Effects of atherogenic diet consumption on lipoproteins in mouse strains C57BL/6 and C3H

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Abstract Inbred mouse strains C57BL/6J (B6) (susceptible) and C3H/HeJ (C3H) (resistant) differ in atherosclerosis susceptibility due to a single gene, *Ath-1*. Plasma lipoproteins from female mice fed chow or an atherogenic diet displayed strain differences in lipoprotein particle sizes and apolipoprotein (apo) composition. High density lipoprotein (HDL) particle sizes were 9.5 ± 0.1 nm for B6 and 10.2 ± 0.1 nm for C3H. No major HDL particle size subclasses were observed. Plasma HDL level in the B6 strain was reduced by the atherogenic diet consumption while the HDL level in the resistant C3H mice was unaffected. The reduction in HDL in the B6 strain was associated with decreases in HDL apolipoproteins A-I (– 34%) and A-II(– 60%). The HDL apoC content in mice fed chow was twofold higher in C3H than B6. Lipoproteins containing apolipoprotein B (VLDL, IDL, LDL) shifted from a preponderance of the B-100 (chow diet) to a preponderance of the B-48 (atherogenic diet). The LDL-particle size distribution was strain-specific with the chow diet but not genetically associated with the *Ath-1* gene. In both strains on each diet, apolipoprotein E was largely distributed in the VLDL, LDL, and HDL fractions. The B6 strain became sixfold elevated in total lipoprotein E content which in the C3H strain was not significantly affected by diet. However, the C3H LDL apoE content was reduced. On both diets, the C3H strain exhibited apolipoprotein E levels comparable to the atherogenic diet-induced levels of the B6 mice. — **Ishida, B. Y., P. J. Blanche, A. V. Nichols, M. Yashar, and B. Paigen.** Effects of atherogenic diet consumption on lipoproteins in mouse strains C57BL/6 and C3H. *J. Lipid Res.* 1991. **32:** 559–568.

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When fed a high fat and high cholesterol diet, inbred mouse strains differ in their susceptibility to atherosclerosis as measured by the formation of atheromatous lesions in the aorta (1, 2). The difference in atherosclerosis susceptibility between strains C57BL/6 (B6) and C3H/HeJ (C3H) is due to a single gene, *Ath-1*, with alleles *s* for susceptibility and *r* for resistance (3, 4). Genetic analysis using recombinant inbred lines and conventional genetic crosses indicates that *Ath-1* maps to chromosome 1 at a distance of 4.9 ± 1.8 (SE) centimorgans from *Apoa2*, the structural gene for apolipoprotein A-II (2–4).

Strain B6 mice differ from strain C3H mice in a reduction of plasma high density lipoprotein cholesterol (HDL-C) when fed an atherogenic diet. Atherogenic diet consumption results in plasma HDL-C levels of 31 ± 6 mg/dl (mean ± SEM) in the susceptible B6 strain (a twofold reduction) and an unchanged level of 62 ± 8 mg/dl in the resistant C3H strain (4). This twofold reduction and strain difference in plasma HDL-C cosegregates with lesion formation, suggesting that HDL-C and atherosclerosis susceptibility are influenced by the same gene or by two closely linked genes separated by no more than 1.7 centimorgans.

Characterization of plasma lipoproteins in two inbred mouse strains, which differ in atherosclerosis susceptibility due to a single gene difference, simplifies the analysis of usually complicated lipoprotein interrelationships encountered in other mammalian models. In this report we compare four lipoprotein classes isolated by ultracentrifugation using GGE to determine the particle size and relative quantities of lipoprotein, and quantitative SDS-PAGE to determine the apolipoprotein composition of each lipoprotein class.

METHODS

Diet

The normal control diet was Purina laboratory chow (#5012, Purina, Richmond, IN) which contained 4% fat.

Abbreviations: apo, apolipoprotein; *Apoa2*, apolipoprotein A-II structural gene; *Ath-1*, atherosclerosis susceptibility gene; B6, C57BL/6J; C3H, C3H/HeJ; VLDL, very low density lipoproteins (d < 1.006 g/ml); IDL, intermediate density lipoproteins (1.006 < d < 1.019 g/ml); LDL, low density lipoproteins (1.019 < d < 1.063 g/ml); HDL, high density lipoproteins (1.063 < d < 1.21 g/ml); LpB, lipoproteins containing apolipoprotein B; GGE, nondenaturing gradient gel electrophoresis; RI, recombinant inbred; SDS-Page, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

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The atherogenic diet described in detail earlier (1) contained 15% fat primarily from cocoa butter, 1.25% cholesterol, and 0.5% cholic acid, and had a polyunsaturated to saturated ratio of 0.7.

Animals

C57BL/6J (B6), C3H/HeJ (C3H), and the set of recombinant inbred (RI) strains derived from these two strains, BXH, were obtained from the Jackson Laboratory, Bar Harbor, ME. Occasionally, strains B6 and C3H were obtained from animal facilities at the University of California, Berkeley, CA, but these mice were never more than two generations removed from Jackson Laboratory mice. Female mice, 2–4 months of age were used throughout these experiments. Animals were housed in a temperature-controlled facility with a 12-h light and dark cycle and consumed diets offered ad libitum.

