Obese Pigs Fed a High Cholesterol Diet from Birth to 2 Months Are Less Susceptible than Lean Pigs to Atherosclerosis\textsuperscript{1,2,3}

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**Abstract** Dietary cholesterol in infancy may alter cholesterol metabolism and the propensity to develop atherosclerosis. This study examined the effects of a 1% cholesterol diet (HC) vs. a no-cholesterol diet (NC) during the first 2 mo of life on pigs selectively bred for leanness or obesity. Three lean and three obese pigs received the no-cholesterol diet, and four lean and four obese pigs received the 1% cholesterol diet from d 1. Lean and obese pigs fed the no-cholesterol diet showed no increase in serum lipid concentrations, nor did they develop atherosclerosis. Obese pigs fed the 1% cholesterol diet developed significantly higher serum total cholesterol (TC) and high density lipoprotein cholesterol (HDL-C) at 35 d than lean pigs fed the 1% cholesterol diet. By d 55, only HDL-C remained significantly higher in the obese pigs, resulting in a higher (P < 0.1) TC/HDL-C ratio in the lean pigs. Atherosclerotic plaque formation in the aorta was more extensive in the lean pigs. Cholesterol synthesis measured in vivo and at termination was equally suppressed in lean and obese pigs fed the 1% cholesterol compared with pigs fed the no-cholesterol diet. We conclude that genetic differences in the response of these lean and obese pigs to a high cholesterol diet render obese pigs less susceptible to atherosclerosis despite higher serum TC concentrations. The persistent elevation of HDL-C in obese pigs is the most likely mechanism of protection. J. Nutr. 126: 564–573, 1996.

**Indexing Key Words:**
- cholesterol
- atherosclerosis
- infant
- obesity
- pigs

Atherosclerosis as it relates to coronary artery disease, myocardial infarction and cerebral vascular disease is the leading cause of morbidity and mortality in the adult populations of industrialized nations. Human autopsy studies have confirmed the presence of fatty streaks in the aortae of many children by 3 y of age similar to the early lesions seen in coronary artery disease (Holman et al. 1958). An elevated level of serum total cholesterol, specifically low density lipoprotein cholesterol (LDL-C),\textsuperscript{5} is associated with an increased incidence of atherosclerosis, whereas high density lipoprotein cholesterol (HDL-C) is protective against atherosclerosis (Castelli et al. 1977, Kannel et al. 1979,

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\textsuperscript{5}Abbreviations used: FSR, Fractional synthesis rate; HC, 1% cholesterol diet; HDL-C, high density lipoprotein cholesterol; HMG-CoA, 3-hydroxy-3 methylglutaryl coenzyme A; LDL-C, low density lipoprotein; NC, no-cholesterol diet; TC, total cholesterol; TG, triglycerides; VLDL-C, very low density lipoprotein cholesterol.
Atherosclerosis in Lean and Obese Pigs

Mahley 1979). The Bogalusa Heart Study of over 3,500 children from a semirural community in Louisiana demonstrated a high correlation between premortem serum cholesterol and LDL-C levels and the extent of aortic fatty streaks, and a moderate correlation between obesity and fatty streaks in the aorta as well as the coronary arteries in 30 white males age 6–30 y at autopsy (Berenson et al. 1992, Newman et al. 1986). A second autopsy study of 390 males age 15–34 y, called the Pathobiological Determinants of Atherosclerosis in Youth (1990) study, also found serum cholesterol (specifically, the sum of LDL-C + VLDL-C) to be positively correlated with the extent of atherosclerosis in the aorta and right coronary arteries of their subjects. HDL-C was negatively associated with atherosclerosis in both studies. Studies such as these have prompted recommendations for moderation in dietary fat and cholesterol consumption beginning at 2 y of age (National Cholesterol Education Program 1992). Because commercial infant formulas contain little cholesterol in comparison with breast milk, information is needed regarding the role of cholesterol in infant nutrition, particularly with respect to its potential role in the regulation of serum cholesterol levels and the development of atherosclerosis.

