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trans-Fatty acids in the diet stimulate atherosclerosis

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Abstract

Epidemiological evidence has associated dietary *trans*-fatty acids (TFAs) with coronary heart disease. It is assumed that TFAs stimulate atherosclerosis, but this has not been proven. The purpose of this study was to determine the effects of consuming 2 concentrations of TFAs obtained from commercially hydrogenated vegetable shortening on atherosclerotic development in the presence or absence of elevated dietary cholesterol. Low-density lipoprotein receptor–deficient mice were fed 1 of 7 experimental diets for 14 weeks: low regular fat (LR), low *trans*-fat (LT), regular high fat, high *trans*-fat (HT), or a diet containing 2% cholesterol with low regular fat (C + LR), low *trans*-fat (C + LT), or high *trans*-fat (C + HT). The extent of lesion development was quantified by *en face* examination of the dissected aortae. Dietary cholesterol supplementation significantly elevated serum cholesterol levels. Surprisingly, this rise was partially attenuated by the addition of TFAs (C + LT and C + HT) in the diet. Serum triglyceride levels were elevated with the higher-fat diets and with the combination of *trans*-fat and cholesterol. Animals consuming TFAs in the absence of dietary cholesterol developed a significantly greater extent of aortic atherosclerotic lesions as compared with control animals (LT > LR and HT > regular high fat). Atherosclerotic lesions were more extensive after cholesterol feeding, but the addition of TFAs to this atherogenic diet did not advance atherosclerotic development further. In summary, TFAs are atherogenic on their own; but they do not stimulate further effects beyond the strongly atherogenic effects of dietary cholesterol.

1. Introduction

Coronary artery atherosclerosis is the principle cause of cardiovascular morbidity and mortality in North America. Nutritional choices, including the source of dietary fatty acids, can significantly impact cardiovascular disease. For example, several recent epidemiological and interventional investigations have revealed a significant positive association between coronary heart disease and the consumption of *trans*-fatty acids (TFAs) [1-6]. TFAs acids have also been found in atherosclerotic lesions and adipose tissue of obese and cardiac patients [7,8]. It has been assumed from these studies that TFAs induce their deleterious effects on cardiovascular disease through an atherogenic action. However, surprisingly, Kritchevsky and colleagues [9-11] have repeatedly found no effect of a high-TFA diet on atherosclerosis. It is possible, therefore, that TFAs may influence cardiovascular health in a manner independent of atherogenesis. Alternatively, it is also possible that some aspects of the studies conducted by Kritchevsky et al [9-11] were suboptimal to detect an effect of the TFA diets. Kritchevsky and colleagues [9-11] always supplemented the diets of their animals with TFAs superimposed on a high-cholesterol diet. It is possible that coadministration of TFAs with dietary cholesterol may mask the atherogenic effects of TFAs. In addition, the low-density lipoprotein receptor–deficient ($LDLr^{-/-}$) mouse may be a better animal model in which TFAs can be studied. It is an excellent representation of human atherosclerotic disease and displays a strong atherogenic response to dietary fats [12,13]. We have hypothesized in the present study, therefore, that consuming commercially hydrogenated TFAs in isolation would induce atherosclerosis and consuming higher levels of TFAs would promote more extensive atherosclerotic development.

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2. Materials and methods

2.1. Animals and dietary interventions

Female C57BL/6J LDLr$^{-/-}$ mice (Jackson Laboratory, Bar Harbor, ME), 5 to 7 weeks old, were randomly assigned to 7 dietary treatment groups of 5 animals. The 7 experimental diets consisted of a base of a delipidated RMH3000 diet from TestDiet (Richmond, IN) with the fat content replaced by 1 of 2 fat sources at 2 concentrations: 4% or 8% regular fat (porcine/soy) and 4% or 8% manufactured partially hydrogenated vegetable shortening (*trans*-fat). The 4% partially hydrogenated vegetable shortening provided 1.4% TFA, mainly in the form of elaidic TFA, to the diets. The 8% partially hydrogenated vegetable shortening provided 2.8% TFA, mainly in the form of elaidic TFA, to the diets. The 7 experimental diets are as follows: (1) low (4%) regular fat chow (LR), (2) low (4%) *trans*-fat chow (LT), (3) high (8%) regular fat chow (HR), (4) high (8%) *trans*-fat chow (HT), (5) 2% cholesterol and low (4%) regular fat chow (C + LR), (6) 2% cholesterol and low (4%) *trans*-fat chow (C + LT), and (7) 2% cholesterol and high (8%) *trans*-fat chow (C + HT). Mice were given 4 g of food daily for 14 weeks. Water was provided ad libitum. Guidelines for the ethical care and treatment of animals from the Canadian Council on Animal Care were strictly followed [14]. The nutritional composition of the experimental diets was analyzed by Norwest Laboratories in Lethbridge, Alberta, for proximate analysis of crude protein, carbohydrate, fat, fiber, ash, and digestible energy.