Analytical procedures

Blood was collected after an overnight fast into tubes containing anticoagulant and antimicrobial agents (2 mM EDTA, 50 μg/ml gentamycin sulfate, 0.05% sodium azide), and plasma was prepared by sedimenting cells by centrifugation at 1800 *g* for 30 min at 5°C. Plasma was stored for 1 week or less at 4°C before analysis. Lipoproteins were isolated by sequential KBr density ultracentrifugation in a Beckman 50Ti rotor (114,000 *g*, 24–48 h, 10°C) (5) and gradient gel electrophoresis (GGE) was performed according to the method of Nichols, Krauss, and Musliner (6). When examining strains B6 and C3H by GGE, lipoproteins were isolated from pooled plasma (three to five mice), and the gels were stained for protein with Coomassie blue G-250 (Bio-Rad Laboratories, Richmond, CA). When the BXH RI strains were examined, the plasma from each mouse was electrophoresed on gradient gels and the lipids were stained with Oil Red O (Sigma Chemical Co., St. Louis, MO). Lipoproteins of $d < 1.063$ g/ml and untreated whole plasma were analyzed on 2–16% gradient gels; HDL fractions were analyzed on 4–30% gradient gels (Pharmacia Fine Chemicals, Piscataway, NJ).

Plasma apolipoprotein A-I was quantified by single radial immunodiffusion assay performed at 37°C for 24 h using 0.05 M Tris (pH 7.4)-buffered 8 M urea diluent for all samples. Plasma apolipoprotein A-I was estimated against a reference calibrator consisting of apolipoprotein A-I purified from delipidated mouse HDL by gel-sieving (1.5 × 200 cm Sephacryl S-200, Pharmacia) and ion exchange-column (DEAE-Sepharose, Pharmacia) chromatography. Purified apolipoprotein A-I electrophoresed as a single Coomassie R-250-staining band of approximately 24 kDa in SDS-PAGE. Gels were calibrated with a mixture of proteins of known molecular weights (LMW standards kit, Bio-Rad Laboratories). Purified apolipoprotein A-I was assayed for protein by a modified method (7) of Lowry et al. (8) using human serum albumin as a calibrator (Cohn Fraction V, Sigma Chemical Co.) and stored at −70°C. Antiserum to apolipoprotein A-I raised in New Zealand White rabbits was monospecific in the radial immunodiffusion assay for mouse apolipoprotein A-I.

Apolipoproteins from ultracentrifugally derived density fractions were analyzed by SDS-PAGE for apolipoproteins A-I, A-II, C, and E by electrophoresis on a linear 3–30% gradient polyacrylamide slab gel using a Protean II electrophoresis cell (Bio-Rad Laboratories). Apolipoproteins B-48 and B-100 were analzyed on 2.5% acrylamide/1% agarose mini-slab gels containing SDS as described by Gabelli and co-workers (9), using a discontinuous buffer system (10). This modification provided increased resolution and allowed for the analysis of 15 samples per gel with sample volumes up to 40 μl. In addition, we found it necessary to solubilize apolipoprotein B-containing samples in the following manner to minimize apolipoprotein B aggregation. Dialyzed lipoprotein fractions (35 μl) were treated with 5 μl SDS (20%, w/v) (Bio-Rad Laboratories) and 3 μl dithiothreitol (10 mM) (Sigma Chemical Co.) for 30 min at room temperature followed by a reaction in the dark with 2 μl iodoacetic acid (50 mM in 0.01 M Tris, pH 7.4) (Sigma Chemical Co.) for 30 min. Finally, 10 μl of buffer, containing 5% SDS, 5% 2-mercaptoethanol, 25% glycerol, and 0.62 M Tris (pH 6.8), was added and samples were boiled for exactly 30 sec just prior to electrophoresis. Coomassie R-250-stained bands containing apolipoproteins A-I, A-II, C, and E were quantified by laser scanning densitometry (LKB, Piscataway, NJ) using corresponding purified mouse apolipoproteins as standards. Use of the appropriate apolipoprotein for calibration was necessary since the chromogenicity of the Coomassie R-250-stained apolipoproteins differed. Calibration curves for each apolipoprotein gave the absorption to mass relationships described in Fig. 5. Human LDL ($1.030 < d < 1.050$ g/ml) as a source of apolipoprotein B-100 was used as a calibrator for both the B-100 and B-48 mouse apolipoproteins. It is known, however, that the chromogenicity values for Coomassie-stained apolipoproteins B-100 and B-48 differ (11). Hence, without a known correction factor, values given for B-48 were quantitated relative to the apolipoprotein B-100 calibrator.

Data analysis

The Student's *t*-test was used to determine statistical differences at the $P < 0.05$ level of significance. All data are presented as mean ± SEM.

RESULTS

Lipoprotein changes in response to diet

In an initial study, the particle size distribution of total plasma lipoproteins was examined on 4–30% nondenaturing gradient gels. Groups of five mice from each strain (B6 and C3H) were fed chow or the atherogenic diet for 4 weeks and killed. Plasma from each group was pooled, and the ultracentrifugal total lipoprotein fraction ($d < 1.210$ g/ml) was isolated. In B6 mice fed the chow diet, a single major peak was observed in the HDL particle-size region, and two considerably smaller peaks were observed in the VLDL and the LDL particle-size regions (**Fig. 1A**). These observations are consistent with previous reports showing that HDL is the major lipoprotein in mice fed chow (12–14). In the B6 strain, consumption of the atherogenic diet resulted in the following major changes in lipoprotein profiles when compared to profiles from mice fed the chow diet: *1*) a single, major peak appeared in the VLDL–LDL-size region; and *2*) the peak area in the HDL-size region was reduced approximately one-half. The decrease in HDL protein-staining peak area is in agreement with the 50% decrease in HDL-C reported earlier for these strains fed an atherogenic diet (4). The total lipoprotein profiles of chow-fed C3H mice displayed a small peak in the VLDL–LDL region, corresponding to human LDL-sized particles, and as in chow-fed B6 mice, a single major peak in the HDL region (Fig. 1B). Consumption of the atherogenic diet by C3H mice resulted in the appearance of two lipoproteins of increased peak height in the VLDL–LDL region, but no significant peak area changes in the HDL region were observed. Similar changes were observed in three separate dietary experiments; the data in Fig. 1A and B are from a single representative experiment.

