Atherosclerosis and Plasma and Liver Lipids in Nine Inbred Strains of Mice

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Nine inbred strains of mice, which are progenitors of recombinant inbred sets, were evaluated for aortic lesion formation and plasma and liver lipid levels. This survey was done to determine if a semi-synthetic high-fat diet could elicit the same extent of diet-induced atherosclerosis as that observed in mice fed a natural ingredient high-fat diet and to discover strain-specific plasma and liver lipid variants for future genetic characterization. Evaluation of aortic lesions after 18 wk of diet consumption showed that strains C57BL/6J, C57L/J, SWR/J and SM/J were susceptible to atherosclerosis and that A/J, AKR/J, C3H/HeJ, DBA/2J and SJL/J were relatively resistant. High-density lipoprotein cholesterol (HDL-C) levels were negatively correlated to lesion formation. Susceptible strains had decreased HDL-C levels when switched from chow to the semi-synthetic high-fat, high cholesterol diet, whereas resistant strains either showed no change or a slight increase in HDL-C levels. The exception to this pattern was found in SM mice, which were susceptible to aortic lesion formation but maintained the same HDL-C level on both chow and high-fat diets. HDL size differed among the strains, and levels of plasma apolipoprotein A-I and A-II correlated with HDL-C levels. Liver damage was not correlated to HDL-C levels or to susceptibility to atherosclerosis. Mice from strain A, which are resistant to atherosclerosis, had evidence of liver damage as observed by elevated levels of plasma alanine aminotransferase activity, by liver histology, by increased liver weight and by exceptionally high hepatic cholesterol content. For all strains, the levels of liver cholesterol and triglycerides were inversely correlated with each other; phospholipids did not vary greatly among strains. No remarkable differences in hepatic fatty acid profile were noted among the strains fed the atherogenic diet, but the fatty acid profile did differ considerably from that found in the diet itself.

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The mouse model is proving to be an invaluable tool for identifying genetic factors that affect heart disease and lipid metabolism because of the extensive genetic characterization of the inbred mouse strains (1), the special tools available, such as congenic, transgenic and recombinant inbred strains, the reproducible method for measuring aortic lesions (2) and the adaptation of methods to quantitate lipids in small blood volumes available from a single mouse. Previously, inbred strains of mice have been shown to differ in susceptibility to atherosclerosis when fed a natural grain diet high in saturated fat and cholesterol. The diet which was used in these studies was not chemically defined, and measurements of combined plasma very low density lipoprotein (VLDL) and low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) which have been shown to be associated with heart disease risk in humans were not done (3-5).

In this report, we sought to further develop the mouse model for the study of heart disease and lipoprotein metabolism. The semi-synthetic atherogenic diet which was designed to produce fatty streak lesions but minimize pathological changes in liver and gallstone formation in strain C57BL/6 is utilized in this study (6). However, cocoa butter was replaced by dairy butter as the fat source. The first question we asked was whether susceptibility to atherosclerosis would remain the same in nine inbred strains of mice fed a semi-synthetic high-fat and cholesterol diet rather than the natural ingredient diet used previously. Four susceptible strains (C57BL/6, C57/L, SWR and SM) and five resistant strains (A, DBA/2, AKR, C3H/He and SJL) were chosen for this study. These inbred strains of mice are progenitors of recombinant inbred sets. Secondly, we measured baseline differences in plasma lipid parameters and subsequent changes in these parameters in response to high-fat feeding in order to identify genetic variants in progenitors that could be further characterized and eventually mapped using recombinant inbred strains. In addition, because the liver is intimately involved in lipoprotein metabolism, the composition and levels of liver lipids were determined. The possible associations of the plasma and liver lipids with atherosclerosis susceptibility were also examined.

MATERIALS AND METHODS

Chemicals and diet. Cholesterol oxidase, bovine pancreas cholesteryl ester hydrolase, peroxidase and the plasma alanine aminotransferase assay kit were from Sigma Chemical Co. (St. Louis, MO); the cholesterol reagent kit No. 23691 was from Boehringer Mannheim (Indianapolis, IN); the triglcyeride reagent set No. 46676 was from Seradyn Inc. (Indianapolis, IN); triglyceride blank blend No. 10021 was from Craig Bioproducts (Streamwood, IL); chemicals for gel electrophoresis were from Bio-Rad Laboratories (Richmond, CA); gradient gels (4-30% polyacrylamide) were from Pharmacia (Piscataway, NJ); Oil red O was from Aldrich Chemical Company (Milwaukee, WI); hematoxylin and light green were from Fisher Scientific Co. (Dallas, TX); gentamycin sulfate was from U.S. Biochemical Corp. (Cleveland, OH); and fatty acid methyl ester mixtures were from Supelco (Bellefonte, PA). Other chemicals utilized were of reagent grade or better. Components for the purified diets were purchased from ICN

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Abbreviations: ALT, alanine aminotransferase; apo A-I, apolipoprotein A-I; apo-II, apolipoprotein A-II; BW, body weight; EDTA, ethylenediaminetetraacetic acid; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; VLDL, very low density lipoprotein.

Biochemicals, Inc. (Cosa Mesa, CA) with the exception of Mazola corn oil (Best Foods, Englewood Cliffs, NJ). Laboratory rat chow (No. 5012) containing 4% fat was obtained from Ralston Purina (St. Louis, MO).

