fats with hydrogenated vegetable oils (22). To date, there are no published studies on *trans*-fatty acid intake or their effects in populations with dietary patterns and lifestyles different from those of developed countries. Because

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[4] Abbreviations used: CHD, coronary heart disease; CI, confidence interval; CVD, cardiovascular disease; FA, fatty acids; FFQ, food-frequency questionnaire; GLC, gas-liquid chromatography; MI, myocardial infarction.

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food products vary widely in their content of total and specific *trans*-isomers (1), obtaining reliable data on intake is difficult. Biomarkers of intake are a good alternative to FFQ because they do not require knowledge of the *trans*-fat content of foods, do not rely on memory or self-reported information and are not subject to interviewer bias (23). In this study we used adipose tissue biomarkers of intake to examine the association between 16:1, 18:1 and 18:2 *trans*-FA and risk of MI.

SUBJECTS AND METHODS

Study population. The catchment area for this study was the 18 counties that compose the metropolitan area of San José, Costa Rica. Eligible case subjects were men and women who were diagnosed as survivors of a first acute MI by two independent cardiologists at any of the three recruiting hospitals in the catchment area (San Juan de Dios Hospital, Calderón Guardia Hospital and México Hospital) between 1994 and 1998 as previously described (24). All cases met the WHO criteria for MI, which require typical symptoms plus either elevations in cardiac enzyme levels or diagnostic changes in the electrocardiogram (25). Cases were ineligible if they *1*) died during hospitalization, *2*) were ≥75 y old on the day of their first MI, 3) were physically or mentally unable to answer the questionnaire, and 4) had a previous hospital admission related to CVD. Enrollment was carried out while cases were in the hospital's step-down unit. To achieve 100% ascertainment, fieldworkers carried out daily visits to the three hospitals.

Cases (n = 530) were matched by age (±5 y), sex and area of residence to population controls (n = 531) randomly identified with the aid of data from the National Census and Statistics Bureau of Costa Rica. 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 unlikely to have undiagnosed CVD due to poor access to medical care. Control subjects were ineligible if they had ever had an MI or if they were physically or mentally unable to answer the questionnaires. All cases and controls were visited in their homes for the collection of dietary and health information, anthropometric measurements and biological specimens. Participation was 97% for cases and 90% for controls. All subjects gave informed consent on documents approved by the Human Subjects Committee of the Harvard School of Public Health and the University of Costa Rica.

Data collection. Trained personnel visited the subjects at their homes for data collection. Subjects provided information on socioeconomic, demographic and health characteristics during an interview. A subcutaneous adipose tissue biopsy was collected from the upper buttock as previously described (26). The samples were stored at −80°C and later shipped to the Harvard School of Public Health for analysis. Energy and nutrient intakes were assessed with an FFQ developed and validated specifically for use among Costa Ricans (26,27). Dietary information obtained by the FFQ was used for validation purposes and to assess confounding by dietary factors that do not have good biomarkers of intake such as saturated fat intake. The fatty acid composition of all major types of fat used for cooking in Costa Rica was determined (Campos, H., unpublished data, 1999–2001) and incorporated into the nutrient calculation.

Fatty acid analysis. Fatty acids were extracted from adipose tissue and analyzed by gas-liquid chromatography (GLC) (26). Briefly, the fatty acids in the in the adipose tissue biopsy were extracted using a hexane/isopropanol (3:2) mixture and esterified with methanol and acetyl chloride. After esterification, the methanol and acetyl chloride were evaporated, and the fatty acid methyl esters were redissolved in isooctane. The methyl esters were quantitated by GLC. Peak retention times and area percentages of total fatty acids were identified by injecting known standards (Nu-Chek-Prep, Elysium, MN), and analyzed with the Agilent Technologies ChemStation A.08.03 software (Agilent Technologies, Inc., Palo Alto, CA). Twelve identical samples were analyzed throughout the study. The CV for 16:1, 18:1 and 18:2 *trans*-FA were 8.4, 15.7 and 6.4%, respectively. We previously showed that adipose tissue *trans*-FA are good biomarkers of *trans*-fatty acid intake in the Costa Rican population (26).

