Familial and Diet-Induced Hypercholesterolemia in Swine
Lipid, ApoB, and ApoA-I Concentrations and Distributions in Plasma and Lipoprotein Subfractions

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Abstract Low levels of high-density lipoproteins (HDLs) may constitute an independent risk factor that may be as important as elevated low-density lipoproteins (LDLs) in coronary artery disease (CAD). Concentrations and distributions of lipids, apolipoprotein (apo) B, and apoA-I in the plasma and lipoprotein subfractions of two groups of swine, one with familial hypercholesterolemia (FHC) and the other with diet-induced hypercholesterolemia (DHC), were examined. Normolipidemic (NL) animals served as controls. All pigs carried the Lpb5 apoB mutation, which is known to influence the formation of atherosclerotic lesions. Mean concentrations of serum total cholesterol in NL, DHC, and FHC were 80.0±9.3, 774.3±54.5, and 316.5±36.1 mg/dL, respectively; HDL cholesterol (HDL-C), 33.5±1.9, 137.0±9.9, and 223±2.2 mg/dL; triglycerides, 33.0±163, 40.3±11.7, and 56.8±7.2 mg/dL; apoB, 35.7±3.1, 142.0±4.8, and 169.3±13.9 mg/dL; and apoA-I, 62.4±9.3, 170.9±6.9, and 42.6±4.8 mg/dL. The distributions of total cholesterol, apoB, and apoA-I in plasma lipoprotein subfractions were also examined. Compared with NL, FHC had fourfold and 4.7-fold increases in total cholesterol and apoB, respectively, distributed in the lower densities (d<1.043 g/mL), and low HDL-C and apoA-I levels, resulting in a high total cholesterol/HDL-C ratio (14.4:1) and elevated triglyceride levels. DHC was characterized by 10-fold and fourfold increases in total cholesterol and apoB, respectively, resulting in an LDL particle highly enriched in cholesterol, a fourfold increase of HDL-C, an almost threefold increase in apoA-I, and a normal triglyceride level. Thus FHC but not DHC exhibited a marked resemblance to familial combined hyperlipidemia, the most common endogenous dyslipidemia in humans. (Arterioscler Thromb. 1994;14:923-930.)

Key Words • swine • familial hypercholesterolemia • diet-induced hypercholesterolemia • plasma cholesterol • HDL cholesterol • apoB • apoA-I • triglycerides • immunochemistry

Hyperlipidemia and low levels of high-density lipoprotein (HDL) cholesterol (HDL-C) represent independent and major risk factors for coronary artery disease (CAD) in humans. In hyperlipidemia elevated cholesterol levels, or specifically, increased low-density lipoprotein (LDL) cholesterol (LDL-C) and apolipoprotein (apo) B are strongly associated with an increased risk for CAD.4-7 Low levels of HDL-C and apoA-I are strongly implicated in the development of CAD, and elevated HDL-C is viewed as antiatherogenic.8-10 The inverse relation between LDL and HDL in CAD seems to have its basis in alternative roles of LDL and HDL in the transport of plasma cholesterol: LDL transports cholesterol from liver and small intestines to peripheral tissues,9 whereas HDL plays a key role in reverse cholesterol transport, from the peripheral tissues to the liver.10

Because approximately 50% of swine with age develop spontaneous aortic lesions resembling the early stages of human atherosclerosis,11 numerous investigators have attempted to accelerate the development of atherosclerosis through diet-induced hypercholesterolemia (DCH) (for review see Reference 12). Feeding swine with various fats alone or with 0.5% to 4.2% cholesterol concentrations13-15 causes variable effects on the development of hypercholesterolemia and atherosclerosis and shows that in swine, as in humans,4 the severity of atherosclerosis rises progressively with the increase of plasma cholesterol levels.16 DHC is always accompanied by marked alterations in plasma lipids, lipoproteins, and apolipoproteins13,15,17-19; triglycerides (TGs) show insignificant changes. Lipoprotein alterations include the appearance of β-migrating very-low-density lipoproteins (β-VLDLs); increases in intermediate-density lipoproteins and LDL; and the appearance of a cholesterol-induced lipoprotein referred to as HDL1,13,18 In severe hypercholesterolemia (total cholesterol [TC] >400 mg/dL), up to 10-fold increases in LDL are accompanied by up to threefold increases in HDL.17,19