Preparation of diets and formation of the diet mix into pellets has been described previously (6). The high saturated fat diet contained 15% dairy 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. Of the 1% cholesterol contained in the diet, 0.03% came from the dairy butter. In order to obtain 15% dairy butter, 18.45 g butter was used/100 g diet because of the 18.9% water content of butter.

Animals. Female C57BL/6J (C57BL/6), C57/LJ (C57/L), SWR/J (SWR), SM/J (SM), A/J (A), AKR/J (AKR), C3H/HeJ (C3H), DBA/2J (DBA/2) and SJL/J (SJL) mice, 4–8-wk-old, were obtained from The Jackson Laboratory (Bar Harbor, ME) and maintained in a room illuminated from 7 a.m. through 7 p.m. All animals were allowed to adapt to the environment for at least two weeks prior to dietary treatment and were provided free access to food and water throughout the experiment. Weight gain was monitored every three weeks, and food intake was monitored at weeks 3, 7 and 14. Six female mice were used from each strain for plasma lipid analysis and three were used for liver lipid analysis. After 18 wk of diet consumption, 0 of 6 mice from strains DBA/2, C57BL/6, SWR, C57L and A, 1 of 6 mice from strains SM, SJL and C3H and 3 of 6 mice from strain AKR had died. The reason for death in strains SM, SJL and C3H is unknown. The AKR mice died from exposure due to a water bottle malfunction.

Collection of blood and tissues. Prior to any blood or tissue collection, mice were fasted by removing their food at 5 p.m. on the day prior to the experiment. Blood was obtained from the tail vein of the mice after the two-week adaptation period for baseline measurements, and again after four weeks of consuming the semi-synthetic high-fat and cholesterol diet. At the termination of the experiment, blood was obtained from the brachial artery. Blood was mixed in chilled tubes with ethylenediaminetetraacetic acid (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 $\times$ *g* at 4°C.

Upon termination of the experiments, the heart and the upper section of the aorta were removed and placed in 0.9% saline at room temperature. After one hour, the heart was trimmed of extraneous tissue and placed in 10% saline buffered formalin for 24 h at room temperature. Livers were blotted, quickly frozen in liquid nitrogen and stored at −70°C for lipid analysis. A portion of the liver was placed in a Bouin's fixative, embedded in paraffin wax, sectioned and stained with hematoxylin and eosin. Necropsy observations concerning the stomach and intestines were recorded.

Plasma lipids, lipoproteins and apolipoproteins. Plasma total cholesterol (TC), free glycerol and total glycerol were determined by commercial colorimetric enzymatic assay. HDL-C was measured after selective precipitation of LDL and VLDL with polyethylene glycol (7). The combined 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 (apo A-I) and apolipoprotein A-II (apo A-II) were estimated by radial immunodiffusion (8). Purified mouse apo A-I and apo A-II served as standards.

Total plasma lipoproteins ($d < 1.21$) were obtained by density ultracentrifugation of pooled plasma from five mice of each strain (9) and subjected to nondenaturing gradient gel electrophoresis (10). Gels were stained with Coomassie blue G-250 to visualize HDL.

Liver lipids and plasma alanine aminotransferase (ALT) activity. Lipids, extracted from livers by the method of Folch *et al.* (11), were assayed for triglyceride (12), cholesterol (13) and phosphorus (14). 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. Plasma ALT activity was measured using the Sigma Kit No. DG591K which is based upon a coupled reaction in which nicotinamide adenine dinucleotide is oxidized.

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

Evaluation of atherosclerotic lesions. Evaluation of aortic lesions has been described in detail by Paigen *et al.* (2). 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 lipid and 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) was used for statistical analysis. 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 $P < 0.01$. Unpaired *t*-test was used when only two groups were compared. Correlation between aortic lesions and lipid parameters measured were tested by linear regression analysis.

RESULTS

Atherosclerosis susceptibility. The susceptibility to lesion formation is similar in the nine inbred strains of mice fed the semi-synthetic high-dairy-fat diet to that reported earlier (5), when these same strains were fed a natural ingredient diet with cocoa butter as the fat source (Fig. 1).

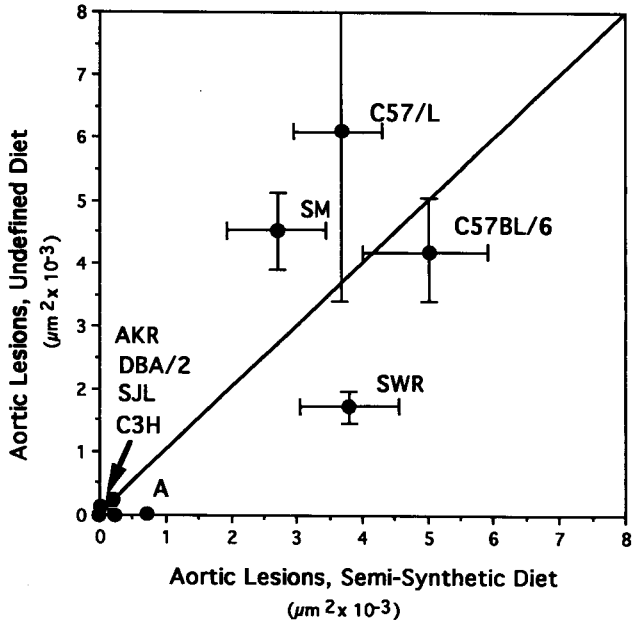

FIG. 1. Comparison of aortic lesions in nine inbred strains fed two different atherogenic diets. The aortic lesion sizes are expressed as the mean size per cross section in $\mu m^2 \times 10^3$. Lesion data for the natural ingredient diet are from Reference 5.