Statistical analysis. Of the 1061 subjects that were recruited, 48 cases and 49 controls were excluded because of missing data. The final sample size consisted of 482 case-control pairs that included 712 men and 252 women. Nutrient intakes were adjusted for total energy intake using regression methods (23). The *trans*-fatty acids in the models below were defined as follows: 16:1 = 16:1(n-7*t*); 18:1 = 18:1(n-7*t*) + 18:1(n-9*t*) + 18:1(n-12*t*); 18:2 = 18:2(n-6*tt*) + 18:2(n-6*ct*) + 18:2(n-6*tc*); Total *trans*- FA = 16:1 + 18:1 + 18:2. Odds ratios (OR) and 95% confidence intervals (CI) of the top quintiles relative to the lowest quintile of total or each adipose tissue *trans*-FA (16:1, 18:1 and 18:2) were estimated using conditional logistic regression (28). Subjects were divided in quintiles and the median value for each quintile was assigned. Tests for trend were performed across quintiles, using the median value for each of the quintiles modeled as a continuous variable. We performed stratified analyses using unconditional logistic regression (with matching variables in the model) to test whether the associations varied by type of oil used for cooking. We tested for the statistical significance of interactions between gender and adipose tissue *trans*-fatty acids using the likelihood ratio test (28).

RESULTS

Cases were more likely to be current smokers than controls and had a higher prevalence of diabetes and hypertension (**Table 1**). Cases were less physically active, had higher waist-to-hip ratio and less income than controls. The distribution of potential confounders by quintiles of total adipose tissue *trans*-fat is shown in **Table 2**. There was a strong positive association between adipose tissue *trans*-FA and income, α-linolenic and linoleic acid and vitamin E, whereas alcohol intake was negatively associated. The same analysis by each adipose tissue *trans*-fatty acid (i.e., 16:1, 18:1 and 18:2) yielded similar results. However, compared with 18:1 and 18:2 *trans*-FA, the correlations between 16:1 *trans*-FA and the variables above were weaker (data not shown). The major source of 18:1 and 18:2 *trans*-FA in Costa Rica is partially hydrogenated soybean oil, which was used by >40% of the subjects. Other important sources of 18:1 and 18:2 *trans*-FA were margarine and baked products. It is notable that ruminant products contain substantial amounts of 18:1 *trans*-FA and were a major source of 16:1 *trans*-FA.

TABLE 1

General characteristics of Costa Rican adult survivors of myocardial infarction and population-based matched controls[1]

	Controls (n = 482)	Cases (n = 482)	P
Age, *y*	57 ± 11	57 ± 10	
Gender, % *women*	26	26	
Body mass index, *kg/m²*	25.9 ± 4.0	26.0 ± 3.9	0.77
Waist-to-hip ratio	0.94 ± 0.07	0.95 ± 0.07	0.0002
Physical activity,[2] *METS*	1.54 ± 0.63	1.46 ± 0.65	0.045
Income, *US$/mo*	570 ± 485	462 ± 436	0.0012
Diabetes, %	12	24	<0.0001
Hypertension, %	27	43	<0.0001
Current smokers,[3] %	27	43	<0.0001
Multivitamin users,[4] %	9.5	7.3	0.18

[1] Values are means ± SD or %, n = 964.
[2] METS, metabolic equivalents.
[3] Smoke ≥1 cigarette/d.
[4] Including supplement users.

TABLE 2

Distribution of potential confounders of Costa Rican population-based controls

	Quintiles of total *trans*-fat in adipose tissue, (mean, g/100 g)[1,2]				
	1 (1.80)	2 (2.46)	3 (3.00)	4 (3.61)	5 (4.61)
Adipose tissue FA,[3] *g/100 g*					
16:1 *trans*-FA	0.07	0.08	0.08	0.09	0.08
18:1 *trans*-FA	0.94	1.34	1.67	2.02	2.57
18:2 *trans*-FA	0.79	1.04	1.23	1.50	1.93
α-Linolenic acid	0.43	0.53	0.51	0.61	0.66
Linoleic acid	10.8	12.6	12.6	14.7	15.9
Eicosapentaenoic acid	0.04	0.05	0.04	0.04	0.04
Docosahexaenoic acid	0.17	0.17	0.16	0.18	0.16
Body mass index, *kg/m²*	26.8	25.8	26.1	25.4	25.1
Waist-to-hip ratio	0.95	0.95	0.94	0.93	0.92
Income, *US$/mo*	391	476	620	663	672
Time living in the house, *y*	26	24	22	24	23
Diabetes, %	10	12	10	19	7
Hypertension, %	36	21	27	28	23
Physical activity,[4] *METS*	1.64	1.66	1.52	1.49	1.45
Current smokers, %	35	31	23	30	23
Multivitamin use, %	3.7	9.0	11.4	8.6	12.5
Daily intake					
Total energy, *MJ/d*	8.87	9.68	10.08	10.07	9.87
Saturated fat, *% energy*	12.4	11.8	11.8	11.3	10.9
Alcohol, *g/d*	12.9	8.0	6.6	5.4	4.4
Vitamin E, *mg/4.18 MJ*	11	15	17	20	25
Folate, *μg/4.18 MJ*	163	159	164	157	157
Fiber, *g/4.18 MJ*	11.5	10.7	10.8	10.0	10.5
Fish intake, *g/d*	15	19	18	19	22

