Effects of dietary fats from animal and plant sources on diet-induced fatty streak lesions in C57BL/6J mice

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Abstract This study was designed to determine the effects of a variety of naturally occurring saturated fats on aortic lesion formation in C57BL/6J mice that are susceptible to diet-induced fatty streak lesions. Groups of female mice were randomly assigned to one of seven treatment groups and were fed diets containing 15% (w/w) hydrogenated coconut oil, hydrogenated soy oil, hydrogenated palm oil, cocoa butter, lard, tallow, or dairy butter, 1% cholesterol, and 0.5% cholic acid. Plasma lipid levels were measured to determine whether lesion formation was related to specific changes in these parameters. Lesions, which were observed in all groups of mice, ranged from 420 to 3220 μm^2/aortic cross section. Lesion area was positively correlated to the percentage of saturated fatty acids contained in the fat sources and the ratio of combined VLDL plus LDL-cholesterol to HDL-cholesterol and inversely correlated to monounsaturated fatty acids content and to HDL-cholesterol levels. Results from this study demonstrate that inbred mice may provide a good model for dissecting the genetic basis for the differential atherogenic responses to diet-induction and for studying the effects of dietary factors on aortic lesion development.—**Nishina, P. M., S. Lowe, J. Verstuyft, J. K. Naggert, F. A. Kuypers, and B. Paigen.** Effects of dietary fats from animal and plant sources on diet-induced fatty streak lesions in C57BL/6J mice. *J. Lipid Res.* 1993. **34:** 1413–1422.

Supplementary key words dietary saturated fats • atherosclerosis • mouse model • plasma lipids

Many studies have explored the relationship between dietary factors, changes in lipoprotein levels, and risk for atherosclerosis, particularly, the regulation of cholesterol and lipoprotein metabolism by type and amount of dietary fat (reviewed in references 1 and 2). In general, diets rich in monounsaturated and polyunsaturated fat decrease plasma cholesterol levels; whereas, diets high in saturated fat, in particular palmitic, myristic, and lauric acids, are positively correlated to plasma cholesterol levels (2–5). Studies in nonhuman primates, rabbits, and other animal models evaluating the development of aortic lesions in response to dietary fat intake and the predictive value of plasma lipid levels for lesion formation have been done (reviewed in references 6–8). However, screening drug or diet therapies for prevention or amelioration of heart disease in larger mammals (i.e., nonhuman primates and pigs) is limited due to their availability or special husbandry needs, and studies of interaction between heritable and environmental factors is difficult in species that have not been specifically developed as a genetic resource (i.e., inbred strains that are genetically defined).

The mouse is a particularly suitable model for the study of interaction between environmental and genetic factors in the development of atherosclerosis because a large number of defined phenotypes in the form of inbred strains are available (9). In addition, its well developed genetics, small size, and short generation time allow for manipulations that cannot be carried out in humans or other animal models. Moreover, a reproducible method for evaluating diet-induced aortic lesions and methods for assessing lipids and lipoproteins in a small plasma volume have been developed (10). *Ath-1, Ath-2,* and *Ath-3,* genes that affect atherosclerosis susceptibility, and other genes that regulate lipoprotein structure and metabolism have been identified in the mouse and described in the literature (11–14).

This study was designed to examine the effects of naturally occurring dietary fats of plant and animal origin on fatty streak lesion development in C57BL/6J mice, a strain which has diet-inducible atherosclerosis-suceptible

Abbreviations: TC, total plasma cholesterol; HDL-C, high density lipoprotein cholesterol; VLDL, very low density lipoprotein; LDL, low density lipoprotein; SO, hydrogenated soy oil; CO, hydrogenated coconut oil; CB, cocoa butter; PO, hydrogenated palm oil; LA, lard; TA, tallow; DB, dairy butter; RID, radial immunodiffusion.

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alleles of *Ath-1, Ath-2,* and *Ath-3* (12–14). Plasma and liver lipid levels were also measured to determine whether different dietary fats would elicit a distinct lipoprotein profile and whether specific changes in that profile were correlated with aortic lesion formation.

MATERIALS AND METHODS

Chemicals and diet

The sources of chemicals were: cholesterol oxidase, bovine pancreas cholesterol ester hydrolase, and peroxidase, Sigma Chemicals Co., St. Louis, MO; cholesterol reagent kit #236691, Boehringer Mannheim, Indianapolis, IN; triglyceride reagent set #46676, Seradyn Inc. Indianapolis, IN; triglyceride blank blend #10021, Craig Bioproducts, Streamwood, IL; chemicals for gel electrophoresis, Bio-Rad Laboratories, Richmond, CA; gradient gels (4–30% polyacrylamide), Pharmacia, Piscataway, NJ; Oil red O, Aldrich Chemical Company, Inc., Milwaukee, WI; hematoxylin and light green, Baxter Scientific, McGaw Park, IL; gentamycin sulfate, US Biochemical Corp., Cleveland, OH; and fatty methyl ester mixtures, Supelco, Bellfonte, PA. Other chemicals used were of reagent grade or better. Components for the purified diets were purchased from ICN Biochemicals, Inc., Costa Mesa, CA with the exception of Mazola corn oil, Best Foods, Englewood Cliffs, NJ. Commercially prepared stock diet, Rodent Chow #5012, Ralston Purina, St. Louis, MO containing 4% fat, was fed to the mice during the environmental adaptation period.