C57BL/6, C57L, SWR and SM are susceptible to lesion formation while the remaining five strains, namely A, AKR, C3H, DBA/2 and SJL, are relatively resistant to atherosclerosis. This difference among strains could not be accounted for by food consumption and weight gain (Table 1) as these parameters were not significantly different from that expected based on the relative sizes of these inbred strains maintained at The Jackson Laboratory. Therefore, the reduction in dietary cholesterol from 1.25% in the undefined diet to 1% in the semi-synthetic diet and the change in the source of fat from cocoa to dairy butter did not affect susceptibility to aortic lesion formation. The intra-strain variability and extent of lesion area was also similar between the two experiments. The one exception was strain SWR, where the lesion area was larger in mice consuming the semi-synthetic rather than the undefined diet (3804 ± 741 *vs.* 1690 ± 280 μm^2, respectively).

TABLE 1

Initial Body Weight, Body Weight Gained and Food Intake of Mice Fed a High-Saturated Fat Diet[a]

Strains	Body weight (g)			Food intake[b] g/d/20 g BW
	Initial	Final	% Normal	
C57BL/6	19.0 ± 0.5	22.4 ± 0.6	100	1.8
C57L	17.4 ± 0.6	24.2 ± 0.9	99	2.7
SWR	18.6 ± 0.6	23.9 ± 0.4	n.a.[c]	2.2
SM	13.8 ± 0.8	20.4 ± 1.0	100	2.7
A	18.0 ± 1.1	21.0 ± 1.0	75[d]	2.3
DBA/2	20.8 ± 0.4	28.7 ± 0.7	104	2.0
AKR	26.9 ± 1.2	44.3 ± 1.6	138	2.2
C3H	21.2 ± 0.8	32.9 ± 1.1	102	2.0
SJL	20.7 ± 0.8	24.9 ± 1.3	92	2.4

[a]Values represent the mean ± SE of at least five animals per group at 4 wk of high-fat diet consumption and at least three animals per group at 18 wk.
[b]Food intake was estimated by weighing food disappearance over a 3- to 5-d period; the values represent the mean of food consumed per 20 g body weight (BW) of mouse per day during weeks 3 through 4 of the experiment.
[c]n.a. = Not available.
[d]This strain lost weight during the last six weeks of the study, presumably because their livers were damaged.

Baseline plasma lipid levels in the nine inbred strains of mice. Significant variation in basal plasma lipids (combined triglyceride and cholesterol) was noted among the strains studied (Fig. 2). Plasma triglyceride levels ranged from 26 ± 3 mg/dL in strain C57/L to 68 ± 5 mg/dL in strain SWR. Plasma cholesterol levels ranged from 45 ± 4 mg/dL in C57/L to 106 ± 4 mg/dL in C3H mice (Fig. 2B). As observed by others (15), the majority of cholesterol in mouse plasma, 71 to 87%, is found in the HDL fraction (Fig. 2C) with 13 to 29% in the VLDL + LDL fraction (Fig. 2D).

Plasma lipid levels in mice fed a diet high in fat and cholesterol for four weeks. Fasting total triglyceride levels did not reflect the high-fat content of the diet (*i.e.* 15% w/w) fed to the mice (Fig. 2A). Triglyceride levels either did not change from basal levels in strains C57BL/6, C57L, SWR, A, AKR, C3H and SJL, or significantly decreased by 38% in SM and 39% in DBA/2.

Unlike plasma triglyceride, total plasma cholesterol levels increased significantly in all strains fed the semi-synthetic atherogenic diet (Fig. 2B). The level of increase ranged from 131 to 295% over baseline values. The majority of the increase in plasma cholesterol could be found in the VLDL + LDL fraction (Fig. 2D). VLDL + LDL-C levels of the nine inbred strains clustered roughly into two groups with C57BL/6, SM, DBA/2, AKR and SJL having levels from 47 ± 4 to 67 ± 19 mg/dL and C57/L, SWR, A and C3H having levels from 94 ± 10 to 132 ± 10 mg/dL (Fig. 2D). HDL-C either decreased significantly from baseline values in strains C57BL/6, C57/L and SWR or showed no significant change in the remaining strains. The decrease in SWR, C57BL/6 and C57/L mice was 19, 32 and 37%, respectively (Fig. 2C).