2.2. Blood collection and analysis

After the dietary intervention, plasma and serum were collected and stored at −80°C until analyzed for fatty acid, triglyceride, and cholesterol content. Plasma triglyceride and cholesterol levels were quantified using commercial enzymatic kits (Thermo Electron, Waltham, MA). Lipids were extracted from plasma (100 μL) and a 1-g sample of ground diet using chloroform-methanol (2:1, vol/vol) [15]. Conversion of the fatty acids to their methyl esters was accomplished using a modification of the method by Park and Goins [16]. Briefly, the extracted lipids (stored in 100 μL of dichloromethane containing the internal standard C11:0) were first esterified using 1 mL of 0.5 N methanolic NaOH at 90°C for 10 minutes under an atmosphere of nitrogen. After a brief cooling period, 1 mL of 14% BF_3-methanol was added; and the contents were heated as described above. The reaction was terminated upon the addition of 1 mL distilled water, and the fatty acid methyl esters (FAMEs) were extracted into 300 μL hexane. Completion of the derivitization reaction was verified using thin-layer chromatography using conditions detailed by Cruz-Hernandez and colleagues [17]. The FAMEs were then analyzed using gas chromatography coupled with flame-ionization detection. The separation methodology is based upon the 150°C gas chromatography temperature program detailed by Kramer and colleagues [18]. The FAMEs were analyzed against an authentic standard, GLC 469A, with the addition of eicosapentaenoic acid (EPA) (Nu-Chek Prep, Elysian, MN). The results for the TFA are presented as a sum of TFA isomers.

2.3. Assessment of atherosclerotic lesion formation

The aorta was cleaned of adventitial tissue and washed in cold phosphate-buffered saline solution before evaluating the tissue for atherosclerotic lesions by *en face* and cross-sectional analysis [19,20]. For *en face* analysis, the aorta from the ascending arch to the iliac bifurcation was cleaned of peripheral tissue, opened longitudinally, pinned flat, and digitally photographed; and the luminal images were analyzed using the Silicon Graphics Imaging software

Table 1
Nutritional composition of the experimental diets and average food intake

Group code	Group name	Dietary fat	Ash	Protein	Fibre	CHO	Fat	Calories (kcal/g)	TFA (% of diet)	TFA (% caloric energy)	Food intake (g/d)
LR	Low regular fat	4% Pork/soy fat	7.0	24.0	4.3	56.1	8.5	3.5	0	0	3.8
LT	Low *trans*-fat	4% Hydrogenated vegetable shortening	7.0	24.2	4.4	56.2	8.2	3.5	1.4	3.2	3.8
HR	High regular fat	8% Pork/soy fat	6.4	22.2	4.2	54.7	12.5	3.6	0	0	3.7
HT	High *trans*-fat	8% Hydrogenated vegetable shortening	6.6	21.7	5.2	54.5	12.0	3.6	2.8	6.4	3.8
C + LR	Cholesterol + low regular fat	2% Cholesterol + 4% pork/soy fat	6.7	23.2	4.3	55.0	10.8	3.6	0	0	3.9
C + LT	Cholesterol + low *trans*-fat	2% Cholesterol + 4% hydrogenated vegetable shortening	6.7	22.9	4.8	54.8	10.8	3.6	1.4	3.2	3.8
C + HT	Cholesterol + high *trans*-fat	2% Cholesterol + 8% hydrogenated vegetable shortening	6.4	22.7	4.2	53.2	13.4	3.7	2.8	6.4	3.8

Ash, protein, fiber, carbohydrates, and fat are represented as a percentage of total nutrients, measured by proximate analysis. The TFA content was estimated based on the addition of 4% or 8% partially hydrogenated shortening, containing 35% TFA content, to the diets. Food intake (grams per day) represents the average amount of food consumed by LDLr$^{-/-}$ mice fed experimental diets over 14 weeks. There was no significant difference in food intake between the groups (n = 15 per group). Protein indicates crude protein; fiber, crude fiber; CHO, carbohydrates; fat, crude fat; calories, digestible energy (kilocalories per gram).

(Sunnyvale, CA) [19,20]. The lesion area index was calculated as the ratio of areas with lesions vs total luminal surface area × 100 (mean ± standard error).

2.4. Statistical analysis

Data are expressed as mean ± standard error. Statistical comparisons were made using 1-way analysis of variance, followed by the Fisher least significant difference test for multiple parametric comparisons using the SigmaStat software (San Jose, CA). Differences between means were considered significant when $P < .05$.