Methods of diet preparation have been described previously (15). The high fat diets contained 15% fat, 50% sucrose, 20% casein, 1% corn oil, 5.07% cellulose, 5% AIN-76 mineral mix, 1% AIN-76 vitamin mix, 1% choline chloride, 0.3% DL-methionine, 0.13% DL-α-tocopherol, 0.5% sodium cholate, and 1% cholesterol. Saturated fats included in the diets were dairy butter, lard, tallow, and hydrogenated coconut, palm, or soy oils. Of the 1% cholesterol contained in the dairy butter diet, 0.03% came from the dairy butter itself. To obtain 15% dairy butter, 18.45 gm butter was used per 100 g diet because of the 18.9% water content of butter. Endogenous cholesterol contained in tallow and lard is negligible (<0.005%), therefore, 1% exogeneous cholesterol was added to these diet mixes. The low fat diet, containing 8% corn oil, fed to the reference group (RG) has been previously described in reference 15.

Animals

An animal use protocol for this experiment was approved by the Institutional Animal Care and Use Committee at Children's Hospital Oakland Research Institute. Female C57BL/6J (B6) mice, 4–8 weeks old, were obtained from The Jackson Laboratory, Bar Harbor, ME, and maintained in a room illuminated from 7 AM through 7 PM. All animals were allowed to adapt to the environment for at least 2 weeks prior to dietary treatment and provided free access to food and water throughout the experiment. Weight gain was monitored every 3 weeks and food intake was monitored at weeks 3, 7, and 14. Eight female mice were used for each dietary treatment group. However, the number of animals used for analysis of plasma lipids, liver lipids, and lesions ranged from five to eight animals.

Collection of blood and tissues

Prior to blood or tissue collection, mice were fasted by removing their food at 5 PM on the day previous to the experiment. Blood was obtained between 7:30 and 9:30 AM from the tail vein of the mice after the 2-week adaptation period for baseline measurements, and again after 4, 10, and 18 weeks of consuming the semi-synthetic high fat and cholesterol diet. Blood was mixed in chilled tubes with EDTA, sodium azide, and gentamycin sulfate at final concentrations of 2 mM, 0.05 mg/ml, and 0.05 mg/ml, respectively. Plasma was obtained by centrifugation of whole blood for 5 min at 12,000 *g* at 4°C. For the evaluation of methods for handling and for storage of plasma for triglyceride measurements, in which assay variability was being tested, plasma samples from 10 mice were immediately pooled and aliquoted into appropriate proportions for the conditions tested (see Fig. 1).

Upon termination of the experiments, the heart and the upper section of the aorta were removed from each animal and placed in 0.9% saline at room temperature. The heart, which continues to contract, is flushed of blood cells. After 1 h, the heart was trimmed of extraneous tissue and placed in 10% phosphate-buffered formalin for 24 h at room temperature. Livers were blotted, quickly frozen in liquid nitrogen, and stored at −70°C for lipid analysis.

Evaluation of methods for handling and storage of plasma for triglyceride determination

Plasma triglyceride concentrations are estimated by subtraction of free glycerol from total glycerol (e.g., free glycerol plus glycerol liberated from triglycerides by lipase contained in the assay mixture). Free glycerol concentrations in mice are quite high and are not in constant proportion to the triglyceride concentration; therefore, free glycerol levels must be determined for each plasma sample. Previously, we noticed that free glycerol levels increased in plasma samples with increasing length of storage causing the triglyceride levels to be underestimated (P. M. Nishina, unpublished results). This is probably due to enzymes (i.e., lipases) which are present and active in the plasma. To determine the optimum conditions to minimize endogenous hydrolysis of triglycerides, aliquots of a pooled plasma sample were stored at various conditions. The concentration of triglyceride at time zero

was normalized to 100%, and degradation of triglyceride to free glycerol and fatty acids were recorded as % triglyceride remaining compared to time zero.

Plasma lipids, lipoproteins, and apolipoproteins

Plasma total cholesterol (TC), free glycerol, and total glycerol were determined by commercial colorimetric enzymatic assay. HDL-cholesterol (HDL-C) was measured after selective precipitation of LDL and VLDL with polyethylene glycol (16). The combined VLDL and LDL-cholesterol (VLDL and LDL-C) was calculated as the difference between TC and HDL-C. Plasma triglyceride concentrations were estimated by subtraction of free glycerol from total glycerol. Lipid measurements are given as mg/dl ± SEM. Plasma concentrations of apolipoprotein A-I (apoA-I) and apolipoprotein A-II (apoA-II) were estimated by radial immunodiffusion (17). Purified mouse apoA-I and apoA-II isolated by column chromatography from ultracentrifugally isolated mouse plasma lipoproteins served as standards (18). VLDL and LDL particles were separated by density ultracentrifugation from plasma pools of two mice each by adjusting the plasma density to 1.006 and 1.063 g/ml in successive runs (19).

Liver lipids

Lipids extracted from livers by the method of Folch, Lees, and Sloane Stanley (20) were assayed for triglyceride (21), cholesterol (22), and phosphorus (23). Phosphorus values were multiplied by a factor of 25 to estimate liver phospholipid content, assuming that phospholipids contain approximately 4% phosphorus by weight based on an average molecular weight for phospholipid of 775.

Fatty acid composition of diet and livers

The fatty acyl group composition of lipid extracts from diet and liver was determined by profiling the long-chain fatty acyl groups after transesterification of the fatty acids (24). Gas-liquid chromatography was performed on a Shimadzu 95A Gas Chromatograph equipped with a 30 m × 0.25 mm fused silica SP 2330 column (Supelco, Bellefonte, PA) and a flame ionization detector. Standard fatty methyl ester mixtures were used to identify the fatty methyl esters > C8 in the samples.