A strain of swine that carries components of at least six domestic breeds and exhibits familial hypercholesterolemia (FHC) when fed a low-fat, low-cholesterol diet20 was developed through multigeneration propagation and selection as a result of long-term studies on immunologically identified LDL polymorphism.21-22 The FHC strain is characterized by elevated cholesterol concentrations (130 to 490 mg/dL) that are probably determined by several yet-unidentified genes.20 FHC is characterized by cholesterol ester-enriched buoyant LDL as well as by the dense LDL present in d<1.063 g/mL20 compared with the LDL of normolipidemic

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Metabolic and in vitro studies reveal that FHC is accompanied by defective binding of buoyant LDL to the LDL receptor, which seems to be functionally normal, as well as by a defect in LDL catabolism. Plasma of FHC swine also exhibits decreased lecithin:cholesterol acyltransferase (LCAT) activity.

The FHC swine is the first animal model to exhibit FHC and to develop atheromatous lesions ranging from fatty streaks to advanced plaques containing necrotic cores, calcification, neovascularization, hemorrhage, and rupture at 3 through 4 years of age, developments that are similar to those in humans. The association of low HDL-C and apoA-I (the major apolipoprotein in HDL) with CAD has been investigated in humans, but data on this relation are not available in experimental animal models. The need for more extensive information on HDL-C, apoA-I, and TGs in FHC and DHC and the availability of a novel animal model for studying causal factors of familial dyslipidemias and their association with atherosclerosis stimulated our interest in this study. In addition, the availability of plasma from DHC and NL swine homozygous for the Lpb allele made possible the first comparison between the two hypercholesterolemias by studying concentrations of two cholesterol parameters (TC and HDL-C), TGs, apoB, and apoA-I and their distributions, relations, and ratios.

Methods

Animals

Seven-month-old male swine were genetically classified according to immunologically defined lipoprotein phenotypes by using 16 apoB, 2 apoU, and 2 apoR epitope-specific alloantibodies and were selected on the basis of their apoB, apoU, and apoR genotypes and TC levels. Twelve swine were divided into three groups of four animals each. Group 1 (NL controls) was homozygous for the Lpb allele. Group 2 (DHC) was identical to group 1 but from 3 months of age was fed for 16 weeks an atherogenic diet composed of Chow supplemented with 1% cholesterol, 20% beef tallow, and 0.75% cholate. DHC animals were housed individually to monitor the consumption of their daily rations. Group 3 (FHC) was derived from a strain of swine originally described as exhibiting inherited hyperlipoproteinemia and hypercholesterolemia. All FHC animals were homozygous for the Lpb, Lpα, and Lpρ alleles. The FHC group and three animals of the control group were from the Immunogenetic Project Herd, University of Wisconsin, Madison, and carried components of at least six domestic breeds. The DHC group and one of the control animals were from the colony at the Francis Owen Blood Research Laboratory, University of North Carolina, Chapel Hill. The latter five animals are part of a larger study on the effect of diet-genotype interaction and von Willebrand disease on atherosclerosis. The animals were treated according to the standards set in “Guide for Care and Use of Laboratory Animals” (National Institutes of Health publication 85-23). All swine, except the four fed the atherogenic diet, were fed ad libitum a standard diet containing 6% fat (3% corn oil and 3% lard); total daily cholesterol intake per animal was 60 to 65 mg.

Plasma and Serum Collection

Blood was drawn from the jugular vein into 0.0045 mol/L Na2EDTA, pH 7.4 (1 mL 10% Na2EDTA/60 mL blood), of animals fasted overnight. The blood was kept on ice and centrifuged immediately, and the plasma was separated from the cells by low-speed centrifugation at 4°C. The following preservatives were added (final concentrations): 0.02% NaN3, 0.13% e-aminon-caproic acid (Sigma Chemical Co A-2504), 8 U/mL aprotinin (Sigma A-6012), and 1 mmol/L phenylmethanesulfonyl fluoride (Sigma P-7626). Blood (10 mL) was collected without anticoagulant for serum, to which 0.02% Na2EDTA and 0.02% NaN3 were added after separation. Plasma (30 mL per animal) and serum samples from the five swine from Chapel Hill were shipped on wet ice to Madison by overnight courier. The isolation of lipoproteins was started within 24 hours.