3. Results

3.1. Experimental diets

The nutritional composition of the 7 experimental diets is listed in Table 1. Three diets were created to serve as internal controls for the 4 diets containing TFAs (LR for LT, HR for HT, and C + HT and C + LR for C + LT and C + HT). The composition of these control diets was identical to the TFA-containing diets, apart from the source of dietary fat. The grain content in each of the diets contributed a small amount of dietary fat. As detailed in Table 1, doubling the amount of added fat increased the dietary fat content by approximately 50%; and adding cholesterol to the diet added approximately 25% more dietary fat. The fat content was therefore highest in the C + HT group. Both the LT and C + LT diets contained 1.4% TFA, which relates to 3.2% of caloric energy as TFA intake. The HT and C + HT diets contained 2.8% TFA, which provides 6.4% of caloric energy as TFA intake. The ash, protein, fiber, and carbohydrate contents of all of the diets were controlled and similar among the groups. Despite an increase in dietary fat in the higher-fat groups, the digestible energy levels provided by the diets were all in a similar range (within 3.5 and 3.7 kcal/gram). Food intake between the groups was similar (Table 1).

3.2. Fatty acid profile of the experimental diets

The experimental diets differed in fatty acid composition (Fig. 1). Noteworthy differences include a general increase in fatty acid levels in experimental diets with higher fat contents (HR, HT, and C + HT) and a general, although not always statistically significant, reduction in saturated

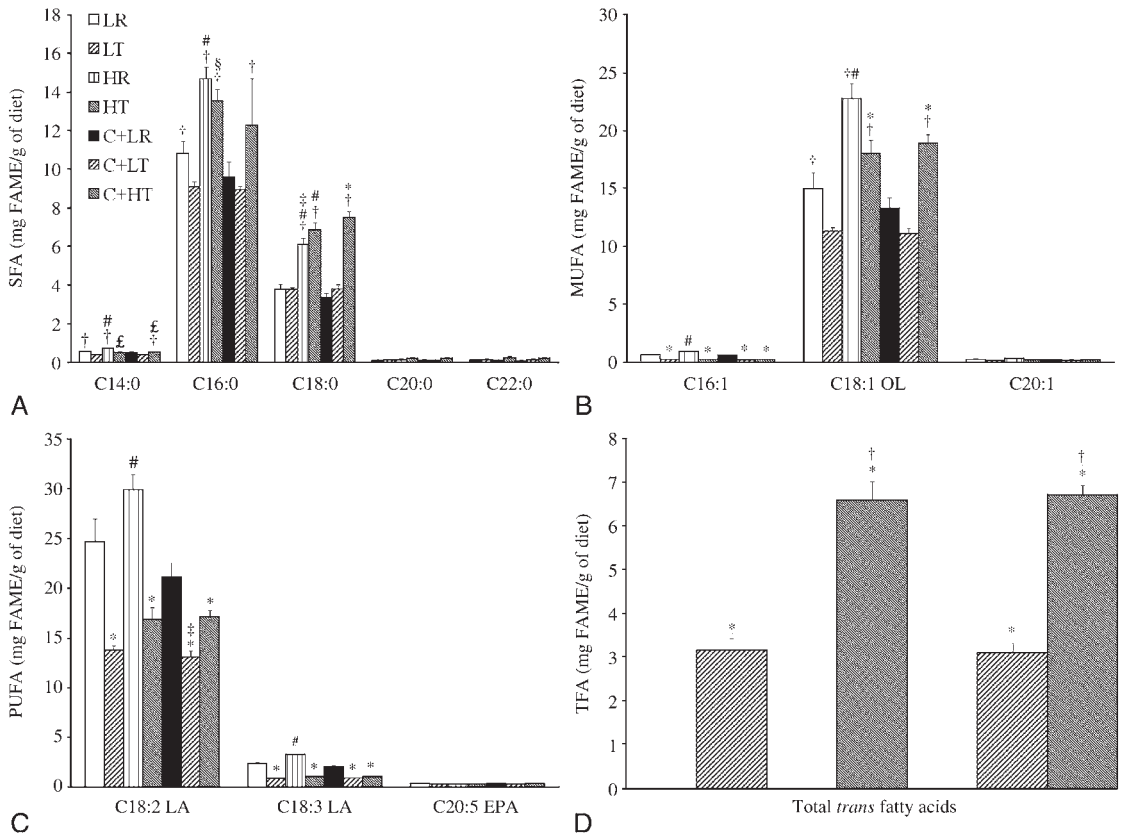

Fig. 1. Fatty acid composition of the experimental diets. Levels of SFA (A), MUFA (B), PUFA (C), and TFA (D) in the experimental diets (milligrams per gram diet). Values are means ± SE; n = 3. *$P < .05$ vs LR, HR, and C + LR groups. $^{\dagger}P < .05$ vs LT and C + LT groups. $^{\#}P < .05$ vs LR and C + LR groups. $^{\ddagger}P < .05$ vs C + HT group. $^{\S}P < .05$ vs C + LR group. $^{\infty}P < .05$ vs LR group. $^{£}P < .05$ vs HR group. Only trace amounts (<0.01 mg/mL) of C13:0, C14:1, C16:1*t*, C17:0, C18:2*tt*, C18:3 γ-linolenic acid, C20:$3^{11,14,17}$, C20:$3^{8,11}$, C22:1, and C22:6 docosahexaenoic acid were detectable (data not shown). OL indicates oleic acid; GLA, γ-linolenic acid; DHA, docosahexaenoic acid.