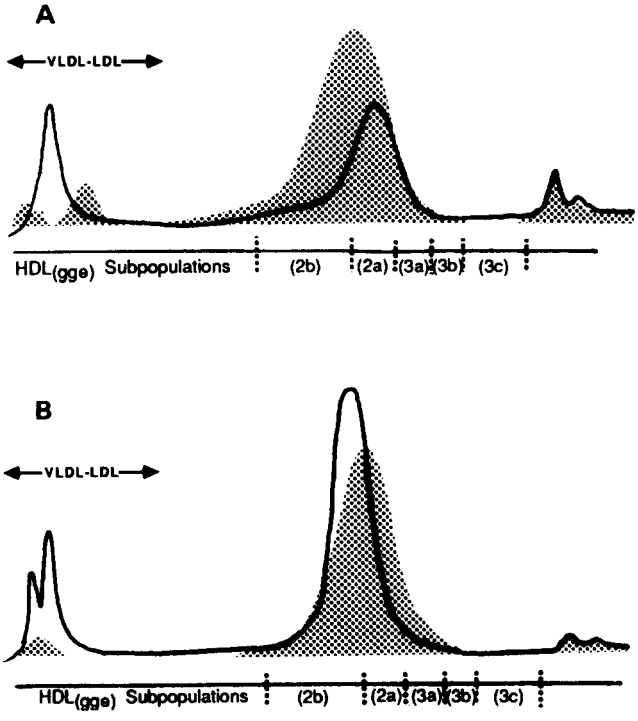

Fig. 1. Pooled lipoproteins ($d < 1.210$ g/ml) from five mice of strains C57BL/6 (B6) (A) and C3H (B) fed chow (shaded area) or an atherogenic diet (solid line) were analyzed by nondenaturing 4–30% polyacrylamide gels and stained for protein with Coomassie G-250. The displayed results are representative scans obtained from three experiments. Migration regions of human VLDL–LDL and HDL subpopulations are indicated. Equivalent quantities of lipoproteins were analyzed from each strain for comparison.

To further dissect the particle size profiles in Fig. 1, the experiment was repeated with VLDL, IDL, LDL, and HDL fractions ultracentrifugally isolated from pooled plasma of each mouse strain fed the chow and the atherogenic diet. The gel scans for each lipoprotein fraction of the two strains are superimposed for comparison in **Fig. 2**. As expected from our initial analysis of the total lipoprotein $d < 1.210$ g/ml fraction (Fig. 1), the VLDL and the IDL fractions contained very small quantities of lipoproteins in the mice fed chow. Lipoprotein concentrations in the VLDL, IDL, and LDL fractions were dramatically increased in both strains fed the atherogenic diet. The largest lipoprotein increase occurred in the VLDL fraction where the elevation was greater in the C3H strain. The LDL fraction of chow-fed animals showed a distinct, relatively monodisperse peak present in B6; on the other hand, the C3H LDL profile appeared bimodal. Upon feeding the atherogenic diet, the two strains became similar in the LDL particle-size profile. The VLDL and the IDL particle sizes were similar in both strains on each diet.

In agreement with Fig. 1, the HDL fraction exhibits major interstrain differences for both chow and atherogenic diets. On chow, C3H has quantitatively more HDL (larger peak area) with mean particle size of 10.1 ± 0.1 nm ($n = 4$). The strain difference becomes even more striking in mice fed the atherogenic diet; the HDL in strain C3H remained high with a small insignificant reduction in particle size to 9.9 ± 0.1 nm, but the peak area in B6 decreased with a concomitant reduction in HDL particle size from 9.6 ± 0.1 nm to 9.1 ± 0.1 nm ($P < 0.05$, $n = 4$).

As shown in Figs. 1 and 2, HDL from mice do not exhibit distinct multicomponent profiles as observed for human HDL by GGE. HDL from chow-fed B6 and C3H strains show essentially single peak profiles of differing sizes ($P < 0.05$), 9.6 ± 0.01 nm and 10.1 ± 0.2 nm, respectively. The peak maxima of these HDL particles are at the boundary located between the human HDL_{2b} and HDL_{2a} size intervals.