Lipoprotein Subfractionation

Subfractionation of LDL and HDL was performed by single-spin and sequential density-gradient ultracentrifugation, respectively. By the single-spin method 27 mL plasma was placed in a 38-mL Quick-Seal tube (Beckman No. 342414), underlaid with a potassium bromide solution of d=1.080 g/mL, centrifuged for 26 hours at 45,000 rpm in a Beckman 50.2 Ti rotor at 10°C, and then separated into five lipoprotein layers: layer 1, d<1.019 g/mL (5 mL); layer 2, d=1.021 to 1.028 g/mL (15 mL); layer 3, d=1.032 to 1.043 g/mL (7 mL); layer 4, d=1.054 to 1.073 g/mL (5 mL); and layer 5, d=1.073 g/mL (5 mL). This method was chosen because of the one-step isolation of LDL subfractions and the 100% recovery of proteins for quantitative analysis. To estimate TC and apoB distribution in subfractions, the layers were diluted without washing to their original plasma concentrations. To obtain lipoprotein subfractions (for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis), 3 mL of layers 1 and 2 and 1 mL of layer 3 were adjusted with solid potassium bromide to d=1.019 g/mL, d=1.028 g/mL, and d=1.043 g/mL, respectively, while layers 4 and 5 were adjusted to d=1.21 g/mL to include HDL. The floating lipoproteins were removed by pipette and dialyzed against several changes of 0.01 mol/L tris(hydroxymethyl)aminomethane (Tris) and Na2EDTA, pH 8.6. Sequential ultracentrifugation was used to isolate VLDL (d<1.006 g/mL) for determination of its electrophoretic mobility (β versus pre-β) and TC. To determine the density distribution of apoA-I, fresh plasma (within 4 hours after blood collection to avoid changes in density distribution and electrophoretic mobility) was adjusted with solid potassium bromide to d=1.087 and d=1.19 g/mL, overlaid with buffer of the respective densities, and ultracentrifuged for 48 hours at 45,000 rpm.

Lipid Measurements

TC, HDL-C, and TG concentrations in serum and TC in lipoprotein subfractions were determined by enzymatic procedures employing colorimetric end points. TC was determined according to Allain et al.,29 HDL-C initially by the dextran sulfate-Mg2+ precipitation method according to Warnick et al.,29 and HDL-C in DHC after initiation of feeding by the phosphotungstic acid-MgCl2 precipitation method according to März and Grossb9 and Assmann et al.37 TG was determined according to a modification of McGowen et al.36 Absorbances were measured in a Beckman DU-65 spectrophotometer at 500 nm for cholesterol and 540 nm for TGs. Samples with high TC, HDL-C, or TG levels were diluted with saline so that their values were in the linear range of the standard curve. All reagents and standards were obtained from Sigma (kits 352 for TC, 352-3 and 352-4 for HDL-C, and 339 for TGs).

SDS-PAGE

Lipoprotein subfractions (layers 1 through 4), not adjusted to the original plasma volume but with comparable volumes for each layer, were analyzed on a 3% to 30% gradient SDS-PAGE (15x17-cm vertical slabs). The following prestained high- and low-molecular-weight-protein standards were used: myosin, 200 kD; phosphorylase, 97.4 kD; bovine serum albumin, 68 kD; ovalbumin, 43 kD; carbonic anhydrase, 29 kD; β-lactoglobulin, 18.4 kD; lysozyme, 14.3 kD; and bovine trypsin inhibitor, 6.2 kD (BRL 6040 SA and 6041 LA). The subfractions (layer 1, 200 μL; layer 2, 16 μL; layer 3, 10 μL;
and layer 4, 2 μL) containing 1% SDS and 1 mmol/L dithiothreitol, were boiled for 5 minutes, mixed with 20 μL sample buffer (0.01 mol/L Tris, 1% glycerol, 1% SDS, and 0.01% bromophenol blue), and applied to the gel slots. Electrophoresis was performed overnight (16 to 18 hours) at room temperature and 40 V. The proteins were stained with 0.2% Coomassie brilliant blue G-250. ApoE, apoC-III, and apoA-I were verified by N-terminal amino acid sequence; they cross-reacted with antibodies to human apolipoproteins. Anti sera to human apoE, C-III, and A-I were generously provided by Dr Walter McConathy, Texas College of Osteopathic Medicine, Fort Worth, Tex.