Evaluation of atherosclerotic lesions

Evaluation of aortic lesions has been described in detail by Paigen et al. (10). Briefly, mouse hearts were fixed, stored in 4% phosphate-buffered formaldehyde, and embedded in 25% gelatin. After removing the lower two-thirds of the heart, the remaining tissue was sectioned on a cryostat at −25°C. Alternate 10-μm sections were saved on slides. Sections were stained with aqueous Oil Red O for neutral lipids, hematoxylin for nuclei and basophilic tissue, and counterstained with light green. Five sections at 80-μm intervals were evaluated for the cross-sectional area of lesions, beginning where the aorta was rounded and valves appeared distinctly through to the endpoint where the valves disappeared, a distance of approximately 350 μm.

Statistical analysis

The Number Cruncher Statistical System, Version 4.21 1/86, Kaysville, UT and Statview, Version 1.0 6/85, Calabasas, CA were used for statistical analyses. Comparison of data for two groups was done by *t*-test. Comparison of data from more than two groups was analyzed by using one-way analysis of variance with Fisher's least significant difference test to determine which means were significantly different at $P < 0.01$. Correlation between aortic lesions and lipid parameters measured was tested by linear regression analysis.

RESULTS

Evaluation of methods for handling and storage of plasma for triglyceride determination

Two hours after blood sampling, 10% and 30% of the triglyceride in plasma was hydrolyzed when samples were stored on ice or at room temperature, respectively. Of the four storage conditions: ice, 4°C, −20°C, and −70°C, immediate freezing and storage of plasma at −70°C led to the least amount of endogenous hydrolysis (e.g., approximately 10%; **Fig. 1**). From these data we conclude that if triglyceride levels cannot be determined within 2 h of sampling, the best procedure for storage and handling is to immediately freeze plasma samples at −70°C and to

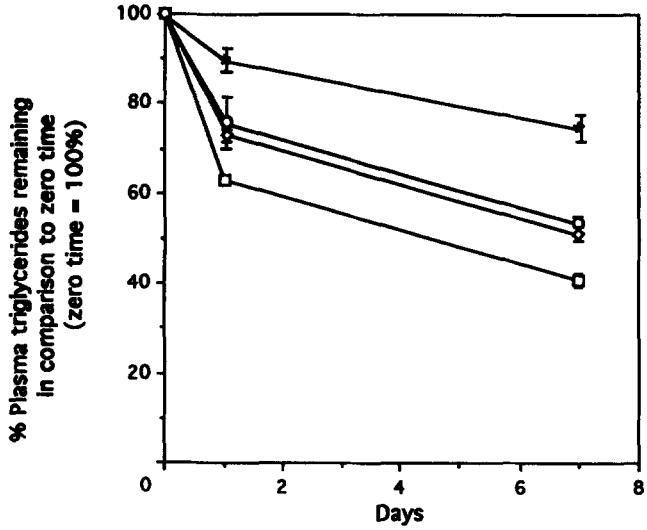

Fig. 1. Effects of handling and storage conditions on plasma triglyceride levels. Percent plasma triglyceride remaining when stored on ice (□), at 4°C (◇), −20°C (○) or −70°C (*) after 1 and 7 days. Four separate pools of plasma were utilized for each point in the graphs.

TABLE 1. Fatty acid profile of fat sources used in diets

Fatty Acid	RG	SO	CO	CB	PO	LA	TA	DB
			weight % of identified fatty acids					
8:0			17					8
10:0			9					11
12:0			49		1			6
14:0			14		2	4	6	12
16:0	10	11	5	25	36	23	23	23
16:1						5	6	3
18:0		10	1	30	3	9	14	7
18:1	21	62	4	37	42	41	41	19
18:2	66	14	1	4	13	11	3	2
20:1	trace	trace	trace			1	1	trace
20:4					2			trace
Unidentified fatty acids	3	3	0	4	1	6	6	9
Total saturated	10	21	95	55	42	36	43	67
Total monounsaturated	21	62	4	37	42	47	48	22
Total polyunsaturated	66	14	1	4	15	11	3	2

Fatty acids are indicated by number of carbon atoms: number of double bonds. No distinction is made on the double bond position. Abbreviations: RG, reference group, low fat diet containing 8% corn oil; SO, hydrogenated soy oil; CO, hydrogenated coconut oil; CB, cocoa butter; PO, hydrogenated palm oil; LA, pork fat; TA, beef fat; DB, dairy butter.

assay for triglycerides within a week. Fresh plasma samples should always be kept on ice, even when aliquoting for assays.

Fatty acid composition of dietary fats, food intake, and body weight changes

Initially, the mice used in this study weighed approximately 19 g. Although there were no statistically significant differences in average body weights among groups at the end of 18 weeks of consuming the high fat diets, body weight gain was variable. Mice consuming the hydrogenated coconut oil diet gained the least weight, 2.8 ± 0.2 g during the 18-week test period, while mice consuming the lard and tallow diets gained the most weight, 5.5 ± 0.3 and 5.2 ± 0.4 g, respectively. Nine to 13 kcal per day was consumed by mice fed the different diets.