Liver lipid levels in mice fed the atherogenic diet for 18 wk. Liver weights varied from 1.2 to 3.9 g/animal. When liver weight was normalized (g/100 g body weight) to account for differences in body size, strain A was exceptional in that its liver was more than twice the size observed in other strains. On gross examination, the livers of the animals from all strains fed the semi-synthetic high-fat diet tended to be paler than normal compared to animals fed laboratory chow. C57L, DBA/2 and SJL had livers that were closest to normal in color. This group was followed by strains C57BL/6, C3H, SM, SWR and AKR, which had paler-colored livers. The livers of strain A were chalky white and rigid. In general, the strains with the lowest levels of hepatic cholesterol were most normal in appearance, and as the hepatic cholesterol content of the liver increased, the livers became paler in color. Histological examination of livers (Fig. 3) were consistent with this interpretation. Lipid accumulation observed in the liver as clear areas in the cytosol was least in C57/L (Fig. 3, Panel B), intermediate in C57BL/6 (Fig. 3, Panel C), and most in strain A (Fig. 3, Panel D). As a gross indicator of liver damage, plasma ALT activity was measured. The

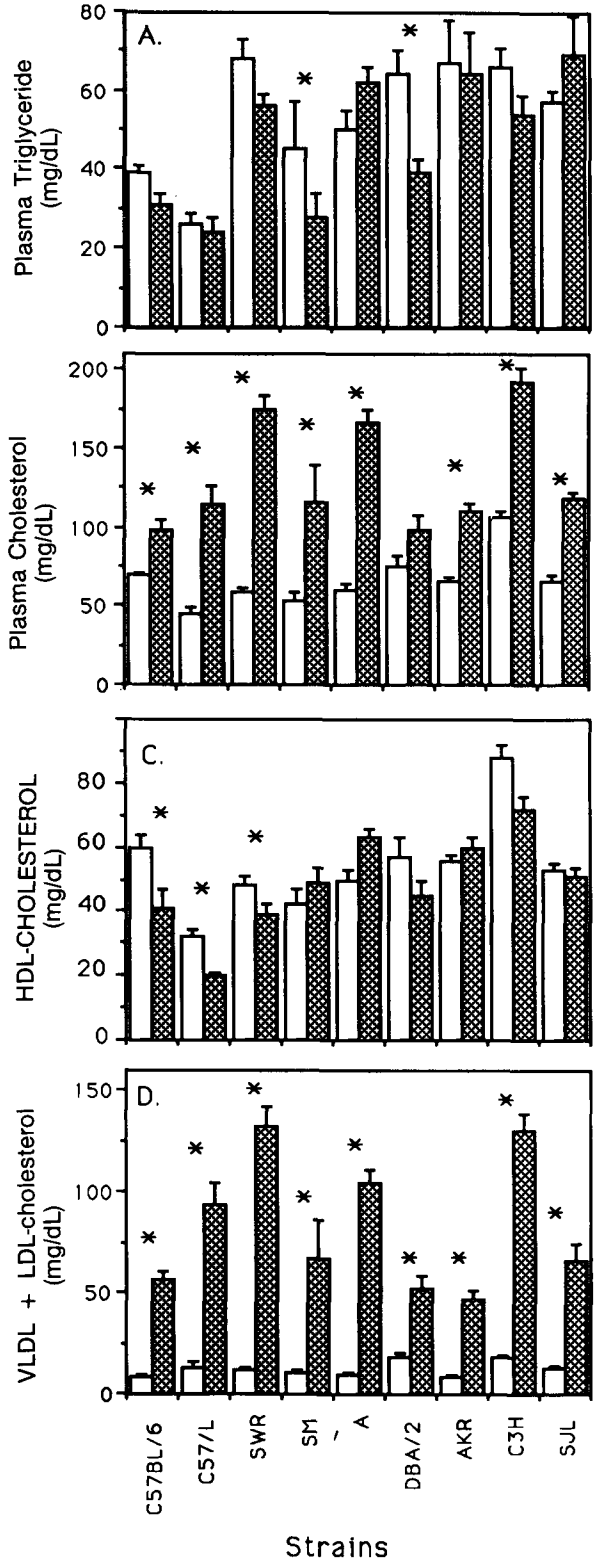

FIG. 2. Comparison of basal and high-fat levels of plasma triglycerides (A), cholesterol (B), high-density lipoprotein (HDL)-cholesterol (C) and combined very low density lipoprotein (VLDL) and low-density lipoprotein (LDL)-cholesterol (D) in nine inbred strains. The solid bars represent mean lipid and lipoprotein levels in mg/dL ± SE in the basal state, and the gray bars represent mean lipid and lipoprotein levels in mg/dL ± SE for animals fed the semi-synthetic atherogenic diet for four weeks. Significance ($P < 0.05$) between basal and high-fat values is indicated by a star above the bars.