Preparation of Antibodies

Isospecific anti-apoB antibodies were produced in sheep (NL563) that had been inoculated with apoB-100 purified by SDS-PAGE from an LDL fraction isolated by ultracentrifugation at d=1.010 to 1.050 g/mL from plasma of an adult Lpb27 pig. Anti-apoA-I antibodies were produced in sheep (2276) that had been inoculated with apoA-I purified by two-dimensional SDS-PAGE from an HDL fraction isolated by ultracentrifugation at d=1.09 to 1.17 g/mL from a young Lpb27 pig. A gel slice containing 15 to 20 μg apoB-100 or the major apoA-I isoform minced and mixed with 1 mL Freund's complete (initially) or incomplete adjuvant was used per injection. Injections were administered subcutaneously and intramuscularly at biweekly intervals. The single radial immunodiffusion39 and double immunodiffusion40 tests in agarose or Noble agar gel, respectively, and immunoblot analysis41 were used to detect the presence of anti-apoB or anti-apoA-I antibodies in the recipient sera; these were collected on the seventh day after each inoculation. Sufficiently strong and specific anti-apoB and anti-apoA-I for the use in this study were obtained after the fourth and seventh inoculations, respectively.

Measurement of ApoB and ApoA-I

The modified20,21 single radial immunodiffusion test39 with 0.18% anti-apoB and 20% anti-apoA-I immune serum, respectively, was used to measure apoB and apoA-I concentrations in sera, plasma, and their ultracentrifuge subfractions. The apoB standard was an LDL fraction of d=1.010 to 1.050 g/mL isolated by ultracentrifugation from plasma from an NL swine of the Lpb27 genotype. The apoA-I standard was obtained from a fraction isolated from the same plasma as apoB at d=1.09 to 1.17 g/mL and was further purified on a 2.5×100-cm Sephadex S-200 (6 mol/L urea, 0.01 mol/L Tris, 0.01% Na2EDTA, and 0.01% NaN3, pH 8.6) sizing column. Both secondary standards were subjected to amino acid analysis, and their protein concentrations were calculated using the Lowry22 method with bovine serum albumin as the primary protein standard.

To establish the regression equation for apoB and apoA-I, both standards were tested four times on three different plates, with 1, 2, 3, and 4 μL for apoB (53 mg/dL) and 4, 6, 8, 10, and 12 μL of a 1:6 dilution for apoA-I (39 mg/dL). The regression equation for apoB was y=−53.370+3.776e−x (r=0.982) and for apoA-I, y=−8.2876+8.6990e−x (r=0.982). Coefficient of variation was 0.4% to 1.4% for intra-assay and 1.3% to 8.6% for interassay. From this, 1.2% for intra-assay and 8% for interassay were adopted as the limit of change; variation between the duplicate values for an experimental sample.

Each experimental sample was tested in duplicate. The plates were photographed and enlarged 10× by an "HS" Opaque 1000 projector to measure the diameter of the precipitation ring. The apoB and apoA-I protein concentrations in each sample were calculated by using the area (x) in the regression equation.
FIG 1. Bar graphs showing mean percent distribution of total plasma cholesterol (top) and apolipoprotein B (bottom) in five lipoprotein subfractions (layer [Ly] 1, d<1.019 g/mL; Ly 2, d=1.021-1.028 g/mL; Ly 3, d=1.032-1.043 g/mL; Ly 4, d=1.054-1.073 g/mL; and Ly 5, d>1.073 g/mL) from plasma of three groups of pigs: normolipidemic (NL), diet-induced hypercholesterolemic (DHC), and familial hypercholesterolemic (FHC).