[1] (*n* = 482).
[2] 16:1 = 16:1(n-7*t*); 18:1 = 18:1(n-7*t*) + 18:1(n-9*t*) + 18:1(n-12*t*); 18:2 = 18:2(n-6*tt*) + 18:2(n-6*ct*) + 18:2(n-6*tc*); total *trans*-fat = 16:1 + 18:1 + 18:2.
[3] FA, fatty acids.
[4] METS, metabolic equivalents.

Total adipose tissue *trans*-fat was associated with increased risk of MI (**Table 3**) after adjusting for sex, age, residence, income, history of diabetes, history of hypertension, physical activity, smoking, years living in the house, adipose tissue α-linolenic acid and intake of alcohol, vitamin E, saturated fat and total energy. Total adipose tissue *trans*-fat comprised ~3% 16:1 *trans*-FA, 55% 18:1 *trans*-FA, and 45% 18:2 *trans*-FA.

The age-, sex- and residence-adjusted OR for 16:1 *trans*-FA showed no association with MI (**Table 4**). However, after adjusting for established risk factors, subjects in the fifth quintile showed a higher risk than those in the first quintile (OR = 2.32; 95% CI, 1.24–4.33, *P*, test for trend = 0.02) (Table 4). Adjustment for adipose tissue α-linolenic acid and other dietary variables strengthened this association. The significant effect observed only in the highest quintile may suggest a threshold effect for 16:1 *trans*-FA (Table 4).

Adipose tissue 18:1 *trans*-FA were not associated with the risk of MI (**Table 5**). Adjustment for nondietary confounders increased the OR for the third and fourth quintiles, but the fifth quintile was not significant and there was no significant

TABLE 3

Odds ratios (OR) and 95% confidence intervals (CI) of myocardial infarction by quintiles of total trans-fatty acid in men and women[1]

	Quintiles of adipose tissue total *trans*-fatty acid, (median, g/100 g)					
	1 (1.84)	2 (2.46)	3 (2.98)	4 (3.57)	5 (4.40)	*P*
Age-, sex- and residence-adjusted OR	1.00	1.05 (0.70–1.59)	0.99 (0.65–1.50)	1.02 (0.68–1.55)	0.93 (0.61–1.41)	0.6762
Multivariate OR[2]	1.00	1.37 (0.78–2.41)	1.84 (1.03–3.31)	1.75 (1.00–3.07)	1.63 (0.88–3.02)	0.1106
Multivariate OR[3,4]	1.00	1.34 (0.73–2.47)	2.05 (1.06–3.98)	2.22 (1.14–4.33)	2.94 (1.36–6.37)	0.0040

[1] Values are OR and 95% CI from conditional logistic regression models.
[2] Adjusted for income, history of diabetes, history of hypertension, physical activity, smoking status, years living in the house and alcohol intake.
[3] Adjusted further for adipose tissue α-linolenic acid and intake of vitamin E, saturated fat and total energy.
[4] Further adjustment for body mass index, walst-to-hip ratio, multivitamin use, folate or fiber intake did not affect the results.