Pigs are widely used as an experimental model for human atherosclerosis due to their spontaneous development of atherosclerosis and the fact that it can be induced by feeding pigs a diet high in lipids (Pond 1982). In addition, there are striking similarities between human and pig lipoproteins and apolipoproteins which modulate cholesterol transport and receptor interaction (Black and Davidson 1989). Because obesity and hypercholesterolemia are associated with more extensive atherosclerosis in young children (Berenson et al. 1992, Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group 1990), populations of genetically obese and lean pigs were used to evaluate the effects of obesity and dietary cholesterol on serum lipid levels and the development of atherosclerosis in infancy. Previous studies in these lean and obese pigs have demonstrated a rapid increase in the serum cholesterol of obese pigs in response to a high fat, high cholesterol diet between 2 and 5 wk of age which is distinct from the more gradual increase seen in the lean pigs (Patterson et al. 1992). The goals of the present experiment were to determine whether the higher cholesterol levels in the obese pigs resulted in more extensive atherosclerosis, and whether the differences in cholesterol levels between lean and obese pigs could be explained by inadequate suppression of cholesterol biosynthesis during high cholesterol feeding.

**MATERIALS AND METHODS**

**Design.** The seven genetically lean piglets (four male and three female) and seven genetically obese piglets (five male and two female) to be used in this 8-wk experiment were removed from their dams on d 1 of life after receiving colostrum. A sample size of four animals per group with differences of 1.0–2.5 SD between treatment groups has been shown previously in neonatal pigs to be adequate to detect the expected differences at P < 0.05. The coefficient of variation (CV) of most of the traits tested is 10–30%. The experimental protocol was approved by the Animal Care and Use Committee, Baylor College of Medicine, to ensure ethical and humane treatment of animals. The parental strains of Yorkshire and Duroc pigs were derived by selection for low or high back-fat thickness for 14 and 18 generations, respectively (Hetzer and Harvey 1967). The current pigs were derived by mating Duroc lean × Yorkshire lean and Duroc obese × Yorkshire obese. The F1 generation formed the basis for the current lean and obese pigs, and both groups have been maintained for many generations with no further genetic selection. The 14 piglets were randomly assigned within genetic groups in a 2 × 2 factorial arrangement of genetic group and diet to receive either a modified Sowena diet (Merrick Foods, Union Center, WI) containing no cholesterol (NC) or the same diet with 1% crystalline cholesterol (USP Grade, ICN Nutritional Biochemicals, Cleveland, OH) added by weight (HC) (Table 1). In each genetic group, four piglets received HC [three male and one female obese; three male and one female lean] and three piglets received NC [two male and one female obese; one male and two female lean]. The concentration of protein (250 g/kg diet) and fat (115 g/kg diet) and the ratio of polyunsaturated to saturated fatty acids (0.18) were essentially the same for both diets. The crystalline cholesterol in the HC diet was added to the dry feed and mixed thoroughly in a twin-shell dryer blender. Liquid diets were prepared by mixing the dry feed with water (4 parts water to 1 part dry feed by weight) in a Waring blender.

Animals were housed in individual cages (1.14 m × 1.14 m) in two separate rooms maintained at a temperature of 30°C. Heat lamps and warming boards placed over half of the cage floor were used during the first 2 wk to increase the temperature within the cages to 34–36°C. Cage size was increased to 2.27 m × 1.14 m once pigs reached a weight of ~15 kg, and the room temperature was gradually decreased to 22–23°C.

