

Intake of *Trans* Fatty Acids and Low-Density Lipoprotein Size in a Costa Rican Population

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Intervention studies show that dietary composition altered low-density lipoprotein (LDL) particle size, but population studies are scarce, and the potential effects of *trans* fatty acids (FA) on LDL size are unknown. *Trans* FA intake has been associated with a more atherogenic lipid profile and increased coronary heart disease (CHD). We examined the association between dietary intake, including *trans* FA and LDL size, in 414 randomly selected subjects living in Puriscal, Costa Rica. Dietary intake was assessed by a validated food frequency questionnaire (FFQ). Women had larger LDL size (Å) compared with men (263 v 261), and large LDL particles were correlated with increased intake (% energy) of protein ($P = .005$), animal fat ($P = .041$), *trans* FA ($P < .0001$), and decreased intake of carbohydrate ($P = .052$) in sex-, age-, and total energy intake-adjusted models. The correlation between *trans* FA intake and large LDL was significant in multivariate models that included dietary and nondietary factors; a 1% difference in *trans* FA was associated with a 2.44 Å increase in LDL size ($P = .004$). In sum, it is possible that the effects of dietary factors, such as intake of *trans* FA on CHD are mediated through their effects on LDL size.

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CARDIOVASCULAR DISEASE is the main cause of death in most Latin American countries¹ and in Hispanics living in the United States.² The highest age-adjusted mortality rates (per 100,000) from cardiovascular disease are found in men and women living in Brazil (534.3 and 374.4, respectively), whereas Costa Rica has the highest cardiovascular disease rates in Central America (229.1 and 175.4, respectively). These numbers are comparable to those found in men and women living in industrialized North American countries, such as Canada (314.8 and 170.4, respectively) and the United States (223.1 and 136.1, respectively).¹

A better understanding of the effect of dietary factors on plasma biomarkers of coronary heart disease (CHD) risk will provide insight into better prevention strategies. Not only low-density lipoprotein (LDL) cholesterol concentrations in plasma, but also the size of these LDL particles may play a role in the development of CHD risk.³ It is known that the size of LDL particles, as well as their concentrations in plasma, increases in response to diets that are high in saturated fat⁴ Interestingly, intake of polyunsaturated fat, plant protein, and crude fiber are also correlated with larger LDL particles.⁵

Trans fatty acid (FA) intake has been associated with an increased risk of CHD.⁶⁻⁸ The effects of *trans* FA intake on plasma lipoproteins may explain, in part, this observed association. Replacement of *cis* FA with *trans* FA in the diet raises LDL cholesterol, triglyceride,^{8,9} and lipoprotein (Lp) (a) plasma concentrations¹⁰ and decreases high-density lipoprotein (HDL) cholesterol.^{8,9} The effect of *trans* FA on LDL size is unknown. This relationship is of interest in view of recent studies showing that large LDL particles are independently associated with increased risk of CHD in case-control¹¹ and prospective¹² studies after the adjustment for established lipid and nonlipid risk factors. These results contrast with those from previous studies showing no association between small LDL and CHD after adjusting for established risk factors.¹³⁻¹⁵ Large LDL particles are large because of the contents of high-cholesterol ester.¹⁶ They preferentially bind to isolated arterial proteoglycan¹⁶ and deliver more cholesterol per particle to cells and connective tissue in the arterial wall.^{17,18}

Although there are very few studies on cardiovascular risk factors in Latin America, it has been suggested that poor dietary habits contribute to CHD in this population.¹⁹⁻²¹ We assessed

dietary intake, including *trans* FA, in a population from Costa Rica and investigated their association with LDL size.

SUBJECTS AND METHODS

Study Population

The study population was randomly selected from the urban and rural areas of Puriscal, Costa Rica in 1988, using maps from the National Census.¹⁹ The Puriscal region extends from the middle to the Pacific coast area of Costa Rica and covers an area of 800 km². There are approximately 26,000 inhabitants in about 150 sparsely distributed localities. Fewer than 500 people reside in each locality. Although not a true urban center, Santiago, the county's capital, is referred to as urban by the Center of Census and Statistics in Costa Rica, and their definition and maps were used in this study. Subjects in the urban and rural areas were selected by stratified random sampling to obtain a similar number of participants in each group. Eligible households were defined as those having 1 man and 1 nonpregnant woman aged 19 to 65 years. A total of 130 such households were randomly selected in the rural and urban areas, respectively. Participation rates were 85% for men and 93% for women. Subjects with triglyceride levels > 400 mg/dL ($n = 10$), glucose levels 126 mg/dL or greater ($n = 9$),²² use of lipid-altering medications ($n = 42$), or missing plasma samples ($n = 1$) were excluded. The final sample size was 202 men and 212 women. All subjects gave informed consent on documents approved at the Institute of Health Research of the University of Costa Rica.