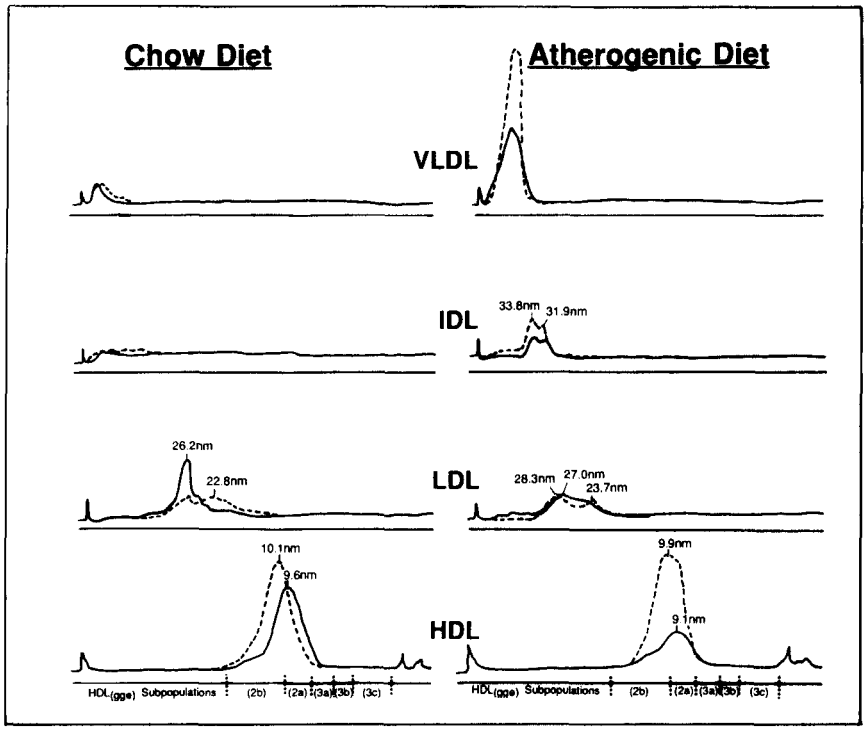

Fig. 2. Pooled plasma lipoproteins from five mice of strains B6 (solid lines) and C3H (dashed lines) on chow and atherogenic diets were isolated by sequential ultracentrifugation and identical plasma equivalents were analyzed on 2–16% gradient gels (VLDL, IDL, LDL) or 4–30% gradient gels (HDL) by protein-staining with Coomassie blue G-250. These scans are representative of three experiments.

In summary, the strain-related differences in lipoprotein GGE patterns include *a*) an atherogenic diet-induced reduction in HDL quantity and particle size for the B6 strain; *b*) a strain difference in HDL particle size; *c*) the presence of distinct LDL-sized particles in chow-fed mice of both strains; and *d*) a larger increase in the VLDL fraction for the C3H strain on the atherogenic diet.

Whether any of the above differences are genetically related to atherosclerosis susceptibility was investigated by examining the set of recombinant inbred (RI) strains derived from B6 and C3H. Each of these 12 RI strains has a unique combination of genes derived from the two parental strains and was previously tested for atherosclerosis susceptibility (4). To determine which of the lipoprotein differences cosegregates with lesion formation, whole plasma from RI strains fed chow was analyzed by GGE. In plasmas from mice fed the atherogenic diet, decreased HDL peak area was found to cosegregate with susceptibility to atherosclerosis (data not shown). This is consistent with previous observations by Paigen et al. (4) who measured HDL lipids by using agarose gels stained with Sudan Black B. The HDL particle size cosegregates with the *Apoa2* gene confirming the work of Lusis et al. (15) who used nondenaturing 5% PAGE to demonstrate HDL particle size differences. This size difference has recently been related to a decreased rate of synthesis of apolipoprotein A-II (16), and is not related to atherosclerosis susceptibility since the atherosclerosis-resistant RI strains BXH-3 and BXH-4 were found to have HDL sizes characteristic of the B6 parent.

The strain-related differences for particles in the LDL density interval were also analyzed. Plasma from chow-fed mice was used in this experiment since the quantity of LDL-sized particles become decreased by the consumption of the atherogenic diet and are poorly resolved from the elevated levels of the VLDL and IDL. In **Fig. 3**, whole plasma from mice fed a chow diet was electrophoresed and stained for lipid while the samples analyzed in Fig. 2 were ultracentrifugally purified lipoproteins stained for protein. These differences in sample preparation may account for the increased peak resolution in Fig. 3. The densitometric scans of the VLDL–LDL region (Fig. 3) indicate two discrete LDL-sized particles present in each mouse strain. Particle diameters of 24.6 and 25.9 nm for the B6 strain, and 23.0 and 25.7 nm for the C3H strain were found. These results suggest that the B6 and C3H strains share a common LDL particle of average size, 25.8 nm, and each contains a strain-specific LDL-sized particle (24.6 and 23.0 nm). Twelve RI inbred strains were analyzed (BXH-2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 14, 19). Of these 12 RI strains, 8 were like the B6 parent (BXH-2, 3, 4, 6, 7, 9, 10, 19); the data

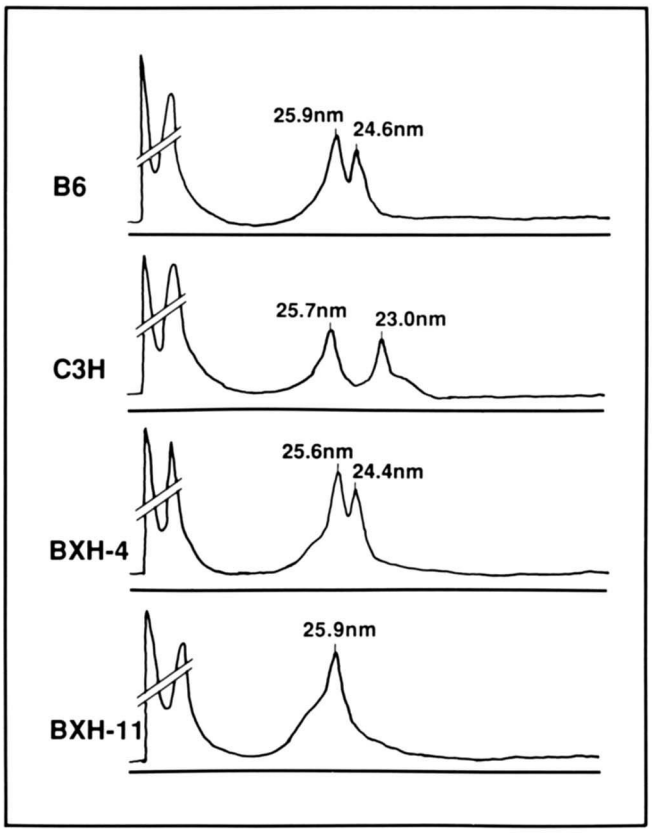

Fig. 3. Three individual mice of each strain were fed a chow diet and whole plasma was electrophoresed on 2–16% polyacrylamide gels and lipid-stained with Oil Red 0. Densitometric profiles with diameters of LDL-sized particles indicated are shown for strains B6, C3H, BXH-4, and BXH-11.