Data on estimated ratios of TC and HDL-C and apoB and apoA-I to each other in the three groups are shown in the Table. The FHC group showed the highest TC/HDL-C ratio, followed by DHC. The ratio in FHC was 2.5-fold higher (P<.001) than in DHC and 6-fold higher (P<.001) than in NL. The TC/apoB ratio was similar for NL and FHC but was much higher for DHC (P<.001). The apoA-I/HDL-C ratios were the same in NL and FHC but the ratio was 32% lower in DHC due to lipid-poor apoA-I particles in the d<1.087 g/mL fraction. The differences between ratios of both NL and FHC to DHC were significant (P<.001).

Distribution of Cholesterol, ApoB, and ApoA-I in Lipoprotein Subfractions

Mean percentage distributions of TC and apoB in the five plasma lipoprotein subfractions of each of the three groups are shown in Fig 1. In the NL group 87.7% of cholesterol was found in layers 3 through 5 (25±2.5%, 39.9±3.3%, and 22.8±5.0%, respectively). Layers 1 and 2 accounted for 3.2±0.9% and 9.2±5.7% of TC, respectively. In DHC 51.8% of TC was present in layer 2, which is the most buoyant LDL subfraction (d=1.021 to 1.028 g/mL). The remaining TC (36.2%) in DHC was present in layers 3 (21.5±5.9%) and 4 (14.7±1.6%), and the rest in layers 1 (5.6±2.6%) and 5 (6.6±1.0%). In the FHC group 81.7% of TC was found in layers 3 (44.9±4.1%) and 2 (36.8±6.2%) and the remainder in layers 4, 5, and 1 (13.5±3.4%, 29±0.9%, and 1.9±0.9%, respectively).

The apoB distribution in the five layers resembled the TC pattern except for layer 5 in NL and layers 4 and 5 in DHC. Most apoB in the NL group was distributed in layers 3 (36.6±5.2%) and 4 (34.0±6.5%), but in DHC the apoB was distributed in layer 2 (63.2±4.0%) and in FHC in layers 2 (32.5±7.0%) and 3 (44.0±3.7%). The remaining apoB was distributed in NL in layers 2 (12.6±4.4%), 5 (9.6±0.7%), and 1 (7.3±1.9%); in DHC in layers 3 (25.7±5.3%), 4 (4.7±1.2%), 1 (4.3±1.6%), and 5 (2.2±0.1%); and in FHC in layers 4 (17.3±5.5%), 1 (3.3±0.8%), and 5 (3.0±0.4%). Significant differences in the ratio of TC to apoB were observed between DHC and both FHC and NL in layers 1 through 3 (P<.001) and in layer 2 between NL and FHC (P<.01).

The distribution of apoA-I in the plasma lipoprotein fractions of NL, DHC, and FHC separated by preparative ultracentrifugation is illustrated in Fig 2. In all three groups the majority of apoA-I was found in the HDL fraction of d=1.087 to 1.19 g/mL, with 94%, 69%, and 85% in NL, DHC, and FHC, respectively. In DHC 8% of apoA-I was present in d<1.087 g/mL, whereas in NL and FHC less than 1% of apoA-I was present in this fraction. Marked differences in apoA-I content were
found in the d>1.19 g/mL fraction among the three groups (DHC, 23%; FHC, 15%; and NL, 5%).

Analysis of Apolipoproteins in Lipoprotein Subfractions by SDS-PAGE

Fig 3 illustrates the distribution of apolipoproteins in lipoprotein subfractions of equivalent layer volumes, obtained from one animal from each group, analyzed on a 3% to 30% gradient SDS-PAGE. Layer 1 of all three groups showed the presence of apolipoproteins corresponding to the major human VLDL apolipoproteins: apoB-100, apoB-48, apoE, apoC-II, apoC-III, and traces of apoA-I. In addition, a minor 22-kD protein corresponding to the major human VLDL apolipoproteins: a 3% to 30% gradient SDS-PAGE. Layer 1 of all three groups showed the presence of apoB and apoA-I, whereas DHC also showed low levels of apoE and traces of apoA-I. In addition, a minor 22-kD protein showing sequence homology with the 25-kD core fragment of the human complement 4-binding protein (Hasler-Rapacz JO, McConathy WJ, Niece RN, Rapacz J, 1992, unpublished data). Layers 2 and 3 of NL and FHC showed mainly apoB-100; the same layers from DHC also showed apoE and apoA-I. Layer 4 from NL and FHC showed the presence of apoB and apoA-I, whereas DHC also showed low levels of apoE and apoA-IV and much higher levels of apoA-I. Apolipoproteins in layer 5 of the three groups were similar to those in layer 4.