Data Collection

Dietary assessment. Dietary information was obtained with a validated semiquantitative food frequency questionnaire (FFQ) developed specifically for use in the Costa Rican population.²³ The FFQ was

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administered by trained interviewers and inquired about intake of 135 food items, as well as 20 vitamin, mineral, and food supplements, types of fat used for cooking and frying, consumption of fried foods in and away from home, and food habits related to meat preparation during the past year. Subjects selected the type of fat or oil that was used most frequently for cooking, frying, and/or baking at home. Nutrient intakes were estimated by multiplying the frequency of each food by its nutrient content and specific portion size, using the US Department of Agriculture (USDA) food composition tables as described.²⁴ The validity and reproducibility of this questionnaire is similar to that in Western populations.²³ The Pearson partial correlation coefficients for saturated fat, monounsaturated fat, and polyunsaturated between the average measurements from 2 FFQs (performed a year apart) and 7-day 24-hour recalls were 0.58, 0.49, and 0.54, and 0.60, 0.47, and 0.59 between the first and second FFQ.²³ This questionnaire was also validated by its ability to predict adipose tissue FA.²⁵ The correlation coefficient for dietary and adipose tissue 18:2 *trans* FA was high ($r = .64$).

Anthropometric measurements. Three trained fieldworkers acquainted with standardized methods took all anthropometric measurements. All measurements were performed in duplicate, and the average was used for analyses. Waist was measured at the smallest horizontal trunk circumference, and hip was measured at the largest horizontal circumference around the hip and buttocks, with nonstretching fiberglass or metal tapes. Height was measured with a steel anthropometer. Weight was measured on a Detecto bathroom scale or a Seca Alpha Model 770 digital scale (Seca, Hamburg, Germany; both calibrated biweekly). Body mass index (BMI) was expressed as weight (kg) divided by height (m) squared.¹⁹

Fitness score. To determine the level of fitness, a modified Harvard step test was performed on a portable wooden 40 cm step. Subjects were asked to maintain a rhythm (76 beats per minute for women, 96 beats per minute for men) for 3 minutes. Pulse rates were taken immediately after the test and at 1, 2, and 3 minutes thereafter. We expressed fitness score as duration of exercise in seconds divided by the sum of pulse at 1 to 3 minutes after the step test, times 100.

Blood samples and blood pressure. Fasting blood samples were drawn into tubes containing 0.1% EDTA and then stored immediately at 4°C. Within 36 hours, they were centrifuged at 2,500 rpm for 20 minutes at 4°C to isolate the plasma. Blood pressure was always measured in the morning while the subject was in a sitting position. All measurements were taken in duplicate by the same registered nurse using a sphygmomanometer. The average of the 2 measurements was used for this analysis.

Lipoprotein Analysis

Plasma cholesterol, triglyceride, and HDL cholesterol levels were measured in the Lipid Metabolism Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA, with an Abbott Diagnostics (Abbott Park, IL) ABA-200 bichromatic analyzer and Abbott A-Gent enzymatic reagents as previously described.²⁶ Total cholesterol, triglyceride, and HDL cholesterol assays were standardized through the Centers for Disease Control Lipid Standardization Program. LDL cholesterol levels were estimated for all subjects with triglyceride levels less than 400 mg/dL by the Friedewald equation. Gradient gel electrophoresis on 2% to 16% non denaturing polyacrylamide gels (PAA 2-16%, Pharmacia, Piscataway, NJ) was performed with frozen plasma specimens to assess LDL size.²⁷ Scanning was performed on an LKB Ultrascan XL laser densitometer (LKB Instruments, Paramus, NJ) interfaced with an AT&T personal computer and a Canon PJ-108A printer by use of the LKB GSXL software for peak integration.

