

Effect of different forms of dietary hydrogenated fats on LDL particle size¹⁻³

Jean-François Mauger, Alice H Lichtenstein, Lynne M Ausman, Susan M Jalbert, Matti Jauhiainen, Christian Ehnholm, and Benoît Lamarche

ABSTRACT

Background: Dietary *trans* fatty acids (FAs), which are formed during the process of hydrogenating vegetable oil, are known to increase plasma LDL-cholesterol concentrations. However, their effect on LDL particle size has yet to be investigated.

Objective: We investigated the effect of *trans* FA consumption on the electrophoretic characteristics of LDL particles.

Design: Eighteen women and 18 men each consumed 5 experimental diets in random order for 35-d periods. Fat represented 30% of total energy intake in each diet, with two-thirds of the fat in the form of semiliquid margarine (0.6 g *trans* FAs/100 g fat), soft margarine (9.4 g *trans* FAs/100 g fat), shortening (13.6 g *trans* FAs/100 g fat), stick margarine (26.1 g *trans* FAs/100 g fat), or butter, which was low in *trans* FAs (2.6 g *trans* FAs/100 g fat) but rich in saturated fat. LDL particle size and distribution were characterized by nondenaturing, 2–16% polyacrylamide gradient gel electrophoresis.

Results: Relative to the LDL particle size observed after consumption of the butter-enriched diet, LDL particle size decreased significantly and in a dose-dependent fashion with increasing amounts of dietary *trans* FAs ($P < 0.001$). Cholesterol concentrations in large ($> 260 \text{ \AA}$) and medium-sized (255–260 \AA) LDL particles also increased proportionately to the amount of *trans* FAs in the diet.

Conclusion: Consumption of dietary *trans* FAs is associated with a deleterious increase in small, dense LDL, which further reinforces the importance of promoting diets low in *trans* FAs to favorably affect the lipoprotein profile. *Am J Clin Nutr* 2003;78:370–5.

KEY WORDS *trans* Fatty acids, hydrogenated fat, LDL particle size, diet, cholesterol

INTRODUCTION

trans Fatty acids are unsaturated fatty acids containing ≥ 1 double bond in the *trans* configuration. Although the conformation of the acyl chain in *trans* fatty acids may be similar to that of saturated fatty acids, the metabolic effects of the 2 classes of fatty acids may be quite different from one another (1). Most dietary *trans* fatty acids are formed during the hydrogenation of vegetable oils (2, 3), a process that converts oily bodies into semiliquid or solid fats for subsequent use in food products.

Studies have consistently shown that the consumption of dietary *trans* fatty acids increases plasma LDL-cholesterol concentrations

(4), and elevated plasma LDL cholesterol is one of the most important risk factors for the development of cardiovascular disease (5–7). This effect was shown to be proportional to dietary *trans* fatty acid intake (8) and is not related to changes in endogenous cholesterol synthesis (9, 10). Moreover, results from a recent population-based case-control study indicated that a high content of *trans* fatty acids in human erythrocyte membranes may be involved in arrhythmias and sudden cardiac death (11).

LDL particles are heterogeneous in size, density, and composition (12). The cholesterol concentration associated with LDL, which is routinely estimated by clinical laboratories, represents only one feature of these atherogenic particles. Accumulating evidence indicates that the size of LDL particles confers an independent risk, with small and dense particles being more atherogenic than are larger, less dense particles (13–15). Recent evidence from the Quebec Cardiovascular Study indicated that increased concentrations of cholesterol in the small LDL subfraction (LDL-C_{<255 Å}) are associated with a marked increase in the risk of cardiovascular disease, even in the presence of relatively normal LDL-cholesterol concentrations (16).

Environmental factors such as the fatty acid content of the diet may modulate the diameter and density of LDL particles to a significant extent. Diets enriched with saturated fatty acids are associated with large and cholesterol-rich LDL particles (17) whereas diets that are low in fat are associated with reduced LDL particle size (17–19). Although the effect of dietary *trans* fatty acids on plasma LDL-cholesterol concentrations is well documented, there is currently no documentation on how dietary *trans* fatty acids modulate LDL particle size and cholesterol distribution among different LDL subclasses. The objective of the present

¹ From the Institute on Nutraceuticals and Functional Foods, Laval University, Quebec (J-FM and BL); the Friedman School of Nutrition Science and Policy (LMA) and the Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging (AHL and SMJ), Tufts University, Boston; and the Department of Molecular Medicine, National Public Health Institute, Helsinki (MJ and CE).