Agarose Gel Immunoelectrophoresis

Fig 4 illustrates electrophoretic mobility of VLDL- and apoA-I-containing lipoproteins identified by anti-apoB and anti-apoA-I, respectively, in the NL, DHC, and FHC swine and a human serum. NL and FHC showed the pre-β-migrating form of VLDL; the DHC group showed β-migrating VLDL. The VLDL (d<1.006 g/mL) cholesterol concentration in NL was 4.23, in FHC 3.37, and in DHC 45.66 mg/dL. Sera and HDL supernatant (serum after selective precipitation of apoB-containing particles used to assay HDL-C) from all three groups showed α-migrating apoA-I, whereas FHC and DHC also showed a β-migrating form of apoA-I that corresponds to the pre-β-migrating lipoproteins carrying apoA-I in human plasma. 45 ApoA-I in normolipidemic human serum migrated a little more slowly than in swine serum (Fig 4) and contained only traces of pre-β apoA-I. The two forms of apoA-I-containing lipoproteins in swine were separated by preparative ultracentrifugation at d=1.19 g/mL into α-migrating apoA-I (d<1.19 g/mL) and β-migrating apoA-I (d>1.19 g/mL) fractions (results not shown).

Discussion

We extended information on plasma lipids, lipoproteins, and the two major apolipoproteins, apoB and apoA-I, in LDL and HDL of three groups of swine (NL, DHC, and FHC). Whereas the NL group is the species reference of normal parameters for the studied lipids, lipoproteins, and apolipoproteins, the two hypercholesterolemic species represent blood plasma lipid disorders with distinctly different etiologies. DHC is produced by physiologically abnormal high levels of exogenous cholesterol that saturate the mechanisms that clear cholesterol, as indicated by increases of LDL and HDL and by the appearance of β-VLDL and HDLc, which may represent HDL overloaded with exogenous cholesterol. 46 FHC, however, is a genetically determined disorder that may result from either oversynthesis or defective metabolism of plasma lipoproteins that transport endogenous cholesterol between body tissues. 47 Consequently, the two cholesterol are transported, at least in part, by different lipoproteins; this difference in transport is reflected in the composition and concentration of their constituents as well as in atherosclerotic lesion composition and development. 30,31

Among similarities observed between DHC and FHC in regard to NL are significant increases in LDL levels, their shift to lower densities, highly elevated TC and apoB levels, and the appearance of β-migrating apoA-I-containing lipoproteins. In spite of these similarities, TC in DHC was significantly higher and shifted further to the lower densities than in FHC. In sharp contrast, apoB was higher in FHC than in DHC, resulting in a
FIG 4. Immunoelectrophoresis in agarose gel of swine plasma lipoprotein fractions, serum, and high-density lipoprotein (HDL) supernatant (serum after selective precipitation of apolipoprotein [apo] B-containing particles used to assay HDL cholesterol) from normohpidemic (NL), diet-induced hypercholesterolemic (DHC), and familial hypercholesterolemic (FHC) swine and human (H) sera and HDL supernatant. B1, Very-low-density lipoprotein (d<1.006 g/mL); B2, plasma fraction of d>1.006 g/mL reacted with antibodies to swine apoB (B); A3, normal serum; A4, HDL supernatant reacted with antibodies to swine apoA-I (A), A5, human serum; and A6, HDL supernatant reacted with antibodies to human apoA-I.