Statistical Analysis

All variables were examined for outliers, and erroneous values were corrected when possible or deleted otherwise. Differences between urban and rural men and women for all parameters were tested using Tukey's multiple comparisons in a general linear model. Differences in categorical variables were evaluated by χ^2 test. Pearson correlation coefficients (r) after adjusting for sex, age, and total energy intake were calculated to determine the associations between LDL size, plasma lipoproteins, dietary intake, and other environmental factors. The intake of FA was analyzed as a percent of total energy (% energy). Log transformed triglyceride, BMI, and total energy intake were used in the analysis. Multiple linear regression models were used to test for trends across *trans* FA quintiles and to determine the effect of *trans* FA on the LDL size, adjusting for other factors. The test for trend was computed by assigning the median value of the corresponding quintile to each subject and entering that variable as a continuous one in the model. To determine the independent effect of *trans* FA (% energy) on LDL size, we simultaneously adjusted for sex, age, and total energy intake, as well as potential confounders, such as BMI, fasting glucose concentration (mg/dL), blood pressure (mm Hg), triglyceride (mg/dL), and HDL cholesterol (mg/dL). The coefficients of dietary *trans* FA from multivariate regression models indicate the estimated increase in LDL particle size (Å) per 1 U increase of *trans* FA (% energy) after adjustment for significant confounders indicated in each model (see Table 4). Analyses were performed with SAS software (version 8, SAS Institute, Cary, NC).

RESULTS

The population general characteristics are shown in Table 1. Table 2 shows macronutrient and specific FA intake by sex and area. *Trans* FA intake was significantly higher in the urban than in the rural area (0.86 v 0.56, $P < .0001$) and in women compared with men (0.77 v 0.66, $P = .002$). Intake (% energy) of protein and saturated, monounsaturated, and polyunsaturated FA were also higher in the urban than the rural area. Subjects obtained more than 60% of total energy from carbohydrate, 9% to 11% from protein, and 26% to 29% from fat. The P:S ratio and cholesterol intake did not differ between the urban and rural area.

LDL particle size was negatively correlated with triglyceride concentrations, BMI, waist to hip ratio, systolic and diastolic blood pressure, fasting glucose (all $P < .05$), and intake of carbohydrate ($P = .052$). LDL particle size was also positively correlated with HDL cholesterol, protein, animal fat, and *trans* FA intake (all $P < .05$) (Table 3).

We examined dietary factors in multivariate analysis to identify independent dietary predictors of LDL size. *Trans* FA intake emerged as the best dietary predictor of LDL size in these analyses. Table 4 shows regression coefficients of *trans* FA and their 95% confidence intervals after the adjustment for potential confounders. The basic model (model 1) including sex, age, and total energy intake showed that a 1% increase in *trans* FA is associated with a 2.07 Å increase in LDL size ($P < .0001$). This correlation was not materially affected after individually adjusting for other lipids and nondietary risk factors that were associated with *trans* FA intake (models 2 and 3). Adjusting for all nondietary variables together (model 5) attenuated the association between *trans* FA intake and LDL size, although it remained statistically significant. The adjustment for other dietary factors that were also correlated with LDL size

Table 1. General Characteristics for Subjects in Urban and Rural Puriscal, Costa Rica

Variable	Men		Women		P Value	
	Urban (n = 99)	Rural (n = 103)	Urban (n = 108)	Rural (n = 104)	Men v Women	Urban v Rural
Age (yr)	39 ± 11	41 ± 13	40 ± 11	39 ± 12	.523	.310
Education level (yr)	9.3 ± 5.0	5.1 ± 2.9*	8.5 ± 4.3	5.0 ± 3.4*	.329	<.0001
Fitness score	53 ± 10	60 ± 11*	37 ± 14†	48 ± 13†	<.0001	<.0001
BMI (kg/m ²)	25.1 ± 3.7	23.8 ± 3.1	26.4 ± 4.0	25.3 ± 4.4	<.0001	<.0001
Waist-to-hip ratio	0.89 ± 0.06	0.89 ± 0.05	0.80 ± 0.05†	0.81 ± 0.04†	<.0001	.756
Systolic blood pressure (mm Hg)	134 ± 16	131 ± 13	127 ± 13†	125 ± 14†	<.0001	.073
Diastolic blood pressure (mm Hg)	83 ± 11	80 ± 10	77 ± 10†	77 ± 9	<.0001	.017
Fasting glucose (mg/dL)	69 ± 12	65 ± 13	69 ± 9	67 ± 14	.611	.014
Total cholesterol (mg/dL)	182 ± 31	173 ± 34	186 ± 31	180 ± 42	.130	.025
LDL cholesterol (mg/dL)	111 ± 30	105 ± 31	116 ± 30	112 ± 34	.050	.080
HDL cholesterol (mg/dL)	39 ± 9	42 ± 9	45 ± 9†	45 ± 10†	<.0001	.080
Triglyceride (mg/dL)	161 ± 79	133 ± 63*	126 ± 62†	119 ± 57	<.0001	.010
LDL size (Å)	261 ± 5	261 ± 5	264 ± 4†	263 ± 5†	<.0001	.220
Hypertension (%)	30	20	14†	13	.004	.260
Current smoker (%)	48	40	9†	10†	<.0001	.721
Oral contraceptive user (%)	-	-	21	15	-	.267
Menopausal status (%)						
Perimenopausal	-	-	10	8	-	.780
Postmenopausal	-	-	21	20		