fatty acids (SFAs) (C14:0 and C16:0), monounsaturated fatty acids (MUFAs) (C16:1, C18:1, and C20:1), and polyunsaturated fatty acids (PUFAs) (C18:2 and C18:3) levels in groups containing TFAs as compared with their control group (LT vs LR, HT vs HR, and C + LT vs C + LR). The regular-fat groups (LR, HR, and L + LR) had minimal levels of TFAs. The addition of commercially hydrogenated vegetable shortening significantly and dose dependently raised the TFA fatty acid content of the experimental diets (LT, C + LT, HT, and C + HT groups) (Fig. 1D).

3.3. Circulating fatty acid profiles

Differences in the plasma fatty acid profile of the animals after the 14-week feeding period were observed (Fig. 2). The cholesterol-supplemented regular diet (C + LR) had the highest levels of plasma SFAs (C16:0 and C18:0), MUFAs (C16:1 and C18:1), and PUFAs (C18:2 linoleic acid [LA], C18:3 α-linolenic acid, C20:4 arachidonic acid [AA], and C20:5 EPA). Surprisingly, this rise was partially attenuated by the addition of TFAs (C + LT and C + HT) in the diet. Cholesterol supplementation on its own (C + LR group) did not elevate circulating plasma TFA levels; however, the addition of TFAs in an atherogenic diet (C + LT and C + HT) had a strong dose-dependent stimulatory effect on plasma TFA levels.

3.4. Circulating cholesterol and triglyceride levels

Serum cholesterol levels were similar among all of the groups at the start of the study (Fig. 3). After 4 weeks of feeding, the addition of dietary cholesterol had a sharp effect on serum cholesterol levels. Substituting the fat source for TFAs partially inhibited the dietary cholesterol-induced rise in serum cholesterol levels. Serum cholesterol levels rose in the groups consuming TFAs and cholesterol (C + LT and C + HT) over the course of the study. After 14 weeks of feeding, the substitution of fat for a TFA source in the diet resulted in lower serum cholesterol levels as compared with the LR group. Increasing the level of fat in the diet resulted in a small rise in serum cholesterol levels (HR vs LR). Including higher levels of TFA in the diet had no effect on serum cholesterol levels (HT vs HR). As expected, the inclusion of cholesterol in the diet induced a significant increase in serum