twofold higher TC/apoB ratio in DHC, suggesting that LDL particles may be significantly less dense in DHC than in FHC. Terpstra et al48 show similar changes in DHC swine, including an increase in the proportion of free and esterified cholesterol in LDL (126% and 12%, respectively) and a reduction of total protein in LDL by 60%. However, the high ratio of TC to apoB in DHC could also be produced in part by additional lipoprotein particles in LDL, namely HDL₄, containing apoA-I, apoC, and apoE but not apoB.13,18,46 Our DHC data agree with studies showing the elevation of apoA-I, apoC-III (the major C peptide in swine49), and apoE; however, in FHC and NL swine the lipoprotein fractions showed much lower levels of the three apolipoproteins. While we did not seek direct evidence for HDL₄ in DHC, plasma fractionated at d=1.087 g/mL showed that about 8% of apoA-I was present in the d<1.087 g/mL fraction in DHC, whereas less than 1% of apoA-I was found in this fraction in NL and FHC. However, in contrast to earlier studies,13,18,46 we also found increases of apoA-I in d>1.19 g/mL (23%) in comparison with 5% in NL and 15% in FHC. The apoA-I/HDL-C ratio was the same for NL and FHC, but 32% lower for DHC, despite the lipid-rich particles in d<1.087 g/mL, likely due to the increase of lipid-poor apoA-I particles in d>1.19 g/mL (β-migrating).

Mahley46 reports that the HDL₄ particle enriched by esterified cholesterol behaved like LDL in at least two ways: it competed equally effectively with ¹²⁵I-LDL for binding and internalization by human fibroblasts,46,50 and it was precipitated by heparin-manganese at the lowest concentration required for LDL-C precipitation.50 Since the heparin-manganese method has been found least reliable for precipitating all apoB-containing particles,35 we used the dextran sulfate-Mg²⁺ and phosphotungstic acid-MgCl₂ precipitation methods, which gave complete precipitation of apoB-containing particles in FHC and NL with comparable results (1% difference between means). Because in DHC lipemic serum the dextran sulfate-Mg²⁺ method did not precipitate all apoB-containing particles, but the phosphotungstic acid-MgCl₂ method did, we used the latter method for HDL-C in DHC.

The most noticeable differences between the DHC and FHC groups were electrophoretic mobility of VLDL and concentrations of VLDL cholesterol, HDL-C, and apoA-I, which were highly elevated in DHC, including the apoA-I/apoB ratio, but low in FHC. VLDL cholesterol and HDL-C in DHC were increased 10- and 4-fold, respectively, over NL, but the level of VLDL cholesterol in FHC did not differ significantly from NL, and HDL-C in DHC was 44% lower than in NL. VLDL cholesterol seems to bear directly on the electrophoretic mobility of swine VLDL; low VLDL cholesterol in NL and FHC showed pre-β-mobility, whereas high VLDL cholesterol in DHC exhibited β-mobility, most likely resulting from the increase of apoB associated with lipoproteins transporting physiologically abnormal high levels of exogenous cholesterol. While our DHC data agree closely with earlier studies13,18 regarding VLDL mobility and VLDL cholesterol concentrations, they differ partially or significantly18 by showing severalfold higher levels of HDL-C and significantly higher levels of apoA-I than NL. In contrast to these earlier studies,13,18 recent DHC data46 and
earlier data observed in Yucatan miniature swine agree with our DHC data by showing significant increases in HDL-C. This increase of HDL-C in DHC and its low levels in FHC correlate with the significant difference of apoA-I/apoB ratios observed between the two groups.

Most observations in humans led to the existing consensus that elevated HDL represents an antiatherogenic factor. In contrast to this and in agreement with our DHC data, Warden et al. show that an increase of atherosclerotic lesions in transgenic mice with overexpressed apoA-II was accompanied by an elevation in HDL-C, suggesting that both the composition and amount of HDL may be important determinants of atherosclerosis. We have shown the development of atherosclerotic lesions by feeding an atherogenic diet to swine of the same origin as the present DHC group. In the present study we showed that the significant increase in HDL-C in DHC was accompanied by a change in the apolipoprotein compositions of HDL, which differ between FHC and NL and may influence lipid transport.