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³ Address reprint requests to B Lamarche, Institute on Nutraceuticals and Functional Foods, Pavillon Paul Comtois, local 2423, Université Laval, Québec, Canada G1K 7P4. E-mail: benoit.lamarche@inaf.ulaval.ca.

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TABLE 1
Baseline characteristics of study participants¹

Characteristic	Women (n = 18)	Men (n = 18)	All subjects (n = 36)
Age (y)	67 ± 4	60 ± 5 ²	63 ± 6
BMI (kg/m ²)	26.6 ± 2.4	28.1 ± 3.4	27.4 ± 3.0
Serum cholesterol (mmol/L)			
Total	6.54 ± 0.83	6.13 ± 0.85	6.34 ± 0.85
VLDL	0.80 ± 0.34	0.72 ± 0.28	0.75 ± 0.31
LDL	4.32 ± 0.78	4.32 ± 0.67	4.32 ± 0.72
HDL	1.37 ± 0.28	1.09 ± 0.23 ²	1.24 ± 0.28
Plasma triacylglycerols (mmol/L)	1.78 ± 0.80	1.56 ± 0.62	1.67 ± 0.72

¹ $\bar{x} \pm SD$.²Significantly different from women, $P < 0.05$ (SAS PROC *t* test).

study was to investigate the effect, relative to that of butter, of commercially available hydrogenated vegetable fats having a wide range of *trans* fatty acid concentrations on LDL particle size and distribution in moderately hypercholesterolemic men and women.

SUBJECTS AND METHODS

Subjects

The study design was described in detail previously (8, 20). For the purpose of the present study, LDL particle size and distribution were measured in 18 men aged 57–73 y and 18 women aged 52–73 y with mild hypercholesterolemia (LDL-cholesterol concentration > 3.36 mmol/L). Participants were recruited from the greater Boston area for this study. The selection criteria included the following: normal kidney, liver, thyroid, and cardiac function; normal fasting glucose concentration; no intake of medications known to affect blood lipid concentrations; and nonsmoker. All females were postmenopausal and were not taking replacement hormones. Subjects receiving β -blocker therapy to treat hypertension were excluded. The characteristics of the subjects at the time of screening are shown in **Table 1**. The Human Investigation Review Committee of New England Medical Center and Tufts University approved the study protocol, and all subjects gave written informed consent.

Experimental design

Each subject was supplied with 5 experimental diets, and the primary fat in each diet came from soybean oil-derived spreads and shortening (hydrogenated fat) or butter (8). Each diet period lasted 35 d, and food was provided to the subjects in a double-blinded way according to a Latin-square design. The subjects were

required to eat all the foods that were provided to them and not to supplement their diet with any other food or drink except water and noncaloric beverages. Caloric intake at baseline was estimated by using the Harris-Benedict formula and was adjusted during the experiment to keep body weight constant. The mean (\pm SD) energy intakes for men and women were 2792 \pm 518 and 2114 \pm 320 kcal, respectively. After day 28 of each dietary phase, 3 fasting blood samples were obtained from each subject for lipid and apolipoprotein determination and for LDL particle size characterization.

Experimental diets

All diets were designed to provide 15% of energy from protein, 55% from carbohydrate, and 30% from fat and included the same foods. The only difference between the diets was the form in which two-thirds of the fat was provided (ie, as soybean oil-based margarines hydrogenated to various extents or as butter) (**Table 2**). These criteria were achieved by first designing a diet containing 10% of energy from fat and then adding the butter or hydrogenated soybean oil to various foods, such as hot cereal, casseroles, and muffins, to increase the fat content of the diet to 30%. All the margarines and the shortening were made from soybean oil except for the semiliquid margarine, which contained some cottonseed oil. This deviation was necessary because no semiliquid margarine made solely with soybean oil was commercially available at the time of the study.

The fat, protein, carbohydrate, and cholesterol content of diet homogenates made from each complete meal cycle was analyzed by Covance Laboratories (Madison, WI), and the fatty acid content of each experimental diet (**Table 2**) was analyzed by Lipton (Baltimore) with the use of capillary gas chromatography.