Four plant fat sources used in the diets were hydrogenated soy oil (SO), hydrogenated coconut oil (CO), cocoa butter (CB), and hydrogenated palm oil (PO) and three animal fat sources included lard (LA, pork fat), tallow (TA, beef fat), and dairy butter (DB). Fatty acid composition of these fat sources indicated that CB, PO, LA, and TA were the most similar in total saturated, monounsaturated and polyunsaturated fat (**Table 1**). However, the actual percentage of a specific type of fatty acid differed among these fats (i.e., 31% of the saturated fatty acids in CB came from stearic acid, whereas it accounted for only 3–15% of the saturated fatty acids in PO, LA, or TA). Marked differences in fatty acid composition of the SO and CO were observed; 78% of soy oil was oleic acid (18:1) and linoleic (18:2) and 75% of CO was predominantly medium chain fatty acids (e.g. < 12 C atoms).

Aortic lesions in C57BL/6 mice fed saturated fat diets

All mice fed the high fat and cholesterol diets for 18 weeks developed fatty streak lesions (**Fig. 2**). The relationships between lesion area and the percentage of saturated, monounsaturated or polyunsaturated fat or P:S ratio of the dietary fats were tested (**Fig. 3**). Lesion area increased with increasing percentage of saturated fat contained in the diet (Fig. 3A). Conversely, as the percentage

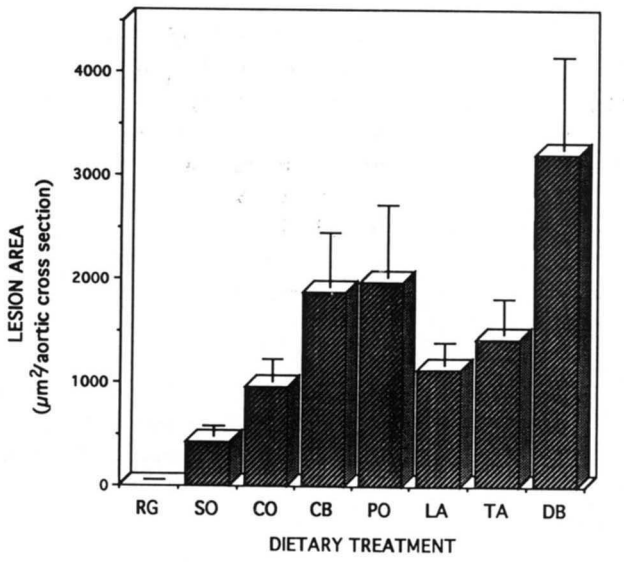

Fig. 2. Comparison of aortic lesions in C57BL/6 mice consuming high fat and cholesterol diets for 18 weeks. The aortic lesion sizes are expressed as the mean size per cross section in μm^2 ± SEM in a minimum of six female mice.

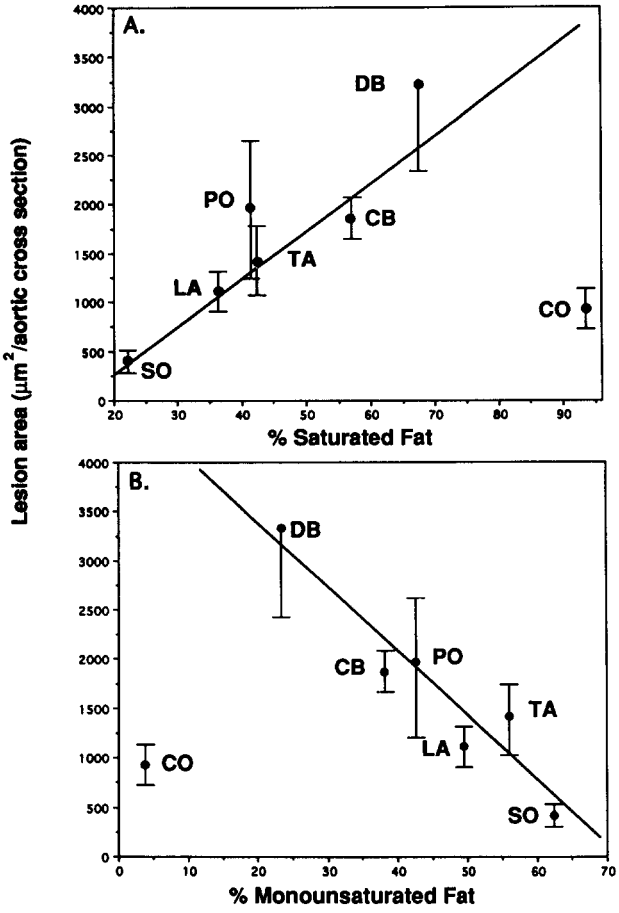

Fig. 3. Relationship between lesion area and fatty acid content of the fat source. Panel A: Lesion area versus % saturated fatty acids. Panel B: Lesion area versus % monounsaturated fatty acids. Results from the CO group were not used to draw the regression line as it appeared to be an outlier in both analyses.

of monounsaturated fat increased, lesion area decreased (Fig. 3B). These relationships were significant when the outlier dietary treatment group of CO was not included (r^2 = 0.90 and r^2 = 0.92, respectively, $P < 0.005$). Lesion area, in this experiment, was not correlated to the percentage of polyunsaturated fat or the P:S ratio of the dietary fat source.