NOTE. Values are mean ± SD for continuous variables and percentage for categorical variables. *P* values were obtained by the general linear model followed by Tukey's multiple comparisons. *P* values for categorical variables were obtained by χ^2 test. Fitness score was defined as duration of exercise in seconds divided by the sum of the pulse rates 1, 2, 3 minutes after cessation of exercise, times 100. Hypertension was defined as a systolic blood pressure > 140 mm Hg and/or diastolic blood pressure > 90 mm Hg. Current smokers were those who smoked one or more cigarettes per day.

*Significantly different ($P < .05$) between urban and rural within the same sex.

†Significantly different ($P < .05$) between men and women within the same area.

Abbreviation: BMI, body mass index.

attenuated the magnitude of the effect to 1.93 Å (model 4). However, protein, carbohydrate, and animal fat did not show an independent association with LDL size when *trans* FA intake was in the model. After adjusting for all nondietary and dietary factors together (model 6), a 1% difference in *trans* FA was associated with a 2.44 Å increase in LDL size.

Figure 1 shows the association between *trans* FA intake and LDL particle size. The LDL size in the highest quintile of *trans* FA (% energy) was statistically different from the lowest quintile after adjustment for significant covariates ($P = .008$). The *P* for trend across quintiles of *trans* FA intake was ($P < .0001$).

Table 2. Intakes of Specific Types of Dietary fat (% energy) and Dietary Cholesterol in Urban and Rural Puriscal, Costa Rica

Variable	Men		Women		P Value	
	Urban (n = 99)	Rural (n = 103)	Urban (n = 108)	Rural (n = 104)	Men v Women	Urban v Rural
% energy						
Protein	10.3 ± 2.5	9.0 ± 2.7*	10.9 ± 3.0	8.9 ± 3.0*	.384	<.0001
Carbohydrate	60 ± 9	65 ± 10*	61 ± 9	66 ± 11*	.318	<.0001
Total fat	29 ± 7	26 ± 7*	29 ± 7	27 ± 8*	.577	<.0001
Animal fat	13 ± 5	10 ± 5*	15 ± 6	10 ± 5*	.096	<.0001
Vegetable fat	16 ± 5	16 ± 6	15 ± 5	17 ± 5*	.345	.017
Saturated fatty acid	12 ± 3.0	11 ± 3.0	12 ± 3.1	11 ± 3.3	.206	.003
Monounsaturated fatty acid	10.1 ± 2	9.2 ± 3	10.5 ± 3	9.5 ± 3*	.136	.001
Polyunsaturated fatty acid	3.3 ± 0.8	3.1 ± 0.9	3.4 ± 1.0	3.2 ± 0.9	.304	.003
<i>Trans</i> fatty acid	0.79 ± 0.30	0.53 ± 0.28*	0.93 ± 0.39†	0.60 ± 0.40*	.002	<.0001
P:S ³	0.30 ± 0.06	0.30 ± 0.09	0.30 ± 0.09	0.30 ± 0.07	.666	.970
Cholesterol (mg/1,000kcal)	128 ± 57	130 ± 88	126 ± 65	106 ± 67	.061	.200

NOTE. Values are mean ± SD. *P* values were obtained by general linear model followed by Tukey's multiple comparisons of the least-square means. P:S means the ratio of polyunsaturated fatty acids to saturated fatty acids.

*Significantly different ($P < .05$) between urban and rural within the same sex.

†Significantly different ($P < .05$) between men and women within the same area.