Biochemical analysis

Fasting blood samples were collected in tubes containing 0.15% EDTA. Serum was separated by centrifugation at 1100 \times g and 4 °C for 10 min. VLDL was isolated from serum by ultracentrifugation at 109 000 \times g and 4 °C for 18 h. Serum and the infranant fraction (density of 1.006 g/mL) were assayed for total cholesterol and triacylglycerol with the use of a bichromatic analyzer (model CCX, Spectrum; Incstar, Stillwater, MN) with enzymatic reagents (21). Serum HDL-cholesterol concentrations were measured in the supernatant fraction after apolipoprotein B-containing lipoprotein was precipitated with the use of dextran-magnesium sulfate. Lipid assays were standardized through the Lipid Standardization Program of the Centers for Disease Control and Prevention (Atlanta).

LDL particle size characterization

Nondenaturing polyacrylamide gradient gel electrophoresis of whole plasma was used to measure LDL particle diameter (16). LDL particle size and distribution were assessed in a blinded

TABLE 2
Fatty acid composition of the fats used in the diets¹

Fatty acid subclass	Butter	Semiliquid margarine	Soft margarine	Shortening	Stick margarine
	<i>g/100 g fat</i>				
Saturated	68.4	16.0	17.6	17.4	16.9
Monounsaturated	24.3	23.4	26.0	37.0	30.8
Polyunsaturated	3.7	59.1	45.0	31.0	24.8
<i>trans</i>	2.6	0.6	9.4	13.6	26.1

¹Based on chemical analysis of the fats and oils.

TABLE 3

Plasma lipid concentrations and electrophoretic characteristics of LDL particles in the 36 study participants after consumption of 5 diets enriched with butter or soybean oil-based margarine¹

Variable	Butter	Semiliquid margarine	Soft margarine	Shortening	Stick margarine	P (ANOVA)
Lipid concentrations						
Triacylglycerols (mmol/L) ²	1.6 ± 0.64 ^{ab}	1.5 ± 0.6 ^b	1.7 ± 0.9 ^{ab}	1.7 ± 0.6 ^{ab}	1.8 ± 0.8 ^a	0.046
Cholesterol (mmol/L)						
Total	6.5 ± 0.9 ^a	5.8 ± 0.8 ^c	6.0 ± 0.7 ^c	6.1 ± 0.8 ^{bc}	6.3 ± 1.0 ^{ab}	<0.0001
LDL	4.6 ± 0.8 ^a	4.0 ± 0.7 ^d	4.1 ± 0.7 ^{cd}	4.2 ± 0.7 ^{bc}	4.3 ± 0.8 ^b	<0.0001
HDL	1.16 ± 0.27 ^a	1.11 ± 0.26 ^{ab}	1.10 ± 0.23 ^{ab}	1.11 ± 0.24 ^{ab}	1.08 ± 0.23 ^b	0.004
Electrophoretic characteristics						
LDL peak particle size (Å)	254.8 ± 4.1 ^a	254.4 ± 4.4 ^{ab}	253.8 ± 4.5 ^{ab,c}	253.2 ± 4.8 ^{bc}	252.6 ± 4.6 ^c	<0.001
LDL integrated size (Å)	256.6 ± 3.1 ^a	255.8 ± 3.0 ^{ab}	256.2 ± 3.5 ^b	255.4 ± 3.3 ^{ab}	255.4 ± 3.2 ^b	0.02
LDL% _{>260 Å} (%)	30.8 ± 9.6	29.9 ± 10.1	31.1 ± 10.7	31.5 ± 11.5	30.1 ± 11.2	0.80
LDL% _{255–260 Å} (%)	20.6 ± 5.1	20.1 ± 5.8	19.5 ± 4.7	21.1 ± 6.0	20.5 ± 7.4	0.59
LDL% _{<255 Å} (%)	48.6 ± 13.3	50.0 ± 14.1	49.4 ± 13.8	47.4 ± 15.7	49.4 ± 16.3	0.80
LDL-C _{>260 Å} (mmol/L)	1.4 ± 0.5 ^a	1.2 ± 0.4 ^b	1.3 ± 0.5 ^{ab}	1.3 ± 0.4 ^{ab}	1.3 ± 0.5 ^{ab}	0.04
LDL-C _{255–260 Å} (mmol/L)	0.9 ± 0.3 ^a	0.8 ± 0.2 ^b	0.8 ± 0.2 ^b	0.9 ± 0.3 ^{ab}	0.9 ± 0.3 ^{ab}	<0.01
LDL-C _{<255 Å} (mmol/L)	2.2 ± 0.8	2.0 ± 0.7	2.0 ± 0.8	2.1 ± 0.8	2.2 ± 1.0	0.16

¹ $\bar{x} \pm SD$. LDL%, relative proportion of LDL having a given diameter; LDL-C, absolute concentration of cholesterol within a given LDL subfraction. Values in the same row with different superscript letters are significantly different, $P < 0.05$ [ANOVA (general linear model) and Tukey's honestly significant difference test].