TABLE 4

Odds ratios (OR) and 95% confidence intervals (CI) of myocardial infarction by quintiles of 16:1 trans-fatty acid in men and women[1]

	Quintiles of adipose tissue 16:1 *trans*-fatty acid, (median, g/100 g)					
	1 (0.044)	2 (0.070)	3 (0.080)	4 (0.096)	5 (0.115)	*P*
Age-, sex- and residence-adjusted OR	1.00	1.09 (0.73–1.62)	1.38 (0.90–2.12)	1.06 (0.68–1.65)	1.42 (0.90–2.24)	0.18
Multivariate OR[2]	1.00	1.20 (0.69–2.07)	1.34 (0.77–2.35)	1.25 (0.68–2.30)	2.32 (1.24–4.33)	0.015
Multivariate OR[3,4]	1.00	1.57 (0.83–2.98)	1.39 (0.73–2.66)	1.34 (0.65–2.79)	2.58 (1.22–5.43)	0.025

[1] Values are OR and 95% CI from conditional logistic regression models.
[2] Adjusted for income, history of diabetes, history of hypertension, physical activity, smoking status, years living in the house and alcohol intake.
[3] Adjusted further for adipose tissue α-linolenic acid and intake of vitamin E, saturated fat and total energy.
[4] Further adjustment for body mass index, waist-to-hip ratio, multivitamin use, folate or fiber intake did not affect the results.

trend. This association is likely to have been due to confounding, because it was completely abolished by further adjustment for dietary variables.

The age-, sex- and residence-adjusted OR for 18:2 *trans*-FA showed no association with MI (**Table 6**). Adjustment for established CVD risk factors increased the OR, but the association remained nonsignificant. Adjusting for dietary variables strengthened this association due mainly to α-linolenic acid. For example, when only α-linolenic acid was added to the model with established CVD risk factors, subjects in the highest quintile showed a higher risk of MI compared with those in the lowest quintile (OR = 4.04; 95% CI, 1.88–8.71, *P*, test for trend <0.0001).

Adjustment for other dietary variables, such as intake of fiber, folate or cholesterol, did not change the results. Analyses using specific isomers within 18:1 or 18:2 *trans*-FA yielded similar results. Subjects in the highest quintile of 18:2 *trans*-FA were at a higher risk of MI than subjects in the lowest quintile, regardless of the primary type of oil used for cooking (i.e., palm oil or soybean oil). We did not find any interaction between gender and adipose tissue *trans*-FA.

DISCUSSION

We assessed the relationship between adipose tissue *trans*-FA and the risk of MI in Costa Rica. We detected a positive association between *trans*-FA and MI. This association was attributed mainly to 18:2 *trans*-FA, which were abundant in both adipose tissue and in partially hydrogenated soybean oil. We did not detect an association between 18:1 *trans*-FA and MI.

The association between total adipose tissue *trans*-fat and MI found in our study is consistent with findings from prospective studies that examined this association using intake data from industrialized nations (8,10–14). In developed countries *trans*-fat derives mainly from margarines, baked products, fast foods and processed foods (9). In contrast, the main source of *trans*-FA in our study was partially hydrogenated soybean oil consumed in homemade meals.

Our data suggest a potential positive association between 16:1 *trans*-FA and MI. These data are consistent with previous studies but these early studies did not control for important confounders (15,29,30). Only the fifth quintile was associated with MI and the trend was weak. Therefore, it is possible that although we adjusted for confounders, some confounding may have remained because the main sources of 16:1 *trans*-FA are also important sources of saturated fat. More studies are required to confirm this result.

Other studies using biomarkers of intake have not found an association between *trans*-FA and CHD (16,17,20), probably because of small sample sizes (16–18). In studies of diet and CHD, a small sample size limits the ability to adjust for dietary confounders such as *cis*-polyunsaturated FA that tend to be highly correlated with *trans*-FA but have opposite effects on MI. However, no association between adipose tissue *trans*-FA and MI was found in the EURAMIC study (20), a large European study on CHD. This association remained nonsig-

TABLE 5

Odds ratios (OR) and 95% confidence intervals (CI) of myocardial infarction by quintiles of 18:1 trans-fatty acid in men and women[1]

	Quintiles of adipose tissue 18:1 *trans*-fatty acid, (median, g/100 g)					
	1 (0.94)	2 (1.31)	3 (1.61)	4 (1.97)	5 (2.54)	*P*
Age-, sex- and residence-adjusted OR	1.00	1.21 (0.80–1.83)	1.09 (0.71–1.67)	1.02 (0.67–1.55)	0.91 (0.60–1.39)	0.38
Multivariate OR[2]	1.00	1.31 (0.75–2.30)	1.89 (1.04–3.44)	2.00 (1.11–3.63)	1.20 (0.66–2.19)	0.54
Multivariate OR[3,4]	1.00	1.14 (0.57–2.26)	1.12 (0.54–2.32)	1.26 (0.58–2.71)	0.75 (0.34–1.65)	0.34

[1] Values are OR and 95% CI from conditional logistic regression models.
[2] Adjusted for income, history of diabetes, history of hypertension, physical activity, smoking status, years living in the house and alcohol intake.
[3] Adjusted further for adipose tissue α-linolenic acid and intake of vitamin E, saturated fat and total energy.
[4] Further adjustment for body mass index, waist-to-hip ratio, multivitamin use, folate or fiber intake did not affect the results.