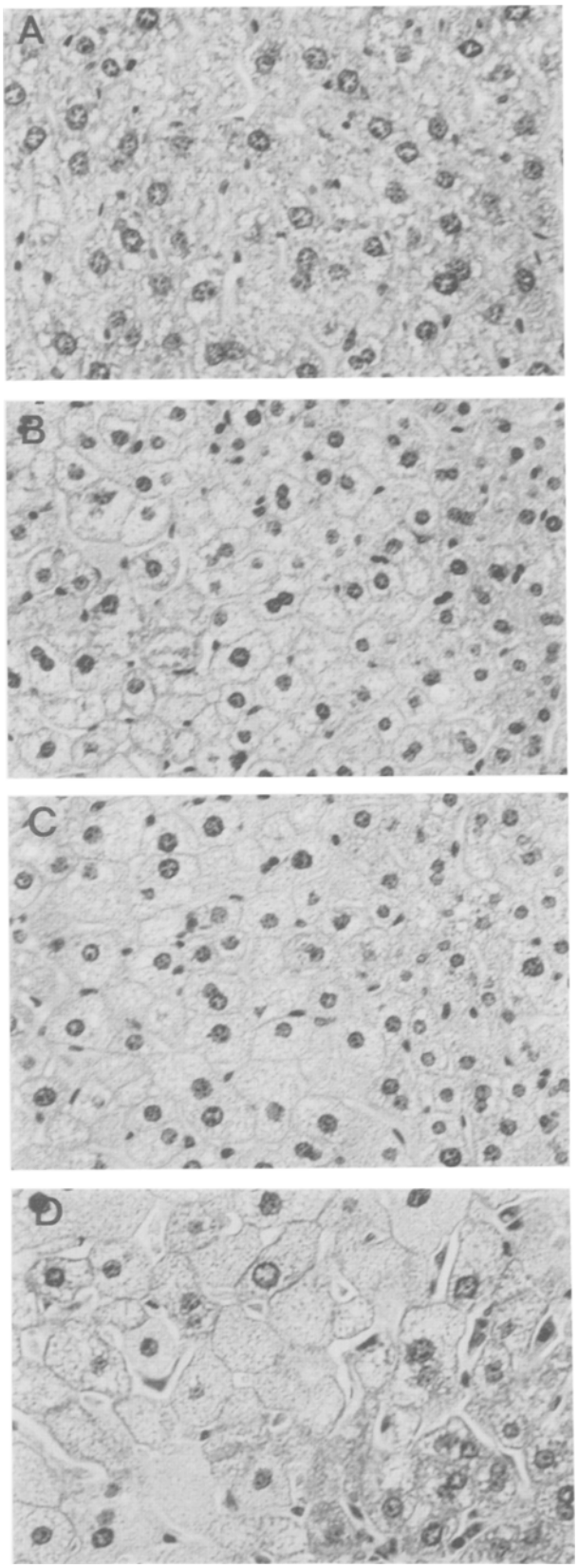

FIG. 3. Liver sections from mice fed chow (A) or the semi-synthetic diet for 7 wk (B–D) stained with hemotoxylin and eosin, 368×. Strain A (A), C57L (B), C57BL/6 (C), and Strain A (D).

TABLE 2

Comparison of Liver Weights and Plasma Alanine Aminotransferase (ALT) Activity in Nine Strains of Mice Fed a High-Saturated Fat Diet (for 18 wk)[a]

Strains	Liver weight		ALT activity[b]
	g/animal	g/100 g BW	μmol/min/mL
C57BL/6	1.5 ± 0.1[c]	6.8 ± 0.4[c,d]	55 ± 12[c,d]
C57L	1.4 ± 0.1[c]	5.9 ± 0.1[c]	29 ± 2[c]
SWR	2.1 ± 0.1[d,e]	8.9 ± 0.3[d]	105 ± 24[d]
SM	1.2 ± 0.1[c]	5.8 ± 0.8[c]	45 ± 16[c,d]
A	3.9 ± 0.1[f]	18.6 ± 0.9[e]	479 ± 28[e]
DBA/2	1.3 ± 0.1[c]	4.4 ± 0.2[c]	67 ± 19[c,d]
AKR	2.6 ± 0.1[e]	5.8 ± 0.2[c]	32 ± 11[c]
C3H	2.1 ± 0.1[d]	6.3 ± 0.2[c,d]	55 ± 8[c,d]
SJL	2.0 ± 0.2[d]	8.2 ± 0.4[d]	68 ± 15[c,d]

[a]Values represent the mean ± SE of at least three animals per group fed a high-saturated fat diet for 18 wk. Values in columns without common superscripts are significantly different, $P < 0.01$. [b]Animals that are fed chow or a low-fat synthetic diet have plasma ALT levels of 16.6 ± 3.1 or 30 ± 4 μmol/min/mL, respectively (Nishina, P.M., unpublished data).

two strains with the highest liver weight/100 g body weight, A and SWR, also had the highest ALT activity (479 and 105 μmol/min/mL, respectively), and both of these strains also had accumulated an extensive number of gallstones. Except for these two strains, plasma ALT levels were not remarkably elevated compared to ALT levels (17 ± 2 μmol/min/mL) measured in chow-fed C57BL/6 mice (Table 2).

Triglyceride, cholesterol and phospholipids were measured in order to determine what lipids were accumulating in the livers (Table 3). Liver cholesterol in C57BL/6 animals fed a low-fat diet is 5 ± 0.2 mg/g liver (6). The liver cholesterol of strains fed the high-fat, high-cholesterol diet ranged from 20 to 63 mg/g liver with strains DBA/2 and C57L accumulating the least, and strains C57BL/6 and A having the most cholesterol. The distribution of cholesterol into free and esterified cholesterol was examined; no significant differences among the strains were observed, and most cholesterol was of the esterified form with only 9 to 13% as free cholesterol (data not shown). Liver triglyceride levels ranged from 3 ± 1 to 24 ± 1 mg/g liver among the different strains. Strain A, which had the highest liver cholesterol concentration, had the lowest concentration of liver triglycerides. The accumulation of liver cholesterol in strain A mice fed a high-fat and high-cholesterol diet has been noted previously (16). Phospholipids, which are necessary for assembly of lipoproteins for secretion from the liver, were less variable than other lipids among strains. Correlation analysis of liver phospholipids, triglyceride or cholesterol showed that concentrations of phospholipids and cholesterol were negatively correlated ($r = -0.90$, $P < 0.01$) among the strains. This suggests that cholesterol may accumulate in livers which have lower concentrations of phospholipid. No correlation was found between plasma lipoproteins and any of the measured hepatic lipid concentrations after 18 wk of receiving the high-fat diet.