²Log₁₀ normalized for analysis.

fashion with all 5 diet phases from each subject obtained on one unique gel. The 5 samples from a single subject were also randomly distributed among the gels' lanes. Gels were prepared in batches of 8 in our laboratory. Plasma sample aliquots (3.5 μ L) were mixed 1:1 with a sampling buffer containing 20% (wt:vol) sucrose and 0.25% (wt:vol) bromophenol blue. A 15-min prerun at 75 V preceded electrophoresis of plasma samples at 150 V for 3 h. Gels were stained with Sudan black (Sigma, St Louis) according to standardized procedures and stored in an acetic acid (9%, vol:vol)–methanol (20%, vol:vol) solution until analysis. Gels were analyzed by using IMAGEMASTER 1-D PRIME computer software (version 3.01; Amersham Pharmacia Biotech, Baie d'Urfé, Canada). LDL particle size was computed on the basis of the relative migration of plasma standards of known diameter. A mean LDL particle size was obtained by integrating the relative contribution of each LDL subclass for a given subject. This integrated LDL particle size corresponded to the weighted mean of all LDL subclasses on the gel. It was calculated as the sum of the diameter of each LDL subspecies multiplied by its relative proportion. Analysis of pooled plasma standards showed that measurement of LDL peak and mean particle size was highly reproducible, with an interassay CV < 2%. The relative proportion of LDL having a diameter < 255 Å (LDL%_{<255 Å}) was ascertained by computing the relative area of the densitometric scan < 255 Å. The absolute concentration of cholesterol within the LDL subfraction characterized by a diameter < 255 Å (ie, LDL-C_{<255 Å}) was calculated by multiplying total plasma LDL-cholesterol concentrations by LDL%_{<255 Å} as previously described (16). A similar approach was used to assess the relative and absolute concentrations of cholesterol in the LDL subfractions with a diameter > 260 Å (LDL%_{>260 Å} and LDL-C_{>260 Å}, respectively) and in those with a diameter between 255 and 260 Å (LDL%_{255–260 Å} and LDL-C_{255–260 Å}, respectively).

Enzyme assays

Cholesterol ester transfer protein (CETP) activity was assayed in plasma after removal of endogenous VLDL and LDL by

phosphotungstate and magnesium chloride precipitation as described previously (22). Transfer of radioactively labeled phosphatidylcholine in phosphatidylcholine-liposomes to HDL₃ was used to quantify phospholipid transfer protein (PLTP) activity in plasma according to the method of Damen et al (23) with minor modifications (24, 25).

Statistical analysis

Data were analyzed by using repeated-measures analysis of variance (general linear model), with the main effect of diet and subject as the repeated measure, followed by Tukey's honestly significant difference test for pairwise comparisons between the 5 experimental diets. Appropriate interaction terms were introduced into the various models to test for distinct effects of the diets between the men and the women. Correlations were investigated by using Pearson and Spearman coefficients for parametric and nonparametric variables, respectively. Statistical analyses were performed by using SAS (version 8.2; SAS Institute Inc, Cary, NC).

RESULTS

The plasma lipid profiles observed in response to the 5 diet periods are presented in **Table 3**. Serum total and LDL-cholesterol concentrations increased progressively with the degree of enrichment of the diet with *trans* fatty acids (from semiliquid to stick margarine). However, total and LDL cholesterol reached the highest concentrations after the subjects consumed the butter-enriched diet. Increased consumption of *trans* fatty acids was also associated with a more subtle but significant increase in serum triacylglycerol concentration.