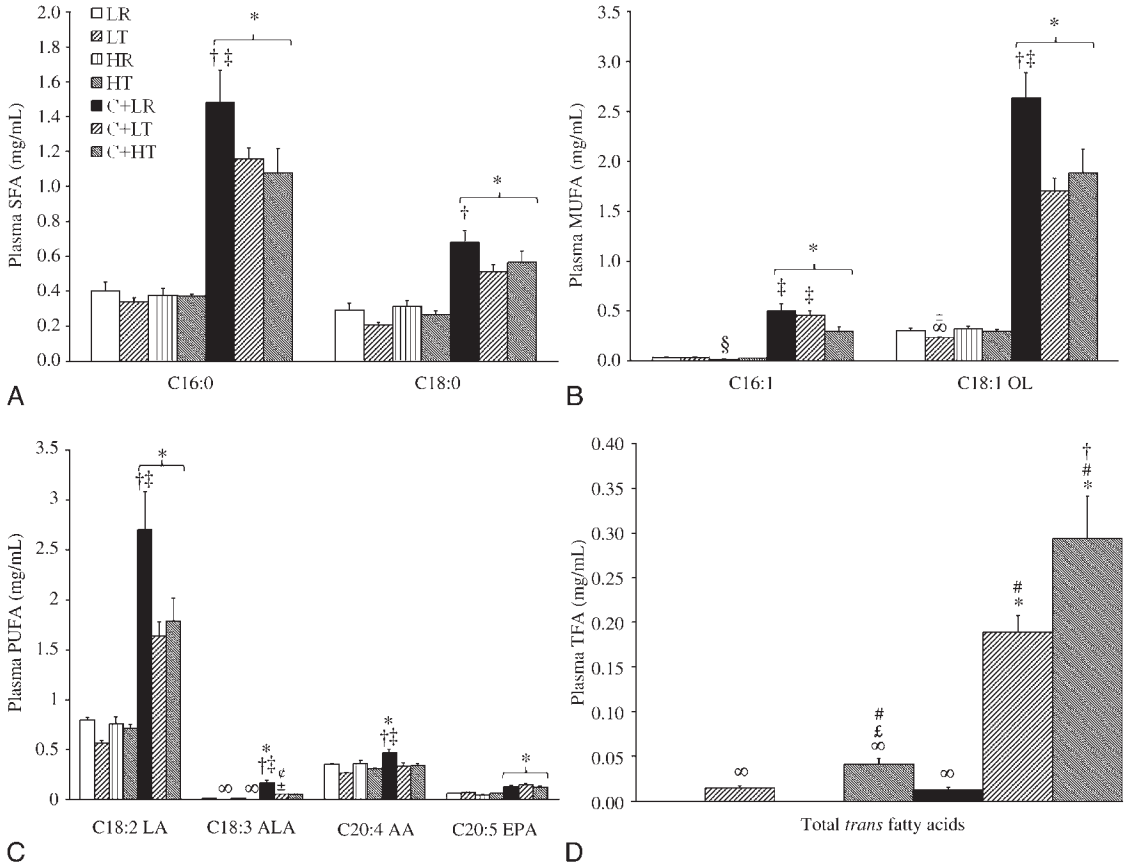

Fig. 2. Serum fatty acid levels in LDLr$^{-/-}$ mice after dietary interventions. Saturated (A), monounsaturated (B), polyunsaturated (C), and *trans* (D) fatty acids were quantitated (milligrams per milliliter) in plasma from LDLr$^{-/-}$ mice after 14 weeks of dietary interventions. Values are means ± SE; n = 4. *$P < .05$ vs LR, LT, HR, and HT groups. $^{\#}P < .05$ vs C + LR group. $^{\dagger}P < .05$ vs C + LT group. $^{\ddagger}P < .05$ vs C + HT group. $^{\infty}P < .05$ vs LR and HR groups. $^{\S}P < .05$ vs LR and LT groups. $^{¢}P < .05$ vs LT group. $^{£}P < .05$ vs HR group. $^{\pm}P < .05$ vs HT group. Only trace amounts (<0.01 mg/mL) of C13:0, C14:0, C14:1, C16:1*t*, C17:0, C18:2*tt*, C18:3 γ-linolenic acid, C20:0, C20:1, C20:3^{11,14,17}, C20:3^{8,11}, C22:0, C22:1, and C22:6 docosahexaenoic acid were detectable (data not shown).

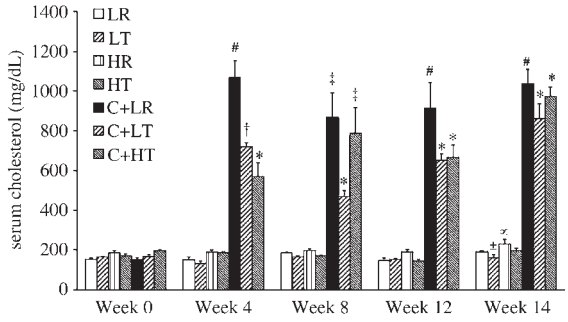

Fig. 3. Serum cholesterol levels in LDLr$^{-/-}$ mice after 0, 4, 8, 12, and 14 weeks of dietary interventions. Values are means ± SE; n = 5 at 0, 4, 8, 12, and 14 weeks. $^{\pm}P < .05$ vs LR group. $^{\infty}P < .05$ vs LR and LT groups. $^{*}P < .05$ vs LR, LT, HR, and HT groups. $^{\dagger}P < .05$ vs LR, LT, HR, HT, and C + HT groups. $^{\ddagger}P < .05$ vs LR, LT, HR, HT, and C + LT groups. $^{\#}P < .05$ vs all groups.

cholesterol levels. The substitution of regular fat for manufactured TFAs in the atherogenic diets resulted in slightly lower serum cholesterol levels at the conclusion of the study. Increasing the dose of TFAs had no effect on serum cholesterol levels.

Substituting the fat source for *trans*-fat had no effect on serum triglyceride levels in the absence of dietary cholesterol (Fig. 4). Consuming TFAs in the presence of dietary cholesterol had a significant impact on serum triglyceride levels (Fig. 4). Triglyceride levels were approximately 1.3 times greater in the C + LT group and approximately 2.5 times greater in the C + HT group vs the C + LR group ($P < .05$).

3.5. Aortic atherosclerotic development

Mice fed a control diet (LR) did not exhibit appreciable atherosclerotic plaque formation (Fig. 5). Including TFAs in the diet from a manufactured hydrogenated vegetable shortening source (LT) stimulated atherosclerotic development on its own, an event not normally observed in the LDLr$^{-/-}$ mouse unless it is fed dietary cholesterol or a strongly atherogenic diet. The TFAs initiated atherosclerotic development in a dose-dependent manner (LT > LR and HT > HR). The inclusion of cholesterol in the diet of the LDLr$^{-/-}$ mice induced a significant atherogenic action in comparison

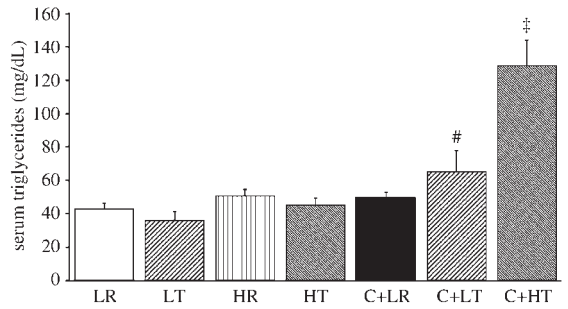

Fig. 4. Serum triglyceride levels in LDLr$^{-/-}$ mice after 14 weeks of dietary interventions. Values are means ± SE; n = 4 to 5. $^{\#}P < .05$ vs LT group. $^{\ddagger}P < .05$ C + HT vs all groups.