TABLE 3

Comparison of Liver Cholesterol, Triglyceride and Phospholipid (mg/g liver) in Nine Strains of Mice Fed a High-Saturated Fat Diet (for 18 wk)[a]

Strains	Cholesterol		Triglyceride	Phospholipid
	Total	Esterified/ free ratio		
C57BL/6	50 ± 4[c]	11 ± 0.5	14 ± 1.4[c,d]	20 ± 0.8[b]
C57L	29 ± 2[b]	7.7 ± 0.5	10 ± 1.9[b,c]	26 ± 1.2[c]
SWR	39 ± 2[c]	8.8 ± 0.6	5.8 ± 0.9[b]	22 ± 0.4[b]
SM	45 ± 6[c]	9.2 ± 0.4	24 ± 1.1[d]	24 ± 2.1[b,c]
A	63 ± 2[d]	7.7 ± 0.5	3.3 ± 0.8[b]	19 ± 0.4[b]
DBA/2	20 ± 1[b]	6.5 ± 0.2	20 ± 1.8[d]	26 ± 0.8[c]
AKR	40 ± 2[c]	8.8 ± 1.0	4 ± 1.7[d]	23 ± 0.3[b,c]
C3H	42 ± 2[c]	9.8 ± 0.3	13 ± 0.6[c]	22 ± 0.4[b,c]
SJL	39 ± 2[c]	8.9 ± 0.4	16 ± 2.6[c,d]	24 ± 0.9[b,c]

[a]Values represent the mean ± SE of at least three animals per group fed a high-saturated fat diet for 18 wk. Values in columns without common superscripts are significantly different, $P < 0.01$, as determined by Fisher's Least Significance Difference test.

In order to determine whether liver fatty acid profiles differed among the nine strains, livers from a minimum of three animals per strain were pooled and analyzed (data not shown[2]). The liver fatty acid profiles did not differ among the strains fed the high-fat diet; approximately 15 to 25% of the fatty acids were saturated, mainly in the form of palmitic acid, 50 to 80% were monounsaturated, mainly in the form of oleic acid, and 5 to 20% were polyunsaturated, mainly in the form of linoleic acid. However, the fatty acid profiles found in livers did differ considerably from those found in the diet where 73% of the fatty acids were saturated, 25% monounsaturated and 2% polyunsaturated.

Relationships between atherosclerosis susceptibility and plasma and liver lipids. Previous studies in mice have shown that the resistance to atherosclerosis cosegregates with elevated levels of HDL-C in crosses or in recombinant inbred strains derived from the susceptible strain C57BL/6 and the resistant strains C3H, BALB/c or A (17-19). In this survey, HDL-C levels in mice receiving the atherogenic diet were inversely related to aortic lesions size ($r^2 = -0.49$, $P < 0.05$). No other significant correlations were observed between aortic lesion formation and plasma or liver lipid levels.

Because of the importance of HDL-C levels in determining resistance to atherosclerosis, the possible biochemical differences in the HDL particles among the inbred strains were examined. The major apolipoproteins associated with these particles, A-I and A-II, were measured. The changes in HDL-C were mirrored by changes in apo A-I and A-II. The correlation among HDL-C, apo A-I and A-II was high; the correlation between HDL-C and apo A-I was 0.79 ($P < 0.0001$, Fig. 4B), between HDL-C and apo A-II was 0.70 ($P < 0.0001$, data not shown) and between apo A-I and apo A-II was 0.64 ($P < 0.0001$, Fig. 4A). Overall, the ratio between these apolipoproteins and HDL-C did not change significantly in inbred mice, whether fed a chow or a high-fat diet. Considerable variation in peak HDL particle sizes occurred among the mouse strains with no obvious correlation to atherosclerosis susceptibility or resistance (data not shown).

[2]A complete liver fatty acid profile of the nine strains of mice fed the semi-synthetic atherogenic diet will be supplied upon request.