TABLE 6

Odds ratios (OR) and 95% confidence intervals (CI) of myocardial infarction by quintiles of 18:2 trans-fatty acid in men and women[1]

	Quintiles of adipose tissue 18:2 *trans*-fatty acids, (median, g/100 g)					
	1 (0.75)	2 (0.98)	3 (1.20)	4 (1.50)	5 (2.04)	*P*
Age-, sex- and residence-adjusted OR	1.00	0.89 (0.58–1.35)	1.02 (0.66–1.58)	0.99 (0.65–1.50)	0.88 (0.58–1.36)	0.70
Multivariate OR[2]	1.00	1.07 (0.61–1.88)	1.60 (0.90–2.85)	1.84 (1.03–3.28)	1.42 (0.77–2.60)	0.15
Multivariate OR[3,4]	1.00	0.96 (0.49–1.89)	2.09 (0.98–4.48)	3.51 (1.49–8.29)	5.05 (1.86–13.72)	0.0005

[1] Values are OR and 95% CI from conditional logistic regression models.
[2] Adjusted for income, history of diabetes, history of hypertension, physical activity, smoking status, years living in the house and alcohol intake.
[3] Adjusted further for adipose tissue α-linolenic acid and intake of vitamin E, saturated fat and total energy.
[4] Further adjustment for body mass index, waist-to-hip ratio, multivitamin use, folate or fiber intake did not affect the results.

nificant even after exclusion of the Spanish centers whose subjects had low adipose tissue *trans*-FA (20).

Our finding of distinct associations between types of *trans*-FA and MI offers some insight into these apparent discrepancies. It has been postulated that the adverse effects of *trans*-FA may be confined to specific isomers (31), and some experimental studies suggest that elaidic acid (18:1n-9*t*) may be more harmful than *trans*-vaccenic acid (18:1n-11*t*) (32). Consistent with our data, epidemiologic studies have also found that increased RBC 18:2, but not 18:1 *trans*-FA is associated with increased risk of primary cardiac arrest (19). These differences in risk are difficult to explain because most intervention studies have focused on total or 18:1 *trans*-FA and data on the effects of 18:2 *trans*-FA on plasma lipids are scarce. Interestingly, the MI risk associated with intake of total *trans*-fat observed in epidemiologic studies is substantially higher than what is predicted from its effects on plasma lipoproteins (9), suggesting that other factors may account for this association, such as lipoprotein[a] (33–39). *Trans*-FA can also impair Δ^6 desaturase activity and decrease eicosanoid production (1,39–41). It is also possible that dietary *trans*-FA may include other isomers with more potent effects than those studied in intervention trials.

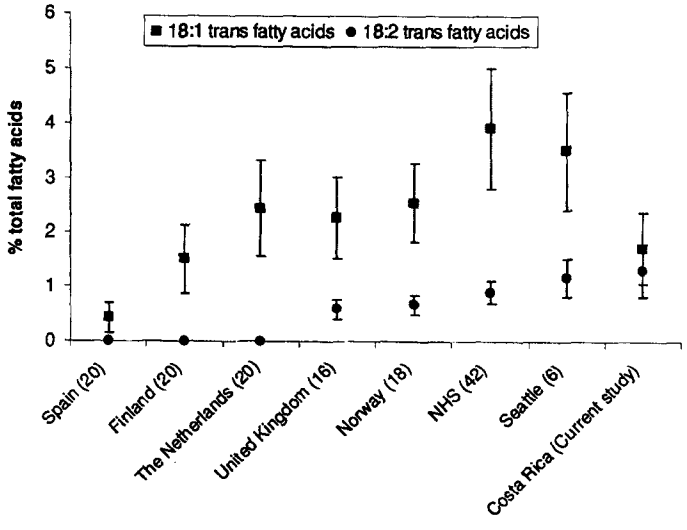

FIGURE 1 Adipose tissue 18:1 and 18:2 *trans*-fatty acid (FA) levels in various countries. Values are means ± SD, n = 48.2. Countries with the minimum (Spain), maximum (The Netherlands) and average (Finland) values in the EURAMIC study (20) were selected. In these countries, 18:2 *trans*-FA were below the detection limit in most samples. NHS, Nurses Health Study (42).