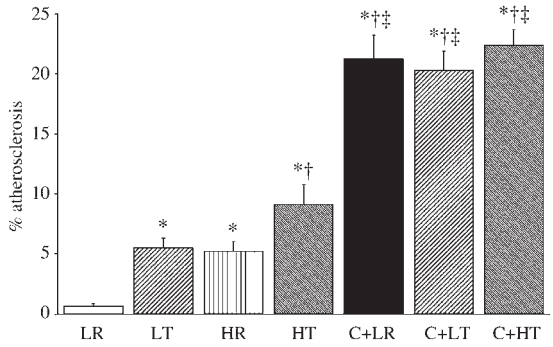

Fig. 5. Extent of aortic atherosclerotic lesions after dietary treatments. The atherosclerotic lesion area was measured as the percentage of total aortic luminal area. The lesion area was measured as the percentage of aortic luminal area covered by atherosclerotic lesions. Values are means ± SE. $^{*}P < .05$ vs LR group. $^{\dagger}P < .05$ vs LT and HR groups. $^{\ddagger}P < .05$ C + LR, C + LT, C + HT vs HR group. n = 5 for each diet type.

with the groups without dietary cholesterol. Substituting the fat source for either a low or high concentration of TFA from vegetable shortening in the presence of dietary cholesterol did not have an additional effect on atherosclerotic development. In the present study, dietary TFAs were directly responsible for the atherogenesis. The higher the circulating TFA was, the more extensive were the atherosclerotic lesions (Fig. 6).

4. Discussion

TFAs are formed during the partial hydrogenation of liquid oils, forming semisolid fats like margarine and shortening. They are also found in fried foods, cookies, donuts, and crackers. The hydrogenation process reduces the number of double bonds in unsaturated fatty acids and thereby changes the original *cis* configuration of the double bonds into a *trans* configuration, thereby resulting in a more linear fatty acid. Epidemiological investigations have

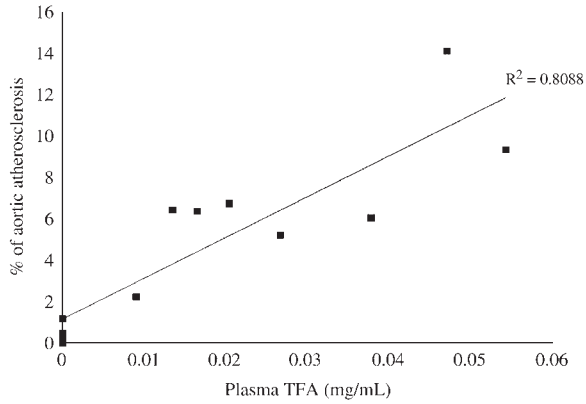

Fig. 6. Relationship of atherosclerosis to circulating TFA concentration. Each data point represents pooled values from 4 mice fed a control diet or 1 supplemented with low or high *trans*-fat. Data are taken from Figs. 2D and Fig. 5 and do not include data from cholesterol-fed animals.

associated diets that contain higher levels of TFAs with a higher incidence of cardiovascular disease [1-6], but there has never been a proven cause-and-effect relationship between dietary TFAs and atherosclerosis [1,3,21]. On the contrary, a high-cholesterol/high-TFA diet administered to rabbits, hamsters, and vervet monkeys did not induce atherogenesis [9-11]. In view of this current lack of mechanistic information, it is somewhat surprising that some governments have restricted TFA content in food products.

We demonstrate for the first time that TFAs have the capacity to directly stimulate atherosclerotic development on their own. Furthermore, the higher the circulating TFA was, the more extensive were the atherosclerotic lesions (Fig. 6). We can conclude from these data, therefore, that circulating TFAs can directly induce atherosclerosis in the LDLr$^{-/-}$ mice. Furthermore, our hypothesis that the effects of dietary cholesterol may have masked the effects of TFAs on their own in the previous works [9-11] appears to have been correct. The LDLr$^{-/-}$ mouse model may have been an ideal choice in which to study our dietary interventions. Genetically modified murine models of atherosclerosis like the LDLr$^{-/-}$ mice have been widely accepted in recent years as ideal in which to mimic human atherosclerotic development [12,13] and only develop lesions when fed a lipid-supplemented atherogenic diet.