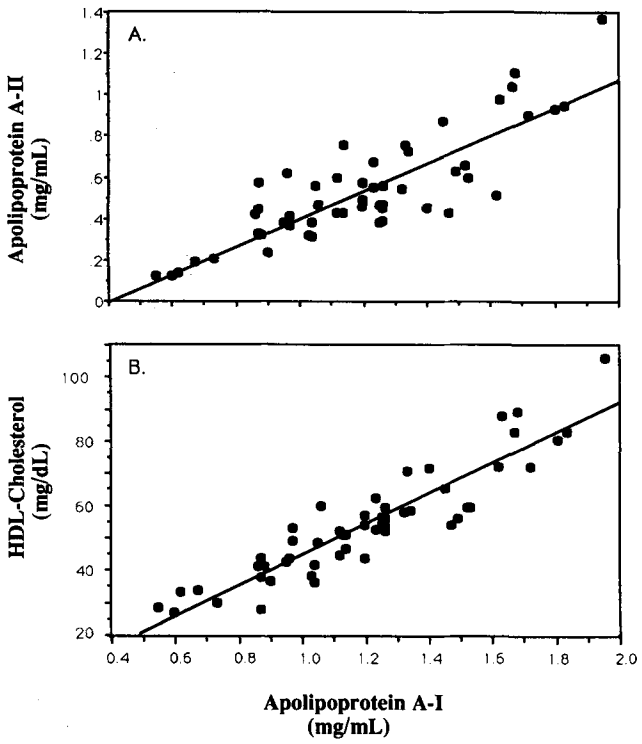

FIG. 4. Correlation analysis of apolipoproteins and high-density lipoprotein (HDL)-cholesterol in mice fed the semi-synthetic diet for four weeks. Panel A. Correlation of apolipoprotein A-II *vs.* apolipoprotein A-I. Panel B. Correlation of HDL-cholesterol *vs.* apolipoprotein A-I.

DISCUSSION

Atherosclerosis susceptibility. The relative susceptibility of nine inbred strains of mice to formation of fatty streak lesions in the aorta did not change when substantial changes were made in the atherogenic diet. The diet which we previously had used contained natural ingredients such as oats and corn, 1.25% cholesterol and cocoa butter as the primary fat source; the diet used in this study was a semi-synthetic diet with 1% cholesterol and dairy butter as the major fat source (5,6). Both diets contain 0.5% sodium cholate (w/w). The fact that the relative susceptibility to lesions did not change with diet indicates that previous conclusions about the genes that affect susceptibility to atherosclerotic lesion formation are applicable to more than one diet. However, it is interesting to note that diet can have an effect on the severity of the disease. Aortic lesion area was twice as large in SWR mice fed the semi-synthetic diet as compared to the undefined atherogenic diet.

The changes in VLDL + LDL-cholesterol and HDL-cholesterol are less than previously reported for C57BL/6 animals fed the natural ingredient atherogenic diet (5). The most likely explanation is the 20% decrease in cholesterol content of the semi-synthetic diet. Consistent with the less dramatic changes in lipoproteins, the length of time which animals needed to be fed the semi-synthetic diet to produce lesions of comparable size as the natural ingredient diet was 18 rather than 14 wk. However, the semi-synthetic diet has considerable advantages over the previous diet because it is defined and dietary components thought to affect development of heart disease can be varied one at a time to determine specific effects. In addition, the semi-synthetic diet produces fewer pathological changes in the liver and a decrease in gallstone formation (6); these pathologies might be further reduced by decreasing the cholesterol and or sodium cholate content of the diet. However, such a reduction would most probably increase the experimental time required to produce aortic lesions.

Liver lipids in response to high-fat diet. Correlation analysis showed no indication of a relationship between liver damage, measured by plasma ALT activity, and a decrease in HDL-C levels or atherosclerosis susceptibility. The disassociation of these two processes is most clearly demonstrated in strain A which shows no drop in HDL-C levels and the greatest liver damage, and strain C57/L, which has the lowest HDL-C and little liver damage when switched from a low- to high-fat diet.

The liver damage observed in these strains is consistent with the histopathology and appears to be associated with the accumulation of cholesterol in the liver. Undoubtedly, the cholesterol accumulated in the liver, in large part, arises from diet consumption. However, *de novo* hepatic cholesterol synthesis may also play a role in Strain A, which exhibits a liver threefold larger than other strains. HMG-CoA reductase activity, the rate-limiting enzyme in cholesterol synthesis, is not diminished when Strain A is switched from a low- to high-fat and cholesterol diet, suggesting that cholesterol synthesis is not down-regulated (Ref. 20; and Paigin, B., Chen, H., and Billheimer, H., unpublished observations). The combination of exogenous and endogenous cholesterol coupled with the lower phospholipid content of the liver may lead to the extreme accumulation of liver cholesterol in Strain A.

Genetic variants in plasma lipids, basal levels and response to high-fat diet. Many aspects of plasma lipids are similar between humans and mice, such as apolipoprotein and lipid composition of the lipoprotein particles and the responses observed with dietary manipulation. Therefore, the mouse model, with its unique advantages for dissecting genetic determinants, may be useful in identifying heritable factors that control lipid metabolism. Differences between humans and mice do occur, such as in the relative proportions of atherogenic lipoprotein particles. In humans, LDL predominates and in mice VLDL predominates (21), presumably because mice lack cholesterol ester transfer activity (22).

The three major observations of this study, which may warrant further investigation, were made when animals were challenged with the high-fat and high-cholesterol diet. The first observation is the lack of change in plasma triglyceride levels in some animals when fed the high-fat diet. A decrease in fasting plasma triglyceride levels would be expected as a result of the dietary fat because long-chain fatty acids inhibit *de novo* lipogenesis (23). This drop occurred in two strains, SM and DBA/2. Srivastava *et al.* (24) also reported similar results; a decrease in plasma triglyceride levels in DBA/2 animals and no change from basal levels in strains C57BL/6, C57/L, SWR and C3H/He. They did, however, see a significant decrease in plasma triglyceride levels in AKR mice when switched from a low fat diet to a semi-synthetic diet containing 21% fat and 2% cholesterol with no cholic acid. The difference in

response among mouse strains to dietary fat and cholesterol intake on plasma triglyceride levels needs to be studied further.