Changes in LDL particle size in response to the degree of hydrogenation of the predominant fat in the diets are also presented in Table 3. Increasing the amount of *trans* fatty acids in the diet produced significant reductions in LDL peak particle size; the smallest LDL particles were observed after consumption of the stick margarine-enriched diet, whereas the largest LDL peak particle size was observed after consumption of the butter-enriched diet. The changes in LDL integrated diameter, which accounts for all

individual LDL particle subclasses within a subject, were more subtle than the changes in LDL peak particle size. Nevertheless, LDL integrated size after consumption of the stick margarine-enriched diet was significantly lower than that after consumption of the butter-enriched diet. There was no significant difference in response between the women and the men (data not shown).

The relative and absolute distributions of cholesterol among small (<255 Å), medium (255–260 Å), and large (>260 Å) LDL after consumption of the diets are also presented in Table 3. In relative terms, the distribution of the different LDL particle subfractions was not significantly altered by the amounts of saturated or *trans* fatty acids in the diet. LDL-C_{>260 Å} and LDL-C_{255–260 Å} concentrations gradually but significantly increased with increasing amounts of *trans* fatty acids in the diet, but the concurrent increase in LDL-C_{<255 Å} concentrations was not significant. Among the diets enriched with soybean oil-based margarines, the lowest LDL-C_{>260 Å} and LDL-C_{<255 Å} concentrations were observed after consumption of the semiliquid margarine-enriched diet, whereas the highest concentrations were achieved after consumption of the stick margarine-enriched diet. Interestingly, consumption of each of the 4 diets enriched with soybean oil-based margarine resulted in lower cholesterol concentrations in medium and large LDL particles than did consumption of the butter-enriched diet.

Plasma triacylglycerol concentrations are known to correlate strongly with variations in LDL particle size (26). Analyses were conducted to see whether the consumption of *trans* fatty acids modulated this relation. The correlations between serum triacylglycerol concentration and LDL peak particle size measured after consumption of each diet were tested by an interaction term in the general linear model analysis. Consumption of various amounts of dietary *trans* fatty acids had no significant effect on the relation between plasma triacylglycerol concentration and LDL peak particle size or LDL-C_{<255 Å} concentration (data not shown).

Analyses were also conducted to examine whether LDL particle size reduction induced by consumption of *trans* fatty acids could be related to changes in CETP or PLTP activity. Dietary *trans* fatty acids had no dose-dependent effect on CETP and PLTP activities. No significant relation was observed between diet-induced variations in CETP and PLTP activities and variation in LDL particle size or variation in the cholesterol content of the different LDL subfractions. No significant differences in CETP and PLTP activities were observed between the men and the women (data not shown).

DISCUSSION

In the present study on the relation between consumption of dietary *trans* fatty acids and both LDL peak particle size and cholesterol distribution among LDL subfractions, 3 main findings were obtained. First, increased consumption of dietary *trans* fatty acids was associated with a dose-dependent reduction in the diameter of LDL particles. Second, the well-characterized increase in LDL-cholesterol concentration associated with *trans* fatty acid consumption can be ascribed to elevated cholesterol concentrations in all LDL subclasses. Third, consumption of *trans* fatty acids did not modulate the association between plasma triacylglycerol concentration and LDL particle size.

trans Fatty acids and LDL particle size

The findings of this study provide further support to the thesis that increased consumption of *trans* fatty acids may accelerate the atherosclerotic process by adversely affecting plasma lipid concentrations (8, 27). Indeed, in addition to promoting an increase in plasma LDL-cholesterol concentration, *trans* fatty acid consump-

tion was associated with a significant reduction in LDL particle size and with a trend toward an increased cholesterol concentration in small LDL (LDL-C_{<255 Å}). It is not possible at this point to determine whether the increase in cholesterol in each LDL subfraction is attributable to an increase in the number of particles in the LDL size range, an increase in the cholesterol content of each individual LDL particle, or both.

Interestingly, compared with the 4 diets enriched in *trans* fatty acids, the diet enriched with saturated fat (butter) was associated with the highest plasma LDL-cholesterol concentrations but, paradoxically, the largest LDL particles. The increase in LDL particle size observed in response to diets high in saturated fat has been documented previously (17). It must be stressed that the effects of a high-fat diet on LDL particle size have generally been compared with those of low fat–high carbohydrate diets, which have been shown previously to reduce LDL particle size, at least under isocaloric conditions (28, 29). In the present study, the proportion of fat in each of the 5 diets was comparable. One study suggested that dietary polyunsaturated fatty acids may reduce LDL particle size independently of saturated fatty acids (30). In the present study, increasing amounts of dietary *trans* fatty acids due to high degrees of hydrogenation of soybean oil in the experimental margarines were matched by diametric reductions in polyunsaturated fatty acids. It is therefore unlikely that the changes in LDL particle size were attributable to differences between the diets in polyunsaturated fatty acid content, thereby reinforcing the notion that *trans* fatty acid consumption per se was largely responsible for the diet-induced deleterious effect on LDL particle size.