We hope that additional studies currently in progress on the effects on atherosclerosis of diet-genotype interaction and von Willebrand disease will provide new information on the observed variations in HDL-C and apolipoprotein composition and their role in atherosclerosis. Preliminary data suggest that the origin of swine and apoB genotypes influence the response to a high-fat and high-cholesterol diet, showing significant variations not only in TC (from 474 to 1396 mg/dL) but also in HDL-C (from 38 to 150 mg/dL). One compositional difference observed among pigs of different origin in response to feeding cholesterol is the appearance of apoA-IV in domestic swine (present study) and Yucatan miniature pigs but not in Hormel miniature pigs.

Investigations of the electrophoretic mobility of apoA-I-containing lipoproteins was stimulated by observations (Hu Z-L, Hasler-Rapacz JO, Rapacz J, 1992, unpublished data) of two forms of apoA-I in FHC swine. Using salt densities, the two electrophoretic forms were separated by preparative ultracentrifugation. Investigations (Hu Z-L, Hasler-Rapacz JO, Rapacz J, 1992, unpublished data) of two forms of apoA-I in FHC swine of the same origin as the present DHC group. In the present study we showed that the significant increase in HDL-C in DHC was accompanied by a change in the apolipoprotein compositions of HDL, which differ between FHC and NL and may influence lipid transport.

We hope that additional studies currently in progress on the effects on atherosclerosis of diet-genotype interaction and von Willebrand disease will provide new information on the observed variations in HDL-C and apolipoprotein composition and their role in atherosclerosis. Preliminary data suggest that the origin of swine and apoB genotypes influence the response to a high-fat and high-cholesterol diet, showing significant variations not only in TC (from 474 to 1396 mg/dL) but also in HDL-C (from 38 to 150 mg/dL). One compositional difference observed among pigs of different origin in response to feeding cholesterol is the appearance of apoA-IV in domestic swine (present study) and Yucatan miniature pigs but not in Hormel miniature pigs.

Investigations of the electrophoretic mobility of apoA-I-containing lipoproteins was stimulated by observations (Hu Z-L, Hasler-Rapacz JO, Rapacz J, 1992, unpublished data) of two forms of apoA-I in FHC swine. Using salt densities, the two electrophoretic forms were separated by preparative ultracentrifugation at ρd = 1.19 g/mL into the α-mobility (ρd < 1.19 g/mL) and β-mobility (ρd > 1.19 g/mL) forms. Further characterization of these two apoA-I forms in swine is in progress. The β-migrating apoA-I in swine plasma corresponds to the pre-β apoA-I-containing lipoproteins in humans and mouse sera, although in human serum lipoproteins containing apoA-I migrate a little more slowly than in swine. Our observations on elevated levels of β-migrating apoA-I in the hypercholesterolemic state agree with observations in humans, in which pre-β apoA-I has been found in hypercholesterolemic, hypertriglyceridemic, and LCAT-deficient plasma. High TC levels in the FHC strain are associated with decreased LCAT activity.

The inclusion of TG measurements in this study was stimulated by earlier observations (J.R. and J.O.H.-R., 1993, unpublished data) that showed that after a period of considerable intravariability fluctuation, from 17 to 225 mg/dL in pigs less than 3 months of age, the TG level stabilized and became considerably higher in some FHC than NL. The present study confirmed this observation but showed that the difference is significant only between FHC and NL, although the TG level in DHC was closer to NL than FHC. In spite of relatively low elevations of TG in FHC, its relevance to lipid disorders linked to atherosclerosis may be by association, as it is in humans, with decreased HDL-C and apoA-I and increased apoB and apo-C-III, which are characteristic of type IIb FHC.

In conclusion, we established new elements in swine dyslipidemias. In FHC these include considerable reductions in HDL-C and apoA-I concentrations and high ratios of TC/HDL-C and apoA-I/apoB; in DHC we showed significant increases in HDL-C. The new elements in the FHC swine are of particular interest, because together with complex types of atherosclerotic lesions, they show correspondence to human familial combined hyperlipidemia, the most frequent type among familial dyslipidemias.

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References


Familial and diet-induced hypercholesterolemia in swine. Lipid, ApoB, and ApoA-I concentrations and distributions in plasma and lipoprotein subfractions.
J O Hasler-Rapacz, T C Nichols, T R Griggs, D A Bellinger and J Rapacz

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