Table 3. Sex, Age, and Total Energy Adjusted Pearson Correlation Coefficients Between LDL Particle Size (Å) and Cardiovascular Disease Risk Factors in Puriscal, Costa Rica

Dependent Variable	LDL Particle Size (Å)	
	r	P
Fitness score	-.033	.532
Body mass index (kg/m ²)	-.257	<.0001
Waist to hip ratio	-.227	<.0001
Systolic blood pressure (mm Hg)	-.136	.006
Diastolic blood pressure (mm Hg)	-.179	.0003
Fasting glucose (mg/dL)	-.104	.034
LDL cholesterol (mg/dL)	.012	.807
HDL cholesterol (mg/dL)	.509	<.0001
Triglyceride (mg/dL)	-.628	<.0001
Dietary factors (% energy)		
Protein	.138	.005
Carbohydrate	-.096	.052
Animal fat	.101	.041
Vegetable fat	.010	.846
Saturated fatty acid	.073	.142
Monounsaturated fatty acid	.085	.086
Polyunsaturated fatty acid	.054	.277
Trans fatty acid	.164	<.0001
Cholesterol (mg/1,000 kcal)	.022	.662

NOTE. Fitness score was defined as duration of exercise in seconds divided by the sum of the pulse rates 1, 2, 3 minutes after cessation of exercise, times 100.

DISCUSSION

We evaluated dietary intake including *trans* FA intake and LDL size in the rural and urban areas of Puriscal, Costa Rica. Mean *trans* FA intake was less than 1% in this population, but higher in the urban than in the rural area (0.86% v 0.56%, $P <$

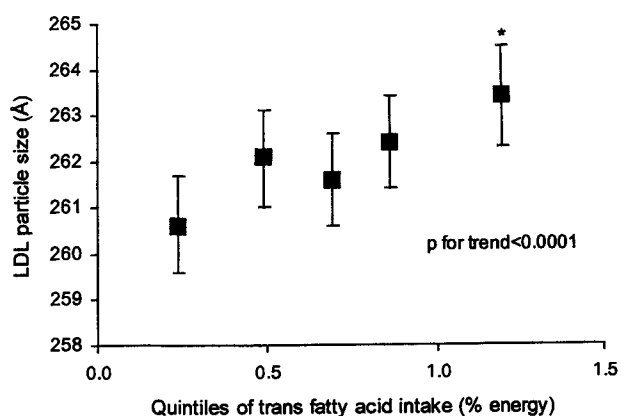


Fig 1. Mean and 95% confidence intervals of LDL particle size (Å) by quintile of *trans* fatty acid intake (% energy) in Puriscal, Costa Rica. Means were adjusted for sex, age, total energy, fitness score, and body mass index. * $P = .008$ compared with the lowest quintile.

.0001) and in women than in men (0.77% v 0.66%, $P = .002$). Despite the low overall intake, increased *trans* FA intake was significantly correlated with larger LDL particles. The association between *trans* FA intake and LDL size remained statistically significant after the adjustment for potential confounders including dietary, lifestyle, and lipid risk factors.

A positive association between *trans* FA and LDL size is of interest. *Trans* FAs are associated with increased risk of CHD.⁶⁻⁸ It is estimated that a 2% increase in *trans* FA intake is associated with 14% to 96% increase in CHD risk. Overall, the effects of *trans* FA on CHD were larger than those expected based on its effects on LDL and HDL cholesterol alone and suggested that other mechanisms may be involved.^{8,28} The

Table 4. Independent Effect of *Trans* Fatty Acid Intake (% energy) on LDL Size in Puriscal, Costa Rica

Adjusted for	β -Coefficient <i>Trans</i> Fatty Acid	95% CI	P
Model 1: Sex, age, and energy intake only	2.07	(0.86, 3.28)	<.0001
Model 2: Variables in model 1 plus			
Body mass index	2.45	(1.31, 3.65)	<.0001
Fitness score	2.44	(1.06, 3.82)	<.0001
Diastolic blood pressure	2.28	(1.09, 3.47)	<.0001
BMI, fitness score, and diastolic blood pressure	2.61	(1.31, 3.91)	<.0001
Model 3: Variables in model 1 plus			
Fasting glucose	2.20	(0.99, 3.40)	<.0001
LDLc	1.99	(0.78, 3.21)	<.0001
HDLc	1.88	(0.84, 2.93)	<.0001
TG	1.51	(0.57, 2.46)	.002
LDLc, HDLc, TG, and fasting glucose	1.34	(0.45, 2.24)	.003
Model 4: Variables in model 1 plus			
Protein (% energy)	1.60	(0.94, 3.10)	.037
Carbohydrate (% energy)	2.00	(0.56, 3.44)	.007
Animal fat (% energy)	2.09	(0.54, 3.64)	.008
Protein, carbohydrate, and animal fat (% energy)	1.93	(0.37, 3.49)	.016
Model 5: All variables in model 1, 2, 3	1.36	(0.34, 2.37)	.009
Model 6: All variables in model 1, 2, 4	2.44	(0.79, 4.08)	.004