Plasma lipids and apolipoproteins associated with the HDL particles

Fasting plasma triglyceride levels, which are normally very low in mice, were not different among mice consuming the various high fat diets (**Table 2**). However, triglyceride levels were approximately 20–25% lower than those of mice in the reference group ($P < 0.01$). After 4 weeks of diet consumption, the reverse was true of total plasma cholesterol levels; mice fed the high fat diets had somewhat higher plasma cholesterol levels than did mice in the reference group that consumed a low fat diet. Statistical significance ($P < 0.01$) was only observed, however, in mice consuming the hydrogenated coconut oil, hydrogenated palm oil, dairy butter, lard, or tallow diets; those consuming hydrogenated soy oil or cocoa butter diets had levels similar to reference group controls. The increase in total cholesterol levels could be attributed mainly to the combined VLDL plus LDL-C fraction. A 2- to 5-fold increase of these lipoproteins compared to levels in low fat-fed mice was observed after 4 weeks of dietary treatment. To determine what proportion of the cholesterol in this combined fraction was attributable to the individual lipoprotein fractions, VLDL (d < 1.006) and LDL (d 1.006–1.063) were separated by sequential ultracentrifugation. Approximately 80% of the cholesterol in the apoB-containing lipoproteins was recovered in the VLDL density range and 20% in the LDL density range. No significant differences in the percentage of cholesterol in each subfraction occurred among the dietary treatment groups tested (i.e., SO, TA, and DB, **Table 3**).

High density lipoprotein cholesterol concentrations ranged from 35 to 75% of the low fat-fed control mice. The greatest reduction in plasma HDL-C was observed in those mice receiving the diet containing tallow as a fat

TABLE 2. Plasma triglyceride, lipoprotein-cholesterol, and apolipoprotein concentrations observed in C57BL/6J mice fed diets made up with different fat sources for 4 weeks

Diet	Triglyceride	Total Cholesterol	HDL-C	VLDL/LDL-C	ApoA-I	ApoA-II
	mg/dl	*mg/dl*	*mg/dl*	*mg/dl*	*mg/ml*	*mg/ml*
RG	29 ± 3^{b}	69 ± 5^{a}	53 ± 3^{c}	15 ± 2^{a}	1.13 ± 0.05^{c}	0.63 ± 0.04^{c}
SO	18 ± 2^{a}	76 ± 4^{a,b}	40 ± 4^{b}	36 ± 4^{a,b}	0.85 ± 0.05^{b}	0.32 ± 0.03^{b}
CO	22 ± 3^{a}	100 ± 4^{b,c}	40 ± 3^{b}	60 ± 5^{b,c}	0.87 ± 0.05^{b}	0.29 ± 0.02^{b}
CB	19 ± 3^{a}	79 ± 5^{a,b}	32 ± 4^{b}	47 ± 3^{b}	0.72 ± 0.07^{a,b}	0.24 ± 0.02^{a,b}
PO	18 ± 2^{a}	104 ± 7^{b,c}	36 ± 4^{b}	54 ± 5^{b}	0.78 ± 0.04^{b}	0.24 ± 0.02^{a,b}
LA	17 ± 1^{a}	106 ± 10^{b,c}	5 ± 5^{b}	71 ± 12^{b,c}	0.80 ± 0.07^{b}	0.26 ± 0.03^{a,b}
TA	18 ± 2^{a}	102 ± 13^{b,c}	18 ± 1^{a}	84 ± 14^{c}	0.52 ± 0.02^{a}	0.18 ± 0.01^{a}
DB	17 ± 1^{a}	114 ± 7^{c}	27 ± 3^{a,b}	88 ± 8^{c}	0.64 ± 0.05^{a,b}	0.21 ± 0.02^{a}

Values represent mean ± SE for five animals. Values in columns without common superscripts are significantly different ($P < 0.01$). Abbreviations: RG, reference group, low fat diet containing 8% corn oil; SO, hydrogenated soy oil; CO, hydrogenated coconut oil; CB, cocoa butter; PO, hydrogenated palm oil; LA, pork fat; TA, beef fat; DB, dairy butter.

TABLE 3. Confirmation of fatty streak lesion development in C57BL/6 mice fed diets containing soy oil, tallow, or dairy butter as a fat source

Diets	Lesion Area[1]	TG	TC	HDL-C	VLDL/LDL-C		
					mg/dl	%C in VLDL[2]	%C in LDL[2]
	μm²/section	*mg/dl*	*mg/dl*	*mg/dl*			
SO	379 ± 125	39 ± 2^b	104 ± 3^a	45 ± 1^b	58 ± 3^a	79 ± 2	20 ± 2
TA	2182 ± 479	30 ± 1^a	131 ± 7^{a,b}	36 ± 3^a	95 ± 8^b	83 ± 3	17 ± 2
DB	5481 ± 891	35 ± 1^b	158 ± 12^b	36 ± 2^a	122 ± 13^b	80 ± 2	20 ± 2

Values represent means ± SE for 10 animals except in columns with superscript 2. Values in columns without common superscripts are significantly different ($P < 0.01$). Abbreviations: SO, hydrogenated soy oil; TA, beef fat; DB, dairy butter.

[1]Lesion area evaluated at 18 weeks of dietary treatment; plasma lipids evaluated at 4 weeks.

[2]Values represent means ± SE of four independent pools of plasma obtained from two different mice per each pool.

source. The two major apolipoproteins associated with the HDL particle, apolipoproteins A-I and A-II, were highly correlated to HDL-C levels in the plasma (r^2 of 0.90 and 0.70, respectively; $P < 0.0001$; Table 2).