for the parental strains and a representative RI strain, BXH 4, are shown in Fig. 3. Four of these RI strains are susceptible to atherosclerosis and 4 are resistant (4) so this strain difference in LDL pattern is not correlated with any differences in atherosclerosis susceptibility. No strain had a pattern like the C3H parent, and 4 strains displayed a nonparental LDL particle size phenotype (BXH-8, 11, 12, 14); the pattern for BXH-11 is shown in Fig. 3. BXH-8 and BXH-11 were similar displaying a single-sized LDL particle. BXH-12 and 14 displayed LDL heterogeneity where no distinct peaks within the LDL particle size range could be distinguished.

Distribution of apolipoproteins among lipoprotein classes

In an attempt to learn more about lipoprotein differences between the B6 and C3H strains and especially differences in response to diet, we determined plasma apolipoprotein levels associated with each of the four lipoprotein density fractions. Quantitation was performed by scanning densitometry of apolipoproteins resolved by SDS-PAGE. Apolipoproteins were quantified against gel calibrators consisting of purified homologous mouse apolipoproteins to avoid problems arising from different apolipoprotein chromogenicities. SDS-PAGE of HDL apolipoproteins is shown in **Fig. 4** to compare the strain differences in apolipoprotein composition and the effect of diet. It is apparent that the major apolipoproteins in HDL consist of apolipoproteins A-I, A-II, C, and E. Despite analyzing HDL under reducing conditions it is known that apoA-II in mice is monomeric due presumably to the absence of cysteine (17, 18), and from Fig. 4, is smaller in apparent molecular weight than apolipoprotein C. The diet-induced reduction in apolipoprotein A-I (lane 2) agrees with the decreased peak area of HDL-sized particles observed for the B6 strain by GGE (Figs. 1 and 2). The SDS-PAGE protein pattern is also in agreement with the GGE data by displaying no decrease in apolipoprotein A-I for the C3H strain. However, the apolipoprotein A-II content appeared significantly decreased in the B6 strain while only slightly decreased in the C3H strain by the atherogenic diet.

The apolipoprotein distribution for the VLDL, IDL, and LDL fractions was similarly determined by quantitative SDS-PAGE and is summarized in **Fig. 5**.

Our primary interest was to determine whether major changes occurred in the apolipoprotein composition of HDL since the HDL lipoprotein class differs significantly between the strains while consuming the atherogenic diet, and the difference is associated with atherosclerosis susceptibility. As expected for strain B6 fed the atherogenic diet, there was a 34% reduction ($P < 0.05$) in mass of apolipoprotein A-I in the HDL fraction that was consistent with the decrease in HDL observed by gradient gel analysis (Figs. 1 and 2). The apolipoprotein A-I level on

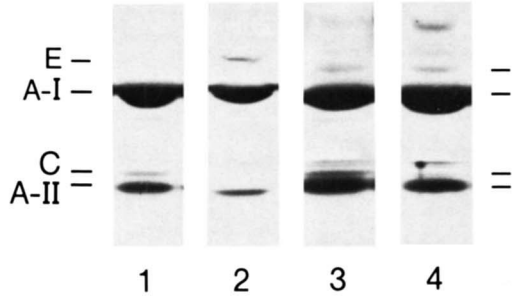

Fig. 4. The relative changes in HDL apolipoproteins are shown by SDS-PAGE. Plasma HDL from B6 and C3H mice fed either chow or atherogenic diet were isolated by sequential ultracentrifugation at a KBr density interval of 1.063–1.210 g/ml. Proteins were resolved on 3–30% polyacrylamide SDS gels and stained with Coomassie R-250. HDL samples were from C57BL/6 fed chow (lane 1) or atherogenic diet (lane 2); C3H fed chow (lane 3) or atherogenic diet (lane 4). HDL samples from chow plasmas applied to the gel contained approximately 10 μg apoA-I. The mobilities of purified mouse apolipoprotein calibrators are indicated. The larger of the two proteins of molecular weight greater than apolipoprotein E in lanes 3 and 4 co-migrated with serum albumin.