The second interesting difference among the strains was the hyper- or hyporesponsiveness observed in plasma cholesterol levels. This difference in response to a high-fat/high-cholesterol diet has been observed in other species such as primates, rabbits and rats (25) as well as in mice (24,26). As in other species, the changes in plasma cholesterol are due to an increase in cholesterol contained in the VLDL and LDL fraction. In our studies, strains C57L and SWR were hyper-responders and AKR and SJL were hyporesponders. These strains are progenitors of recombinant inbred sets, C57L × AKR and SWR × SJL. The responsiveness of combined VLDL and LDL to dietary lipid in these recombinant inbred sets could be used to identify the genetic determinants involved.

The third observation of interest was the failure of the HDL-C levels to decrease in SM mice fed the semi-synthetic atherogenic diet. A hallmark of susceptibility for aortic lesion development in mice studied thus far is a marked reduction in plasma HDL-C levels (17,18). SM mice, which were susceptible to aortic lesions, had basal HDL-C levels of 42 ± 5 mg/dL and of 59 ± 5 mg/dL after four weeks of consuming the atherogenic diet. Normally, the lack of change or slight increase in HDL-C levels upon high-fat feeding is associated with atherosclerosis-resistant strains of mice such as C3H or A.

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REFERENCES

1. Lyons, M.F., and Searle, A.G. (eds.) (1989) *Genetic Variants and Strains of the Laboratory Mouse*, 2nd edn., pp. 632-648, Oxford University Press, New York.
2. Paigen, B., Morrow, A., Holmes, P.A., Mitchell, D., and Williams, R.A. (1987) *Atherosclerosis 68*, 231-240.
3. Roberts, A., and Thompson, J. (1977) *Progr. Biochem. Pharmacol. 13*, 298-308.
4. Paigen, B., Morrow, A., Brandon, C., Mitchell, D., and Holmes, P. (1985) *Atherosclerosis 57*, 65-73.
5. Paigen, B., Ishida, B.Y., Verstuyft, J., Winters, R.B., and Albee, D. (1990) *Arteriosclerosis 10*, 316-323.
6. Nishina, P.M., Verstuyft, J., and Paigen, B. (1990) *J. Lipid Res. 31*, 859-869.
7. Izzo, C., Grillo, F., and Murado, E. (1981) *Clin. Chem. 27*, 371-374.
8. Mancini, G., Carbonara, A.O., and Heremans, J.F. (1965) *Immunochem. 2*, 235-254.
9. Lindgren, F.T. (1975) in *Analyses of Lipids and Lipoproteins* (Perkins, E.G., ed.) pp. 204-224, American Oil Chemists' Society, Champaign.
10. Blanche, P.G., Gong, G.L., Forte, T.M. and Nichols, A.U. (1981) *Biochim. Biophys. Acta 665*, 408-419.
11. Folch, J., Lees, M., and Sloane-Stanley, G.H. (1957) *J. Biol. Chem. 226*, 497-509.
12. Fletcher, M.J. (1968) *Clin. Chim. Acta 22*, 393-397.
13. Allain, C.C., Poon, L.S., Chang, C.S.G., Richmond, W., and Fu, P.C. (1974) *Clin. Chem. 20*, 470-475.
14. Rouser, G. (1970) *Lipids 5*, 494-496.
15. Aubert, R., Perdereau, D., Roubiscoul, M., Herzog, J., and Lemonnier, D. (1988) *Lipids 23*, 48-54.
16. Stewart-Phillips, J.L., Lough, J., and Skamene, E. (1989) *Clin. Invest. Med. 12*, 121-126.
17. Paigen, B., Mitchell, D., Reue, K., Morrow, A., Lusis, A.J., and LeBoeuf, R.C. (1987) *Proc. Natl. Acad. Sci. USA 84*, 3763-3767.
18. Paigen, B., Nesbitt, M.N., Mitchell, D., Albee, D., and LeBoeuf, R.C. (1989) *Genetics 122*, 163-168.
19. Rubin, E.M., Krauss, R.M., Spangler, E.A., Verstuyft, J.G., and Clift, S.M. (1991) *Nature 353*, 265-269.
20. Hwa, J.J., Zollman, S., Warden, C.H., Taylor, B.A., Edwards, P.A., Fogelman, A.M., and Lusis, A.J. (1992) *J. Lipid Res. 33*, 711-725.
21. Ishida, B.Y., Blanche, P.J., Nichols, A.V., and Yashar, M. (1991) *J. Lipid Res. 32*, 559-568.
22. Jiao, S., Cole, T., Kitchens, T.T., Pfleger, B., and Schonfeld, G. (1990) *Metabolism 39*, 155-160.
23. Woollett, L.A., Spady, B.K., and Dietschy, J.M. (1989) *J. Clin. Invest. 84*, 119-128.
24. Srivastava, R.A.K., Jiao, S., Tang, J., Pfleger, B.A., Kitchens, R.T., and Schonfeild, G. (1991) *Biochim. Biophys. Acta 1086*, 29-43.
25. Beynen, A.C., Katan, M.B., and Van Zutphen, L.F.M. (1987) *Adv. Lipid Res. 22*, 115-171.
26. Lusis, A.J., Taylor, B.A., Quon, D., Zollman, S., and LeBoeuf, R.C. (1987) *J. Biol. Chem. 262*, 7594-7604.

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