The mechanism whereby TFAs induce atherosclerosis is unclear. Because they resemble SFAs with respect to their linear configuration, they may act very much like them in the cardiovascular system. Conversely, some factors may be ruled out as being mechanistically involved. For example, the atherogenesis in the LT and HT groups was stimulated without any increases in circulating cholesterol or triglyceride levels. The TFAs can exhibit detrimental cellular effects in humans unrelated to cholesterol [22-24]. With the majority of cholesterol carried in the high-density lipoprotein fraction in mice, it is unlikely that the high-density lipoprotein fraction is changed by the TFA diet. Furthermore, no changes in circulating SFAs, MUFAs, or PUFAs would explain the increase in atherosclerotic lesions in the LT and HT groups in comparison with their respective control groups. The atherogenic effect of TFAs on their own was also achieved without a change in weight gain. Alternatively, it is possible that TFAs may augment atherosclerosis through a stimulation of inflammatory pathways as has been shown using other dietary interventions in the LDLr$^{-/-}$ mouse [19,25]. TFAs adversely affect markers of inflammation in epidemiological and clinical trials [26-30]. However, although we examined several inflammatory markers in aortic tissue (mac-3, interleukin-6, HSP60) and plasma (tumor necrosis factor–α) in the present study, no changes were detected.

The dosage of TFAs used in our study has physiologic implications in humans. The low dose of commercially hydrogenated vegetable shortening in this study (LT and C + LT) provided 1.4% TFA, which is equivalent to 3.2% of caloric energy. The higher dose of hydrogenated vegetable shortening (HT and C + HT) provided 2.8% TFA, which is equivalent to 6.4% of caloric energy. Estimates of TFA intake range from 0.7 to 28.7 g TFA per person per day, with the average daily TFA intake in North America being 5.8 g or 2.6% of calories [31]. The TFA dosage provided to the mice in this study, therefore, was similar in energetic load to previous experimental reports [19,24,32] and similar to the average human TFA consumption values [31].

In summary, our data define the mechanism whereby commercially hydrogenated TFAs contribute independently to cardiovascular disease. Our data have relevance to the current levels of TFA ingestion in North America today. The dose dependency of the atherogenic effects is further cause for concern and lends credence to efforts to reduce its presence in foods available to the public today.

Acknowledgment

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References

[1] Willett WC, Stampfer MJ, Manson JE, Colditz GA, Speizer FE, Rosner BA, et al. Intake of *trans*-fatty acids and risk of coronary heart disease among women. Lancet 1993;341:581-5.

[2] Willett WC. *trans*-Fatty acids and cardiovascular disease-epidemiological data. Atheroscler Suppl 2006;7:5-8.

[3] Ascherio A, Hennekens CH, Buring JE, Master C, Stampfer MJ, Willett WC. *trans*-Fatty acids intake and risk of myocardial infarction. Circulation 1994;89:94-101.

[4] Baylin A, Kabagambe EK, Ascherio A, Spiegelman D, Campos H. High 18:2 *trans*-fatty acids in adipose tissue are associated with increased risk of nonfatal acute myocardial infarction in costa rican adults. J Nutr 2003;133:1186-91.

[5] Pedersen JI, Ringstad J, Almendingen K, Haugen TS, Stensvold I, Thelle DS. Adipose tissue fatty acids and risk of myocardial infarction —a case-control study. Eur J Clin Nutr 2000;54:618-25.

[6] Clifton PM, Keogh JB, Noakes M. *trans*-Fatty acids in adipose tissue and the food supply are associated with myocardial infarction. J Nutr 2004;134:874-9.

[7] Bortolotto JW, Reis C, Ferreira A, Costa S, Mottin CC, Souto AA, et al. Higher content of *trans*-fatty acids in abdominal visceral fat of morbidly obese individuals undergoing bariatric surgery compared to non-obese subjects. Obes Surg 2005;15:1265-70.

[8] Dlouhy P, Tvrzicka E, Stankova B, Vecka M, Zak A, Straka Z, et al. Higher content of 18:1 *trans*-fatty acids in subcutaneous fat of persons with coronarographically documented atherosclerosis of the coronary arteries. Ann Nutr Metab 2003;47:302-5.

[9] Ruttenberg H, Davidson LM, Little NA, Klurfeld DM, Kritchevsky D. Influence of *trans*-unsaturated fats on experimental atherosclerosis in rabbits. J Nutr 1983;113:835-44.