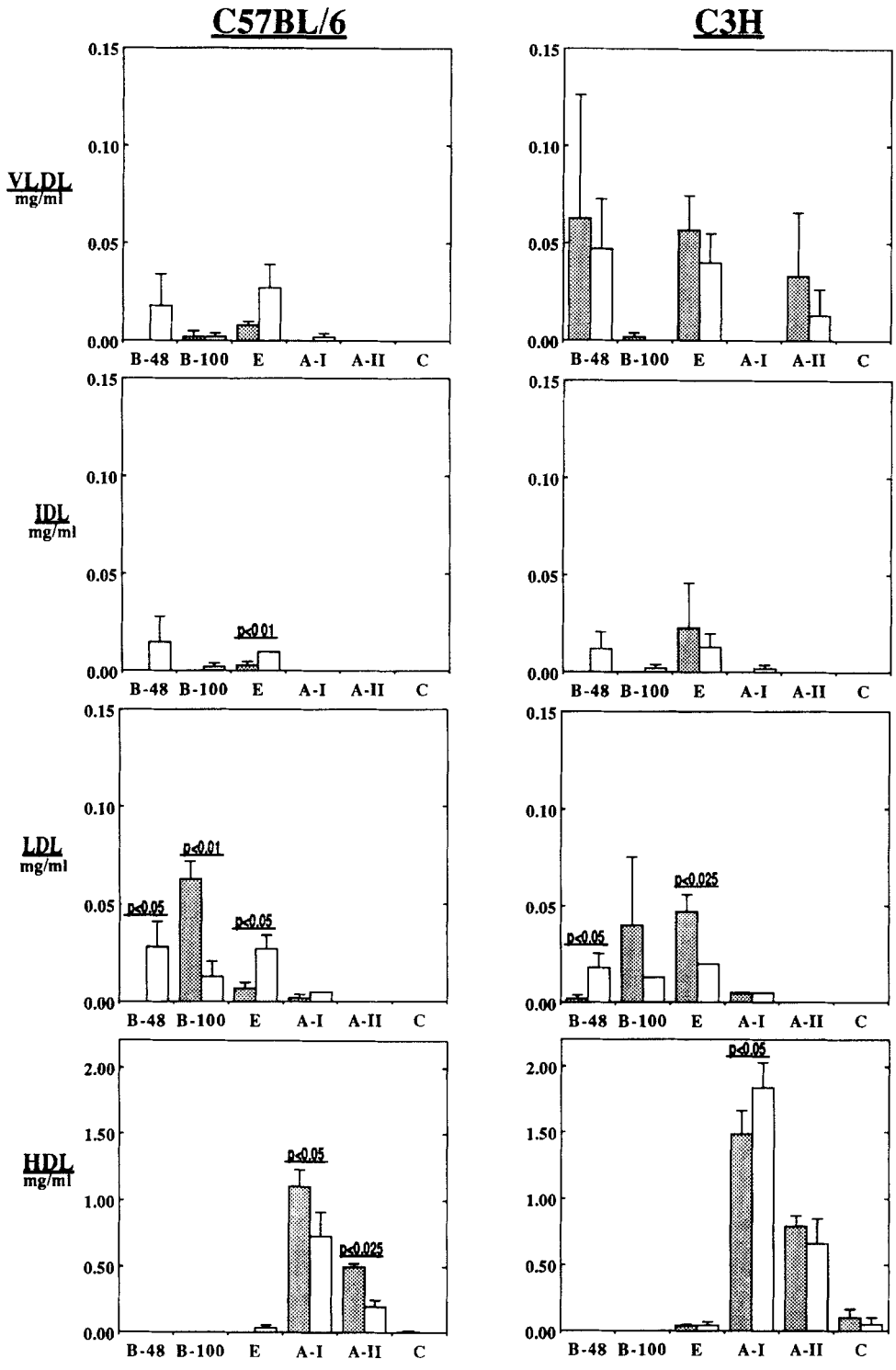

Fig. 5. Plasma apolipoprotein levels associated with ultracentrifugally isolated lipoproteins were analyzed by quantitative densitometry of Coomassie blue R-250-stained SDS-PAGE gels. Apolipoproteins B-48 and B-100 were electrophoresed on composite gels comprised of 2.5% acrylamide stabilized with 1% agarose, and those containing apolipoproteins A-I, A-II, C, and E were electrophoresed as described in Fig. 4. The apolipoprotein levels for B6 (left column) and C3H (right column) are shown for each lipoprotein fraction isolated from plasma of mice fed chow (filled bars) and atherogenic (open bars) diets; apolipoprotein levels represent mean plasma values (mg/ml) with error bars indicating the SEM (n = 3). Statistical significance for paired apolipoprotein values for the same strain on different diets is indicated. Calibration curves (1–10 μg), linear regression equations (peak area$_{OD \times mm}$ = (m) μg + b), and correlation coefficients (r) were determined for each apolipoprotein resulting in the following constants: A-I (m = 0.269, b = 0.081, r = 0.999 ± 0.001) (n = 12); A-II (m = 0.049, b = 0.030, r = 0.987 ± 0.003) (n = 8); B-100 (m = 0.188, b = 0.13, r = 0.992 ± 0.003) (n = 14); C (m = 0.146, b = 0.061, r = 0.998 ± 0.001) (n = 8); and E (m = 0.154, b = 0.007, r = 0.999 ± 0.001) (n = 10).

chow diet in strain C3H was higher than in strain B6, and on the atherogenic diet was significantly increased ($P < 0.01$) to a level nearly threefold greater than that of the B6 strain. The distribution of apolipoprotein A-I was limited primarily (96–99%) to the HDL fraction for both strains regardless of diet. In response to the atherogenic diet, apolipoprotein A-II in the C3H strain was not significantly decreased while B6 levels were reduced 60% ($P < 0.025$). the calculated A-II:A-I molar ratios in HDL for B6 were significantly reduced ($P < 0.025$):1.60 ± 0.13 (chow, n = 4) and 0.99 ± 0.14 (atherogenic diet, n = 3), and not statistically different between diets for the C3H strain: 1.88 ± 0.19 (chow, n = 3) and 1.35 ± 0.50 (atherogenic diet, n = 3). The difference in the A-II:A-I molar ratios was also significant between chow-fed C3H and the atherogenic-fed B6 mice ($P < 0.01$).