It may be argued that the changes in LDL particle size observed in response to increasing amounts of *trans* fatty acids may be too subtle to be of clinical importance. There are arguments, however, against that view. First, it has been calculated that each 1-Å decrease in particle diameter corresponded to a loss of several molecules at the surface of the LDL macromolecular complex (31). These adaptations have been shown to modify the tertiary conformation of apolipoprotein B, which is required to maintain the homeostatic surface pressure of the particle, thereby affecting the chemical and physiologic properties of LDL (31). Second, on the basis of data from the Quebec Cardiovascular Study, we recently reported that a small shift in the LDL particle diameter toward smaller particles (< rather than >256 Å) translated into a highly significant 2.2-fold increase in the 5-y rate of ischemic heart disease (IHD) in men (13). In the present study, increasing amounts of dietary *trans* fatty acids were associated with a mean change in LDL peak particle size of 2.1 Å [from 254.8 ± 4.1 (\bar{x} ± SD) to 252.7 ± 4.6 Å]. If sustained over time, this unfavorable reduction in LDL peak particle size would relate to an increase of ≈11% in the 5-y risk of IHD (on the basis of data from the Quebec Cardiovascular Study). These arguments support the notion that even a subtle reduction in LDL peak particle size, such as the one observed in response to dietary *trans* fatty acids, may have important clinical implications.

trans Fatty acids and LDL-cholesterol distribution

The data from the present study also indicate that the increase in plasma LDL-cholesterol concentration associated with an increased dietary *trans* fatty acid content cannot be ascribed to a specific LDL subspecies (small or large) but is instead observed throughout the distribution of LDL particle size.

Recent data from the Quebec Cardiovascular Study suggested that among a series of LDL characteristics, the cholesterol content of small LDL particles (LDL-C_{<255 Å}) was the strongest predictor of IHD risk in men. Data also suggested that each 0.5-mmol/L increase in the cholesterol content of small LDL particles translates into a 5% increase in the 5-y risk of IHD (16). Although not significant from a statistical point of view, the increase in LDL-C_{<255 Å} concentration with increasing amounts of dietary *trans* fatty acids in the present study, combined with the significant reduction in LDL peak particle size and with the elevation in LDL-C_{>260 Å} concentration, is likely to be an important modulator of the effect of hydrogenated fats on the risk of IHD.

As discussed previously, the significant reduction in LDL peak particle size induced by consumption of dietary *trans* fatty acids was associated with a concurrent but more subtle elevation in serum triacylglycerol concentration. Our analysis showed that within each diet, the subjects with the highest plasma triacylglycerol concentrations were also characterized by having the smallest LDL peak particle size. These data suggest that increasing intakes of *trans* fatty acids do not alter or modify the metabolic association between plasma triacylglycerol concentration and LDL particle size.

Mechanisms

The effect of dietary *trans* fatty acids on CETP and PLTP activities after consumption of the stick margarine-enriched diet and the semiliquid margarine-enriched diet was reported previously for participants of the present study (20). With different amounts of dietary *trans* fatty acids, the diet-induced variation in plasma CETP and PLTP activities was not related to concurrent variations in LDL particle size or cholesterol distribution among the various LDL subclasses. Intravascular hepatic lipase activity has been shown to be an important determinant of LDL particle size (32). To the best of our knowledge, there is no evidence yet available to suggest that the deleterious effect of dietary *trans* fatty acids on LDL particle size could be mediated by changes in hepatic lipase activity. Future studies are needed to clarify this issue.

In conclusion, data from the present study support the concept that consumption of *trans* fatty acids has a deleterious effect on LDL particle size, thus further contributing to the overall effect of *trans* fatty acids on cardiovascular disease. These data reinforce the importance of promoting diets that are low in saturated fat and that contain a minimal quantity of *trans* fatty acids from hydrogenated fat in order to favorably affect the lipoprotein profile and thus contribute to a reduced risk of cardiovascular disease. 

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