We compared the levels of adipose tissue 18:1 and 18:2 *trans*-FA in several populations. Adipose tissue *trans*-FA levels varied widely across populations (**Fig. 1**). The highest levels (18:1 and 18:2 *trans*-FA) and higher variability were found in the United States where three prospective studies found an association between total *trans*-fat intake and MI (10–13). In contrast, adipose tissue 18:2 *trans*-FA were undetectable in some of the EURAMIC study populations and adipose tissue 18:1 *trans*-FA levels in The Netherlands (the country with the highest values) were still lower than those in the United States. Compared with the United States and Europe, Costa Rica had the highest adipose tissue 18:2 *trans*-FA but 18:1 *trans*-FA were relatively low and spanned a narrower range. Considering the low 18:2-levels in adipose tissue (~1% of total fat), these data suggest that 18:2 *trans*-FA may be more atherogenic than 18:1 *trans*-FA. The lack of an association between 18:1 *trans*-FA and MI in Costa Rica may be due to the low levels of 18:1 in this population; therefore the effects on plasma lipoproteins are expected to be negligible (9). Almost all 18:2 *trans*-isomers in the Costa Rican population derive from partially hydrogenated soybean oil and margarines, whereas 18:1 *trans*-isomers reflect a mixture of vegetable oils and animal fats. Health policies regarding the *trans*-fatty acid content of vegetable oils used in developing countries should be established and implemented. In particular, we recommend that vegetable oils used in processed foods and for cooking should not be hydrogenated.

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LITERATURE CITED

1. Expert Panel on *Trans* Fatty Acids and Coronary Heart Disease (1995) *Trans* fatty acids and coronary heart disease risk. Am. J. Clin. Nutr. 62: 655S–708S; discussion 518S–626S.
2. Ascherio, A. & Willett, W. C. (1997) Health effects of *trans* fatty acids. Am. J. Clin. Nutr. 66: 1006S–1010S.
3. Shapiro, S. (1997) Do *trans* fatty acids increase the risk of coronary artery disease? A critique of the epidemiologic evidence. Am. J. Clin. Nutr. 66: 1011S–1017S.
4. Lichtenstein, A. H. (1997) *Trans* fatty acids, plasma lipid levels, and risk of developing cardiovascular disease. A statement for healthcare professionals from the American Heart Association. Circulation 95: 2588–2590.
5. Sommerfeld, M. (1983) *Trans* unsaturated fatty acids in natural products and processed foods. Prog. Lipid Res. 22: 221–233.
6. Lemaitre, R. N., King, I. B., Patterson, R. E., Psaty, B. M., Kestin, M. &

Heckbert, S. R. (1998) Assessment of *trans*-fatty acid intake with a food frequency questionnaire and validation with adipose tissue levels of *trans*-fatty acids. Am. J. Epidemiol. 148: 1085–1093.

7. Hulshof, K. F., van Erp-Baart, M. A., Anttolainen, M., Becker, W., Church, S. M., Couet, C., Hermann-Kunz, E., Kesteloot, H., Leth, T., Martins, I., Moreiras, O., Moschandreas, J., Pizzoferrato, L., Rimestad, A. H., Thorgeirsdottir, H., van Amelsvoort, J. M., Aro, A., Kafatos, A. G., Lanzmann-Petithory, D. & van Poppel, G. (1999) Intake of fatty acids in western Europe with emphasis on *trans* fatty acids: the TRANSFAIR Study. Eur. J. Clin. Nutr. 53: 143–157.

8. Oomen, C. M., Ocke, M. C., Feskens, E. J., van Erp-Baart, M. A., Kok, F. J. & Kromhout, D. (2001) Association between *trans* fatty acid intake and 10-year risk of coronary heart disease in the Zutphen Elderly Study: a prospective population-based study. Lancet 357: 746–751.

9. Ascherio, A., Katan, M. B., Zock, P. L., Stampfer, M. J. & Willett, W. C. (1999) *Trans* fatty acids and coronary heart disease. N. Engl. J. Med. 340: 1994–1998.