In comparing lipid values of mice fed diets containing plant (SO, CO, CB, and PO) versus animal fat (LA, TA, and DB) sources in Table 2, combined VLDL plus LDL-C levels (50 ± 3 versus 81 ± 6 mg/dl) were significantly higher in mice consuming animal fats ($P < 0.005$) and HDL-C levels were significantly lower (37 ± 2 versus 27 ± 3 mg/dl; $P < 0.005$). No significant correlation between the percentage of saturated, monounsaturated or polyunsaturated fats and the lipid or lipoprotein parameters measured was observed. However, combined VLDL plus LDL-C tended toward positive correlation with the percentage of saturated fat and inverse relationship with the percentage of monounsaturated fat contained in the fat source. An inverse relationship was also observed between the percentage of saturated fat and HDL-C levels.

The relationship between the size of fatty streak lesions in mice fed different dietary fats and lipid parameters was tested (**Fig. 4**). No correlation between fatty streak lesion area and plasma triglyceride or plasma cholesterol was observed. However, increasing HDL-C levels were associated with decreasing lesion size in all dietary treatments except the TA-fed animals. When this outlier group was excluded from the analysis, lesion area and HDL-C levels were inversely and significantly correlated (r^2 = 0.74; $P < 0.05$). In the first experiment (Table 2), HDL-C levels for TA-fed mice were low, however, when measured in the group done to confirm lesions (Table 3), HDL-C levels of TA-fed mice fell well within the range of the regression line. The variability in HDL-C levels between these two experiments may be due to batch difference in the beef tallow used in the diet. Combined VLDL plus LDL-C levels were positively but not significantly correlated to lesion area. However, the ratio of VLDL plus LDL-C to HDL-C was positively and significantly correlated to lesion area, that is, the higher the ratio, the greater the lesion area observed (Fig. 4B; r^2 = 0.72, $P < 0.05$; the TA treatment group was excluded from the analysis).

Liver lipids after 18 weeks of consuming the high fat or low fat diets

The livers of animals consuming the high fat diets were larger than those from mice receiving the low fat diet (**Table 4**). A 6- to 8-fold accumulation of cholesterol in the livers of high fat-fed in comparison to low fat, no cholesterol-fed mice was observed. In the livers from the high fat- and cholesterol-fed mice, most of the cholesterol was in the esterified form while in the low fat-fed mice two-thirds was esterified and one-third was in free form. Liver triglyceride concentrations were significantly lower in the low fat-fed mice but no differences in triglyceride accumulation were observed among treatment groups with the different fat sources. The inverse was true of phospholipid concentrations, as low fat-fed mice had significantly higher phospholipid levels than high fat-fed mice ($P < 0.01$).

Fatty acid composition of the livers of mice fed the different diets was determined (data not shown). Despite the large differences in fatty acid composition of the fats fed to the mice, the liver fatty acid composition among groups was quite similar, suggesting a large capacity for adapting to dietary intake. Approximately 15–25% of the fatty acids were saturated, mainly in the form of palmitic acid, 50–80% were monounsaturated, mainly in the form of oleic acid, and 5–20% were polyunsaturated, mainly in the form of linoleic acid.

Because the liver is centrally involved in lipid and lipoprotein metabolism, the relationship between liver lipids and plasma lipids was examined. Liver cholesterol content was positively correlated to total cholesterol and to combined VLDL plus LDL cholesterol (r^2 = 0.66, $P < 0.025$ and r^2 = 0.74, $P < 0.01$, respectively) but inversely related to plasma HDL-C levels (r^2 = 0.56,

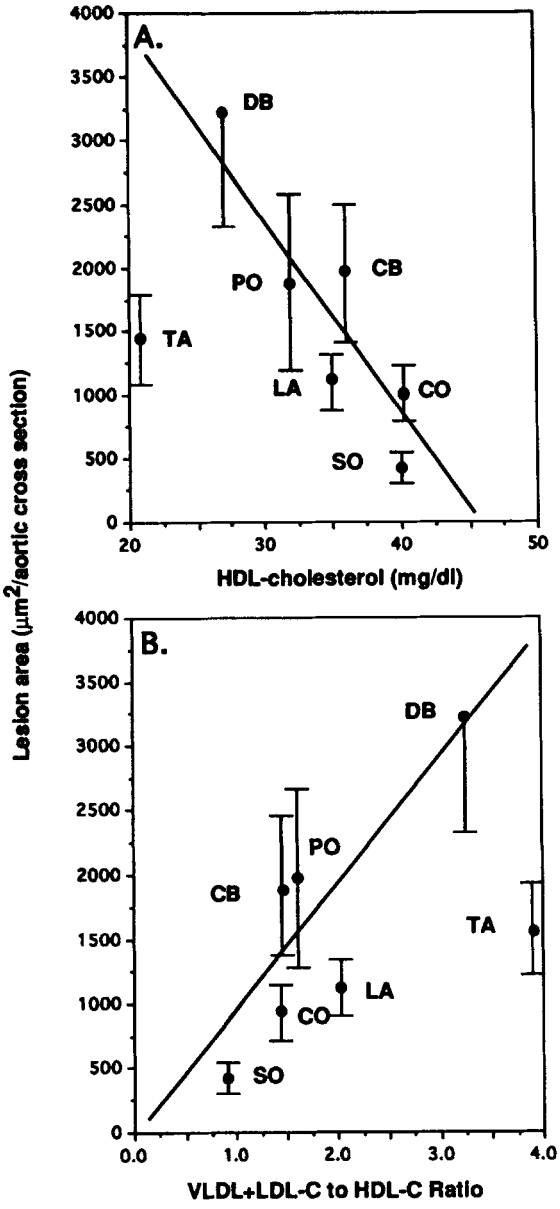

Fig. 4. Relationship between lesion area and plasma lipoprotein components. Panel A: Lesion area versus HDL-C. Panel B: Lesion area versus VLDL + LDL-C:HDL-C ratio. The result from the TA group was not used to draw the regression line as it appeared to be an outlier in both analyses. Subsequent testing in TA-fed animals showed that HDL-C levels fell well within the range of the regression line.