A major strain difference in apolipoproteins was seen in the level and distribution of apolipoprotein E. The C3H strain on both diets had substantially higher levels of total apolipoprotein E than the B6 strain consuming chow ($P < 0.05$). In both strains regardless of diet, apolipoprotein E was largely distributed among the VLDL, LDL, and HDL fractions. The IDL fraction contained a smaller proportion of the total apolipoprotein E. In general, the mouse strains responded differently in apolipoprotein E levels to the atherogenic diet. The total lipoprotein levels of apolipoprotein E were not significantly affected by the atherosclerotic diet in the C3H strain, however, a sixfold increase was observed in the B6 strain ($P < 0.025$). This significant increase occurred in the IDL ($P < 0.01$) and LDL ($P < 0.05$) lipoprotein fractions, and resulted in a total apolipoprotein E level similar to that of the C3H strain. In the C3H strain, the atherogenic diet consumption influenced a trend of decreasing apolipoprotein E content in the VLDL, IDL, and the LDL fractions which was statistically significant in the LDL fraction ($P < 0.025$). Thus, the apolipoprotein E levels in the apoB-containing lipoproteins were oppositely affected by the atherogenic diet: increasing in the B6 and decreasing in the C3H strains. The HDL apolipoprotein E content was not significantly affected by diet.

Compared to the B6 strain, a small amount of apolipoprotein A-II was consistently present in the VLDL fraction of the C3H strain (<0.07 mg/ml). As in the HDL fraction the atherogenic diet reduced the apolipoprotein A-II level associated with the VLDL fraction. It is possible that the presence of apolipoprotein A-II in VLDL is not strain-specific, but was observed since the C3H strain is more abundant in this apolipoprotein.

The atherogenic diet response in lipoproteins of d <1.063 mg/ml was apparent in the level and distribution of apolipoproteins B-48 and B-100. Plasma apolipoprotein B-100 levels, present primarily in the LDL fraction, were similar on chow and decreased ($P < 0.01$) to similar levels in both B6 and C3H on atherogenic diet. On the other hand, the apolipoprotein B-48 content of LDL was increased by the atherogenic diet in both the B6 and the C3H strains ($P < 0.05$), however, the distribution among d < 1.063 g/ml lipoproteins differed between strains on both diets. While the chow diet levels of apolipoprotein B-100 (VLDL + IDL + LDL) were similar for B6 and C3H, apolipoprotein B-48 was undetectable in B6 while considerably higher in the C3H strain (0.077 mg/ml). Apolipoprotein B-48 was distributed equally among the VLDL, IDL, and LDL fractions isolated from the B6 strain on the atherogenic diet, while most of the B-48 was associated with the VLDL fraction in C3H plasma from both atherogenic and chow-fed animals.

DISCUSSION

In this study, we describe changes in the apolipoprotein composition of plasma lipoproteins from the C3H and B6 mouse strains after prolonged consumption of an atherogenic diet. In agreement with previous reports evaluating these mouse strains, a major effect of atherogenic diet consumption on the plasma lipoprotein profile is a reduction in the quantity of HDL. Past studies have documented that the susceptible B6 strain has a quantitatively lower plasma HDL cholesterol (HDL-C) level in comparison to the resistant C3H strain when mice of each strain are fed an atherogenic diet (4). This quantitative difference has been shown by genetic crosses to segregate in a Mendelian fashion as a single gene (1–3). Consistent with the general hypothesis that high levels of HDL are protective, HDL-C levels are not reduced in the nonsusceptible C3H strain upon the consumption of a diet enriched in saturated fat and cholesterol.

The marked reduction in B6 HDL is accompanied by a major reduction in both apolipoproteins A-I and A-II. These reductions do not occur in strain C3H; in fact, a significant increase was observed as noted in previous studies for both plasma HDL-C and apolipoprotein A-I (19–21) during consumption of an atherogenic diet. Apolipoprotein C was detectable only in the HDL fraction. B6 HDL apolipoprotein C levels were considerably lower than in the C3H HDL. In mice consuming the atherogenic diet, apolipoprotein C in the B6 HDL fraction was undetectable. The total apolipoprotein E response to the atherogenic diet was strain-dependent increasing sixfold in the B6 strain while unchanged in the C3H strain. This strain-dependent response is similar to that found in two other related inbred strains C57BR and CBA (14) which, like their genetically related B6 and C3H strains, are susceptible and resistant, respectively, to diet-induced atherosclerosis (14, 19).

Apolipoprotein E was significantly elevated in the IDL and LDL fractions of the B6 strain, and reduced in the

LDL fraction of the C3H strain. Apolipoprotein E levels in B6 mice were lower than in the C3H mice when fed chow, and on the atherogenic diet the B6 and C3H strains became equivalent in total apolipoprotein E level and lipoprotein distribution. The etiology of this differential apolipoprotein E response is unclear and may involve the regulation of both the B/E and E receptors. It is possible that apolipoprotein E receptor activity may be down-regulated by the atherogenic diet to effect a higher plasma level of apolipoprotein E in the B6 strain. It is possible that the high unesterified cholesterol content of the atherogenic diet may have induced macrophages to increase the secretion of apolipoprotein E-containing lipoproteins (22, 23). Elevated levels of lipoproteins containing both apolipoprotein B-48 and E may be highly atherogenic but the C3H strain, which has high levels of these lipoproteins on both chow and atherogenic diets, may avoid atherosclerosis by balancing the plasma lipoprotein profile with high levels of HDL. The B/E receptor activity may be up-regulated in both strains as suggested by decreased plasma levels of LDL apolipoprotein B-100. In an earlier study, other investigators have reported on the effect of the same atherogenic diet on total plasma levels of apolipoproteins B-100, B-48, and E for the B6 and C3H strains (24). Our data agree with the reported fourfold total apolipoprotein B-48 increase in the B6 strain in response to the consumption of atherogenic diet, and the absence of change in the C3H strain. On the other hand our data are discordant in the apolipoprotein B-100 and E responses. While we observed reductions for B-100 in both strains, the other study found reductions only in the C3H strain. Similarly, the present study found dramatic increases for plasma apolipoprotein E in the B6 strain while the other study did not note any significant changes. Methodological differences may explain the discordant findings between the present study which analyzed lipoprotein fractions and the other study which analyzed whole plasma by an immunoblotting technique. Since we sought to determine the apolipoprotein distribution among lipoprotein classes, it was necessary to use purified lipoprotein fractions. It is possible that losses in apolipoproteins, especially apolipoprotein E, may have occurred during the ultracentrifugal preparation of these fractions. However, since selective losses would have to result to explain the dietary and strain differences that we observed, and since our total plasma apolipoprotein E values compare favorably with those reported in the other study, we feel that no major losses in apolipoprotein E resulted.