10. Willett, W. C., Stampfer, M. J., Manson, J. E., Colditz, G. A., Speizer, F. E., Rosner, B. A., Sampson, L. A. & Hennekens, C. H. (1993) Intake of *trans* fatty acids and risk of coronary heart disease among women. Lancet 341: 581–585.

11. Ascherio, A., Rimm, E. B., Giovannucci, E. L., Spiegelman, D., Stampfer, M. & Willett, W. C. (1996) Dietary fat and risk of coronary heart disease in men: cohort follow up study in the United States. Br. Med. J. 313: 84–90.

12. Hu, F. B., Stampfer, M. J., Manson, J. E., Rimm, E., Colditz, G. A., Rosner, B. A., Hennekens, C. H. & Willett, W. C. (1997) Dietary fat intake and the risk of coronary heart disease in women. N. Engl. J. Med. 337: 1491–1499.

13. Gillman, M. W., Cupples, L. A., Gagnon, D., Millen, B. E., Ellison, R. C. & Castelli, W. P. (1997) Margarine intake and subsequent coronary heart disease in men. Epidemiology 8: 144–149.

14. Pietinen, P., Ascherio, A., Korhonen, P., Hartman, A. M., Willett, W. C., Albanes, D. & Virtamo, J. (1997) Intake of fatty acids and risk of coronary heart disease in a cohort of Finnish men. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. Am. J. Epidemiol 145: 876–887.

15. Siguel, E. N. & Lerman, R. H. (1993) *Trans*-fatty acid patterns in patients with angiographically documented coronary artery disease. Am. J. Cardiol. 71: 916–920.

16. Roberts, T. L., Wood, D. A., Riemersma, R. A., Gallagher, P. J. & Lampe, F. C. (1995) *Trans* isomers of oleic and linoleic acids in adipose tissue and sudden cardiac death. Lancet 345: 278–282.

17. van de Vijver, L. P., van Poppel, G., van Houwelingen, A., Kruyssen, D. A. & Hornstra, G. (1996) *Trans* unsaturated fatty acids in plasma phospholipids and coronary heart disease: a case-control study. Atherosclerosis 126: 155–161.

18. Pedersen, J. I., Ringstad, J., Almendingen, K., Haugen, T. S., Stensvold, I. & Thelle, D. S. (2000) Adipose tissue fatty acids and risk of myocardial infarction: a case-control study. Eur. J. Clin. Nutr. 54: 618–625.

19. Lemaitre, R. N., King, I. B., Raghunathan, T. E., Pearce, R. M., Weinmann, S., Knopp, R. H., Copass, M. K., Cobb, L. A. & Siscovick, D. S. (2002) Cell membrane *trans*-fatty acids and the risk of primary cardiac arrest. Circulation 105: 697–701.

20. Aro, A., Kardinaal, A. F., Salminen, I., Kark, J. D., Riemersma, R. A., Delgado-Rodriguez, M., Gomez-Aracena, J., Huttunen, J. K., Kohlmeier, L., Martin, B. C. & et al. (1995) Adipose tissue isomeric *trans* fatty acids and risk of myocardial infarction in nine countries: the EURAMIC study. Lancet 345: 273–278.

21. Pan American Health Organization (1998) Health in the Americas, Publication no. 569. PAHO, Washington, D. C.

22. Uauy, R., Albala, C. & Kain, J. (2001) Obesity trends in Latin America: transiting from under- to overweight. J. Nutr. 131: 893S–899S.

23. Willett, W. (1998) Nutritional Epidemiology. Oxford University Press, New York, NY.

24. Campos, H. & Siles, X. (2000) Siesta and the risk of coronary heart disease: results from a population-based, case-control study in Costa Rica. Int. J. Epidemiol. 29: 429–437.

25. Tunstall-Pedoe, H., Kuulasmaa, K., Amouyel, P., Arveiler, D., Rajakangas, A. M. & Pajak, A. (1994) Myocardial infarction and coronary deaths in the World Health Organization MONICA Project. Registration procedures, event rates, and case-fatality rates in 38 populations from 21 countries in four continents. Circulation 90: 583–612.

26. Baylin, A., Kabagambe, E. K., Siles, X. & Campos, H. (2002) Adipose tissue biomarkers of fatty acid intake. Am. J. Clin. Nutr. 76: 750–757.

27. Kabagambe, E. K., Baylin, A., Allan, D. A., Siles, X., Spiegelman, D. & Campos, H. (2001) Application of the method of triads to evaluate the performance of food frequency questionnaires and biomarkers as indicators of long-term dietary intake. Am. J. Epidemiol. 154: 1126–1135.