$P < 0.05$). Conversely, liver phospholipid content was inversely related to plasma VLDL plus LDL-C levels ($r^2 = 0.51$, $P < 0.05$) but positively correlated to plasma HDL-C and triglyceride levels ($r^2 = 0.64$, $P < 0.025$ and $r^2 = 0.77$, $P < 0.005$, respectively).

DISCUSSION

Mouse as a model for atherosclerosis

Understanding the genetic basis and the environmental factors that influence coronary artery disease, the leading cause of death in developed countries, is extremely important for designing appropriate preventive measures and therapeutic intervention strategies. From a practical viewpoint, the mouse is an ideal animal model for such studies because it is genetically defined, readily available, inexpensive to maintain, and reproduces easily. Results from this study suggest that mice may also serve as a good model for studying diet-induced alteration in plasma lipids and atherogenicity of dietary fats as results closely resembled those reported for humans and other species (6–8). However, as with any model system for human disease, some differences do exist and must be acknowledged when interpreting or extrapolating data to humans. For example, while the distribution of macronutrients used in our diets is similar to that observed in human diets (i.e., 34% fat, 47% carbohydrate, and 19% protein by caloric intake), sodium cholate and a high concentration of cholesterol are added to the diet. Based on previous and ongoing studies in our laboratory, fatty streak lesions still occur when lower levels of cholesterol and sodium cholate and different sources of carbohydrate are used; however, the length of the experiments needs to be extended considerably (15; unpublished observations). Thus, added high levels of cholesterol and cholic acid appear primarily to accelerate the disease process. Further evidence that the aortic lesions in mice are not an artifact of sodium cholate and high cholesterol feeding comes from the observation that apolipoprotein E null mutants fed a commercially prepared stock diet (low fat low cholesterol, no cholic acid) do develop fatty streak lesions spontaneously (25, 26).

Effects of high fat diets on lesion formation

Epidemiological studies have suggested that mortality from coronary artery disease (CAD) is positively correlated with saturated fat intake and negatively related to monounsaturated fat intake (5). Subsequent studies in experimental animal models such as the pig or rabbit have, in general, supported this suggestion but individual studies have shown variable responses to diet-induced hypercholesterolemia and atherogenesis. For example, Bragdon, Seller and Stevenson (27) reported that the frequency and severity of aortic lesions in pigs fed a corn oil or a butter diet were similar. In contrast, Reiser, Sorrels, and Williams (28) reported that diets containing 2% cholesterol with unsaturated fats led to a more severe atherosclerosis than with saturated fats. The variability in results has been attributed to the use of pigs that were not

TABLE 4. Comparison of liver cholesterol, triglyceride, and phospholipid in C57BL/6 mice fed diets made with different fat sources for 18 weeks

Diets	Liver Weight	Cholesterol				Triglyceride	Phospholipid
		Total	Free	Esterified	Esterified / Free		
	g	*mg/g wet weight*				*mg/g wet weight*	
RG	1.1 ± 0.05^{a}	3.9 ± 0.6^{a}	1.4 ± 0.1^{a}	2.5 ± 0.5^{a}	1.8 ± 0.2^{a}	5.6 ± 0.6^{a}	27 ± 1.3^{c}
SO	1.4 ± 0.03^{b}	22 ± 2.0^{b}	3.1 ± 0.4^{b}	19 ± 1.8^{b}	6.3 ± 0.4^{b,c,d}	15 ± 1.5^{b}	21 ± 0.5^{b}
CO	1.3 ± 0.09^{a,b}	31 ± 1.3^{d}	3.3 ± 0.4^{b,c}	28 ± 1.6^{c,d}	9.2 ± 1.7^{d}	15 ± 4.5^{b}	20 ± 1.0^{a,b}
CB	1.7 ± 0.05^{c}	28 ± 1.8^{c}	5.1 ± 0.1^{d,e}	23 ± 1.8^{c}	4.5 ± 0.3^{a,b}	10 ± 0.9^{a,b}	17 ± 0.7^{a}
PO	1.5 ± 0.03^{b,c}	35 ± 1.0^{d}	5.6 ± 0.2^{e}	29 ± 2.0^{d}	5.3 ± 0.2^{b,c}	13 ± 1.8^{a,b}	18 ± 0.4^{a,b}
LA	1.6 ± 0.06^{c}	33 ± 2.0^{c,d}	4.7 ± 0.4^{c,d,e}	29 ± 2.0^{d}	6.4 ± 0.8^{b,c,d}	13 ± 1.0^{a,b}	18 ± 0.3^{a,b}
TA	1.5 ± 0.04^{b,c}	32 ± 0.9^{c,d}	4.2 ± 0.3^{b,c,d}	28 ± 0.7^{c,d}	6.8 ± 0.3^{b,c,d}	13 ± 1.4^{a,b}	17 ± 0.5^{a}
DB	1.4 ± 0.04^{b}	34 ± 1.5^{c,d}	4.1 ± 0.4^{b,c,d}	30 ± 1.7^{d}	7.6 ± 1.0^{c,d}	12 ± 1.5^{a,b}	20 ± 0.4^{a,b}