NOTE. Values are the regression coefficients and 95% confidence intervals. Fitness score was defined as duration of exercise in seconds divided by the sum of the pulse rates 1, 2, 3 minutes after cessation of exercise, times 100.

Abbreviations: LDLc, low-density lipoprotein cholesterol; HDLc, high-density lipoprotein cholesterol; TG, triglyceride.

positive association between *trans* FA intake and large LDL size in our study suggests an additional mechanism for the potential atherogenicity of *trans* FA. Large LDL particles are associated with increased risk of CHD in case-control¹¹ and prospective¹² studies, after adjusting for established lipid and nonlipid risk factors. Among American Indian communities, large LDL was associated with higher coronary disease mortality.²⁹ Large LDL particles are thought to be large because of high-cholesteryl ester content, and they preferentially bind to isolated arterial proteoglycan¹⁶ and deliver more cholesterol per particle to cells and connective tissue in the arterial wall.^{17,18} In nonhuman primates, a diet high in saturated fat and cholesterol increases LDL size, and the magnitude of this increase is strongly associated with severity of atherosclerosis.¹⁷ It is possible that the effects of *trans* FA on LDL size are similar to those found for saturated fat in nonhuman primates. These studies contrast numerous previous studies on the predominance of small LDL size and CHD.^{13-15,30,31} Small LDL is a characteristic trait of the metabolic syndrome³² and does have potentially deleterious properties, such as reduced affinity for the LDL receptor,³³ longer residence time in plasma,³⁴ increased susceptibility to oxidation,³⁵ and deleterious effects on the function of vascular cells.³ It is surprising, however, that small LDL size has not emerged as an independent cardiovascular risk factor.^{13-15,30,31} It is possible that the atherogenic characteristics, *in vivo*, may not be worse than the harmful properties of large LDL.

The effect of *trans* FA on LDL size could be mediated by cholesteryl ester transfer protein (CETP). In rabbits, a diet high in *trans* FA increases LDL cholesterol compared with *cis* FA, whereas the opposite effects are observed in rats. These differential effects could be attributed to the presence of CETP in rabbit, but not in rat plasma.³⁶ In humans, a diet rich in *trans* FA increases CETP activity.³⁷ It has been suggested that CETP

plays an important role in converting small LDL particles to large and homogeneous LDL particles by transferring cholesteryl-esters from HDL.³⁸

Trans FA intake in this population (0.71% energy) is relatively low compared with the US population (2% to 3% energy),^{6,7,39} but similar to other Mediterranean countries, such as Spain (0.76% energy).⁴⁰ As a percent of total fat, *trans* FA intake (2.5%) is also lower than in the US (7.4%).³⁹ These low intake levels are probably due to a low consumption of margarine and fast foods prepared with partially hydrogenated oils. Most of the studied population used palm oil as the main fat used for cooking and prepared the foods at home. More recent study in the metropolitan area of Costa Rica shows that *trans* FA intake from partially hydrogenated soybean oil has increased (1% to 2% energy, 3.3% of total fat).²⁵

We did not find a significant correlation between *trans* FA intake and plasma LDL and HDL cholesterol levels. This is not unexpected given the large range of intake (3% to 10%) used in many intervention studies.⁸ No association between these plasma lipids and *trans* FA was found in a study that compared small differences in *trans* FA intake (0.55% v 0.91% energy).⁴¹ Cross-cultural comparisons in Europe did not find an association between LDL cholesterol and *trans* FA intake.⁴⁰ The investigators attributed this negative result to the low intake (<1 % energy) in the European countries studied.⁴⁰

In conclusion, we found a significant correlation between increased *trans* FA intake and larger LDL particles. Our data suggest that the effects of dietary factors, such as intake of *trans* FA on CHD are mediated through their effects on LDL size.

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