[10] Kritchevsky D, Davidson LM, Weight M, Kriek NP, du Plessis JP. Effect of *trans*-unsaturated fats on experimental atherosclerosis in vervet monkeys. Atherosclerosis 1984;51:123-33.
[11] Nicolosi RJ, Wilson TA, Rogers EJ, Kritchevsky D. Effects of specific fatty acids (8:0, 14:0, *cis*-18:1, *trans*-18:1) on plasma lipoproteins, early atherogenic potential, and LDL oxidative properties in the hamster. J Lipid Res 1998;39:1972-80.
[12] Moghadasian MH, Frohlich JJ, McManus BM. Advances in experimental dyslipidemia and atherosclerosis. Lab Invest 2001;81:1173-83.
[13] Breslow JL. Mouse models of atherosclerosis. Science 1996;272: 685-8.
[14] Olfert ED, Cross BM, McWilliam AA, editors. Canadian Council on Animal Care guide to the care and use of experimental animals, 2nd ed., vol 1. Ottawa: Bradda Printing Services Inc.; 1993. p. 298.
[15] Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 1957; 226:497-509.
[16] Park PW, Goins RE. In situ preparation of fatty acid methyl esters for analysis of fatty acid composition in foods. J Food Sci 1994;59:1262-6.
[17] Cruz-Hernandez C, Deng Z, Zhou J, Hill AR, Yurawecz MP, Delmonte P, et al. Methods for analysis of conjugated linoleic acids and *trans*-18: 1 isomers in dairy fats by using a combination of gas chromatography, silver-ion thin-layer chromatography/gas chromatography, and silver-ion liquid chromatography. J AOAC Int 2004;87:545-62.
[18] Kramer JK, Hernandez M, Cruz-Hernandez C, Kraft J, Dugan ME. Combining results of two GC separations partly achieves determination of all *cis*- and *trans*-16:1, 18:1, 18:2 and 18:3 except CLA isomers of milk fat as demonstrated using Ag-ion SPE fractionation. Lipids 2008;43:259-73.
[19] Dupasquier CM, Dibrov E, Kneesh AL, Cheung PK, Lee KG, Alexander HK, et al. Dietary flaxseed inhibits atherosclerosis in the LDL receptor–deficient mouse in part through antiproliferative and anti-inflammatory actions. Am J Physiol Heart Circ Physiol 2007;293: H2394-402.
[20] Hu H, Pierce GN, Zhong G. The atherogenic effects of chlamydia are dependent on serum cholesterol and specific to *Chlamydia pneumoniae*. J Clin Invest 1999;103:747-53.
[21] Oh K, Hu FB, Manson JE, Stampfer MJ, Willett WC. Dietary fat intake and risk of coronary heart disease in women: 20 years of follow-up of the nurses' health study. Am J Epidemiol 2005;161:672-9.
[22] Zapolska-Downar D, Kosmider A, Naruszewicz M. *trans*-Fatty acids induce apoptosis in human endothelial cells. J Physiol Pharmacol 2005;56:611-25.
[23] Mitmesser SH, Carr TP. *trans*-Fatty acids alter the lipid composition and size of apoB-100–containing lipoproteins secreted by HepG2 cells. J Nutr Biochem 2005;16:178-83.
[24] Cassagno N, Palos-Pinto A, Costet P, Breilh D, Darmon M, Berard AM. Low amounts of *trans* 18:1 fatty acids elevate plasma triacylglycerols but not cholesterol and alter the cellular defence to oxidative stress in mice. Br J Nutr 2005;94:346-52.
[25] Wang S, Wu D, Matthan NR, Lamon-Fava S, Lecker JL, Lichtenstein AH. Reduction in dietary omega-6 polyunsaturated fatty acids: eicosapentaenoic acid plus docosahexaenoic acid ratio minimizes atherosclerotic lesion formation and inflammatory response in the LDL receptor null mouse. Atherosclerosis 2008.
[26] Baer DJ, Judd JT, Clevidence BA, Tracy RP. Dietary fatty acids affect plasma markers of inflammation in healthy men fed controlled diets: a randomized crossover study. Am J Clin Nutr 2004;79:969-73.
[27] Mozaffarian D, Rimm EB, King IB, Lawler RL, McDonald GB, Levy WC. *trans*-Fatty acids and systemic inflammation in heart failure. Am J Clin Nutr 2004;80:1521-5.
[28] Lopez-Garcia E, Schulze MB, Meigs JB, Manson JE, Rifai N, Stampfer MJ, et al. Consumption of *trans*-fatty acids is related to plasma biomarkers of inflammation and endothelial dysfunction. J Nutr 2005;135:562-6.
[29] Han SN, Leka LS, Lichtenstein AH, Ausman LM, Schaefer EJ, Meydani SN. Effect of hydrogenated and saturated, relative to polyunsaturated, fat on immune and inflammatory responses of adults with moderate hypercholesterolemia. J Lipid Res 2002;43: 445-52.
[30] Hodgson JM, Wahlqvist ML, Boxall JA, Balazs ND. Platelet *trans*-fatty acids in relation to angiographically assessed coronary artery disease. Atherosclerosis 1996;120:147-54.
[31] Food labeling: *trans*-fatty acids in nutrition labeling, nutrient content claims, and health claims. Final rule. Fed Regist 2003;68: 41433-506.
[32] Rudel LL, Kelley K, Sawyer JK, Shah R, Wilson MD. Dietary monounsaturated fatty acids promote aortic atherosclerosis in LDL receptor-null, human ApoB100-overexpressing transgenic mice. Arterioscler Thromb Vasc Biol 1998;18:1818-27.