Values represent the mean ± SE of at least three animals per group fed a high saturated fat diet for 18 weeks. Values in columns without common superscripts are significantly different, $P < 0.01$ as determined by Fisher's Least Significance Difference test. Abbreviations: RG, reference group, low fat diet containing 8% corn oil; SO, hydrogenated soy oil; CO, hydrogenated coconut oil; CB, cocoa butter; PO, hydrogenated palm oil; LA, pork fat; TA, beef fat; DB, dairy butter.

genetically defined (7). Severity of diet-induced atherosclerosis in rabbits has been shown to be inversely related to the degree of fatty acid unsaturation (29). Kritchevsky (30) reported that the extent of atherosclerosis in rabbits was greatest for coconut oil and least for corn oil. However, he also observed that atherosclerosis was more severe when diets were devoid of fat but high in cholesterol (30).

A variety of dietary fats used singly or in combination at different concentrations within a diet and the use of different levels of dietary cholesterol make comparison of these studies difficult. As no one study had systematically evaluated the atherogenicity of several dietary fats in genetically defined animals such as mice, atherosclerosis-susceptible C57BL/6J mice were fed atherogenic diets containing different dietary fats to assess their effects on aortic lesion formation (31). Commonly consumed fats from plant and animal sources were selected to cover a range of saturated, monounsaturated, and polyunsaturated compositions. A large range of responses in aortic lesion formation was observed, despite the fact that all diets contained 15% fat (w/w) and 1% cholesterol. However, as reported in the human epidemiological studies, the range of responses was positively correlated to the percentage of saturated fatty acids and inversely related to the percentage of monounsaturated fatty acids in the fat source provided in the diet. Among those receiving the plant fats, lesion area was smallest in mice consuming the hydrogenated soy oil diet (420 ± 108 μm^2/section) and largest in mice consuming the hydrogenated palm oil and cocoa butter diets (1,900 ± 530 μm^2/section). Among mice consuming saturated fats of animal origin, lesion area from least to largest was lard, tallow, and dairy butter diets. When all dietary treatment groups are compared, animals receiving the dairy butter diet developed the largest aortic lesion area. To confirm this finding, 6 months later, second sets of C57BL/6 mice were fed a dairy butter, hydrogenated soy oil, or tallow diet. Lesion areas were in the same relative order as before; DB with the largest, TA with intermediate, and SO with the smallest lesions (Table 3).

The one exception in which aortic lesion area did not correspond to % saturation or monounsaturation was mice receiving the hydrogenated coconut oil diet. Plasma lipids and lipoproteins predicted lesion area for CO more closely than fatty acid composition of the diet. This result could be explained by the fact that the saturated fats in coconut oil are in large part composed of medium chain fatty acids, which have not been associated with the deleterious effects of longer chain fatty acids such as palmitic acid. Although we did not measure oxidative modification of lipoprotein particles in our study, a possible explanation for the protective nature of monounsaturated fatty acids was offered by Bonanome et al. (32). Their experimental data suggested that plasma LDL isolated from individuals consuming diets rich in monounsaturated fatty acids were more resistant to oxidative modification, thus lowering the atherogenicity of the LDL particle.

Effects of high fat diets on plasma and liver lipids

As it is not feasible to directly measure lesions in individuals for screening purposes, serum lipid levels are often used to predict the atherogenic nature of dietary manipulations. In humans, an atherogenic profile includes elevated serum total cholesterol, LDL-C, and

triglyceride levels and reduced concentrations of HDL-C (33). A large range of diet-induced elevation in plasma total cholesterol and combined VLDL and LDL-C and a reduction in HDL-C were observed in mice consuming the high fat, high cholesterol diets. For example, plasma cholesterol levels in animals receiving the hydrogenated soy oil and cocoa butter diets were not significantly different from those observed in the reference group controls. This is presumably due to the predominantly monounsaturated nature of the soy oil and the high stearic acid content of the cocoa butter diet. Others have shown that stearic acid does not affect plasma lipids as much as do other long chain saturated fatty acids (34). The rapid conversion of stearic acid to oleic acid is suggested as the possible reason for its neutral effects (35). However, aortic lesions in CB fed mice were comparable to those consuming CO, PO, LA, and TA.

The most significant plasma lipoprotein predictor of lesion formation, in this study, was HDL-cholesterol, which was inversely related to fatty streak lesion area. In C57BL/6J mice, HDL-C levels are determined by two genes *Ath-1* and *Ath-2*. The type of dietary fat obviously interacted with these genetic components and elicited a range of responses in these mice. Although a statistically significant relationship between the fatty acids and HDL-C levels was not observed, it was noted that HDL-C, apoA-I, and apoA-II levels were positively correlated to the monounsaturated fat content and negatively correlated to the saturated fat content of the fat sources. Osada et al. (36) showed that rats fed monounsaturated fats had higher liver apoA-I mRNA levels when compared to corn oil- or coconut oil-fed rats. Perhaps the positive effects of monounsaturated fatty acids are mediated through plasma lipoproteins, such as HDL and LDL. Unlike in studies of nonhuman primates, an inverse relationship between polyunsaturated fatty acids and HDL levels was not observed (37). This may have been due to the very low levels of polyunsaturated fatty acids contained in the diets.

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