In recent studies by Jiao et al. (25, 26), LDL particle size heterogeneity was documented in numerous inbred mouse and RI strains by gel permeation chromatography. In comparison to the bimodal size distribution found in our current study, these investigators reported a single LDL particle-size for each inbred strain. The ability to resolve LDL particle-size subclasses in the two different methodologies used in the studies may explain the discrepant findings. In fact, our study confirms their findings of strain heterogeneity in LDL size, and that the LDL particle-size for the B6 strain is larger than that of the C3H strain. Our data further suggest that the LDL particle-size (and size heterogeneity) is not a characteristic of the *Ath-1* gene but is regulated by at least two other undefined genes. The other study (25) found no correlations between LDL particle-sizes and plasma or lipoprotein cholesterol level. The data from all three studies suggest that LDL particle-sizes are unrelated to atherosclerosis susceptibility among inbred mouse strains. In our study LDL of the C3H strain contained larger amounts of apolipoprotein E than the B6 strain. This difference could influence the strain-specific LDL particle-size distribution.

It was surprising to find that major apolipoprotein compositional changes did not result in the appearance of additional HDL-size subclasses in the B6 strain despite the significant diet-induced reductions of the A-II:A-I molar ratio. Similarly, an elevation of apolipoprotein A-I in the C3H strain did not affect the HDL-size species. In fact, each strain revealed a single, homogeneous HDL particle-size distribution regardless of diet. Our results, however, are discordant with recent data of other investigators that suggest the presence of three major HDL particle-size classes in strains B6 and Balb/c (20). The nature of this discrepancy is unknown but may be explained by differences in analytical methods. The latter study analyzed whole plasma stained with a lipophilic dye by GGE. On the other hand, the HDL particle diameters and the single-sized distribution found in this study are in excellent agreement with two other published studies (14, 25) both of which analyzed ultracentrifugally purified HDL by GGE and stained for protein. While the A-II:A-I molar ratio was similar, the B6 and C3H strains differed in the level of these apolipoproteins when consuming the chow diet, and suggests that the strain difference in mean HDL particle-size is not directly related to absolute levels of apolipoproteins A-I and A-II. Rather, it has been suggested by others that the HDL particle-size difference is due to a gene called *Hdl-1* (15), and that the genetic basis of atherosclerosis rendered by the *Ath-1* gene is not explained on the basis of differences in HDL particle-sizes (4). In a survey of inbred mouse strains the atherosclerosis-resistant Peru strain has an HDL particle-size even smaller than B6 (15) and may compensate for its physically smaller apolar core by increasing the total number of HDL particles to effect a higher plasma HDL-C level.

A stoichiometric increase in HDL apolipoproteins is likely to accompany increasing amounts of HDL particles for structural reasons, but it is not yet clear whether the apolipoprotein composition influences the physical

characteristics and metabolic role of HDL. In this study, marked reductions in apolipoproteins A-I and A-II, which compose the major structural proteins of HDL, were correlated with a 0.3-nm particle diameter reduction in the B6 strain consuming the atherogenic diet. The small change in particle size, however, may be less metabolically significant than the total apolipoprotein A-I, A-II, and HDL reductions. It appears that the reduction in the plasma HDL-C pool size by atherogenic diet consumption may result from the modulation of HDL synthetic and catabolic pathways. Increased HDL catabolism may explain the reduced levels in the B6 strain since equivalent apolipoprotein A-I synthetic rates were reported for the B6 and the Balb/c strains (20). The latter strain, like the C3H strain, is not susceptible to diet-induced atherosclerosis (19). The HDL compositional changes in apolipoproteins E and C were very small, and may involve changes in a minor HDL subclass in the mouse. The lower A-II level in plasma B6 mice is probably explained by a strain difference in the translational efficiency of apolipoprotein A-II mRNA (20).

In response to the atherogenic diet, we found the lipoproteins of d < 1.063 g/ml of both B6 and C3H altered in their content of apolipoprotein B-species. Thus, apolipoprotein B-100 is largely replaced by apolipoprotein B-48, suggesting the appearance of new lipoproteins. The substantially greater elevation of lipoproteins containing B-48 and E in the VLDL, IDL, and LDL fractions in the B6 strain together with a decrease in HDL may render this strain susceptible to atherosclerosis while a strain such as C3H is nonsusceptible and maintains a relatively unchanged lipoprotein profile while consuming an atherogenic diet.

This study supports a hypothesis that the presence of apolipoprotein B-48- and E-containing lipoproteins and reduced HDL levels may be major contributing factors in diet-induced atherosclerosis in the B6 mouse. The phenotypic similarities of this mouse strain with the human hypoalphalipoproteinemia phenotype make it an attractive experimental animal model to investigate possible mechanisms affecting HDL levels and compositions.

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