28. Hosmer, D. W. & Lemeshow, S. (2000) Applied Logistic Regression, 2nd ed. John Wiley & Sons, New York, NY.

29. Thomas, L. H., Winter, J. A. & Scott, R. G. (1983) Concentration of 18:1 and 16:1 transunsaturated fatty acids in the adipose body tissue of decedents dying of ischaemic heart disease compared with controls: analysis by gas liquid chromatography. J. Epidemiol. Community Health 37: 16–21.

30. Thomas, L. H. & Winter, J. A. (1987) Ischaemic heart disease and consumption of hydrogenated marine oils. Hum. Nutr. Food. Sci. Nutr. 41F: 153–165.

31. Khosla, P. & Hayes, K. C. (1996) Dietary *trans*-monounsaturated fatty acids negatively impact plasma lipids in humans: critical review of the evidence. J. Am. Coll. Nutr. 15: 325–339.

32. Rosenthal, M. D. & Doloresco, M. A. (1984) The effects of *trans* fatty acids on fatty acyl delta 5 desaturation by human skin fibroblasts. Lipids 19: 869–874.

33. Nestel, P., Noakes, M., Belling, B., McArthur, R., Clifton, P., Janus, E. & Abbey, M. (1992) Plasma lipoprotein lipid and Lp[a] changes with substitution of elaidic acid for oleic acid in the diet. J. Lipid. Res. 33: 1029–1036.

34. Mensink, R. P., Zock, P. L., Katan, M. B. & Hornstra, G. (1992) Effect of dietary *cis* and *trans* fatty acids on serum lipoprotein[a] levels in humans. J. Lipid. Res. 33: 1493–1501.

35. Almendingen, K., Jordal, O., Kierulf, P., Sandstad, B. & Pedersen, J. I. (1995) Effects of partially hydrogenated fish oil, partially hydrogenated soybean oil, and butter on serum lipoproteins and Lp[a] in men. J. Lipid. Res. 36: 1370–1384.

36. Aro, A., Jauhiainen, M., Partanen, R., Salminen, I. & Mutanen, M. (1997) Stearic acid, *trans* fatty acids, and dairy fat: effects on serum and lipoprotein lipids, apolipoproteins, lipoprotein(a), and lipid transfer proteins in healthy subjects. Am. J. Clin. Nutr. 65: 1419–1426.

37. Clevidence, B. A., Judd, J. T., Schaefer, E. J., Jenner, J. L., Lichtenstein, A. H., Muesing, R. A., Wittes, J. & Sunkin, M. E. (1997) Plasma lipoprotein (a) levels in men and women consuming diets enriched in saturated, *cis*-, or *trans*-monounsaturated fatty acids. Arterioscler. Thromb. Vasc. Biol. 17: 1657–1661.

38. Sundram, K., Ismail, A., Hayes, K. C., Jeyamalar, R. & Pathmanathan, R. (1997) *Trans* (elaidic) fatty acids adversely affect the lipoprotein profile relative to specific saturated fatty acids in humans. J. Nutr. 127: 514S–520S.

39. Lichtenstein, A. H., Ausman, L. M., Jalbert, S. M. & Schaefer, E. J. (1999) Effects of different forms of dietary hydrogenated fats on serum lipoprotein cholesterol levels. N. Engl. J. Med. 340: 1933–1940.

40. Hill, E. G., Johnson, S. B., Lawson, L. D., Mahfouz, M. M. & Holman, R. T. (1982) Perturbation of the metabolism of essential fatty acids by dietary partially hydrogenated vegetable oil. Proc. Natl. Acad. Sci. U.S.A. 79: 953–957.

41. Kinsella, J. E., Bruckner, G., Mai, J. & Shimp, J. (1981) Metabolism of *trans* fatty acids with emphasis on the effects of *trans*, *trans*-octadecadienoate on lipid composition, essential fatty acid, and prostaglandins: an overview. Am. J. Clin. Nutr. 34: 2307–2318.

42. Garland, M., Sacks, F. M., Colditz, G. A., Rimm, E. B., Sampson, L. A., Willett, W. C. & Hunter, D. J. (1998) The relation between dietary intake and adipose tissue composition of selected fatty acids in US women. Am. J. Clin. Nutr. 67: 25–30.