**Feeding.** Piglets were offered 60 mL (12 g dry feed) of liquid diet in stainless steel bowls four times per day for the first 2 d of life. The volume of liquid was gradually increased from d 3 to d 4, and the ratio of water to dry feed was gradually decreased from d 4 to d 5. Water was made continuously available through a nipple water dispenser attached to the rear of the cage starting on d 5. Stainless steel feeders were attached to the cages, and feedings were divided between the feeders and bowls for d 1. By d 6, all piglets had been trained to eat dry feed ad libitum from the feeders. Daily feed intake was estimated, but it was not possible to quan-
The composition of dry diets is shown in Table 1. Coconut oil, corn oil, soybean flour, whey protein concentrate, calcium caseinate, whey, dicarboxyl phosphate, propylene glycol, Soweena Pig Krave Extra, Vitamin Premix, mineral premix, choline chloride, antioxidant/mold inhibitor, neomycin sulfate, and cholesterol (USP grade) were used in the diets. The amount of each ingredient was varied to produce diets with different cholesterol levels. The diets were formulated as described by Wong et al. (1991). In the presence of deuterium oxide, the rate of cholesterol synthesis can be estimated from the increase in deuterium enrichment of erythrocyte-free cholesterol. On day 35, a loading dose of 500 mg/kg \( \text{H}_2\text{O} \) (99.8 atom % \(^2\text{H}\) from Merck Sharp and Dohme, St. Louis, MO) was administered by subcutaneous injection into the fat in the neck behind the ear. From day 36 through the time of necropsy, a maintenance dose of 70 mg/kg was injected in the same fashion. Daily post-dose blood samples were collected on day 36 through day 38. Red blood cells from these samples were disrupted by sonication using hexane-isopropanol 4:1 (v/v) to separate the lipids. Neutral lipids were isolated from the crude lipid extract using a 0.5-g silica solid phase extraction column (Altech Associates, Deerfield, IL). Cholesterol was purified by HPLC using a Waters Delta-prep 5000 preparative HPLC system with a Waters Model 490 programmable diode array detector (Waters Associates, Milford, MA), and a FOXY peak separator/fraction collector (Isco, Lincoln, NE). Approximately 3–5 mg of cholesterol isolated from each sample was transferred in a small volume of hexane to a quartz tube (Quartz Scientific, Freeport Harbor, OH) containing ~1 g cupric oxide. Hexane was removed and the tube was sealed under vacuum. The sealed tube was heated to 850°C for 1 h. The \( \text{CO}_2 \) and \( \text{H}_2\text{O} \) produced from the combustion of cholesterol in the sealed tube were then frozen with liquid nitrogen. The \( \text{CO}_2 \) was removed, and the \( \text{H}_2\text{O} \) was transferred to a reduction vessel and was reduced to \( \text{H}_2 \) gas for isotope ratio measurement as described by Wong et al. (1987). The \(^2\text{H}:\text{H} \) ratio of the hydrogen gas was measured with a Finnigan Delta-E gas-isotope-ratio mass spectrometer (Finnigan MAT, San Jose, CA) and expressed in \(^2\text{H} \) units as follows:

\[
^{2}\text{H}, \, 0/00 = (R_S/R_{WS} - 1) \times 10^3
\]

where \( R_S = ^2\text{H}:\text{H} \) ratio of sample and \( R_{WS} = ^2\text{H}:\text{H} \) ratio of laboratory standard.

A single pool model was used to compute the fractional synthesis rate of cholesterol from the mean \(^2\text{H} \) enrichment of body water \( ^2\text{H}_{\text{water}} \) and the rise in \(^2\text{H} \) enrichment of erythrocyte cholesterol \( ^2\text{H}_{\text{cholesterol}} \) according to the following equation:

\[
2\text{H}_{\text{cholesterol}} = 0.4783 \times 2\text{H}_{\text{water}} \times (1 - e^{-k})
\]

The constant 0.4783 is the fractional number of hydrogen atoms in cholesterol (22 out of 46 total hydrogen atoms) which are derived from body water during biosynthesis. The constant \( k \) (d\(^{-1}\)) is the rate constant for the incorporation of \(^2\text{H} \) into the erythrocyte cholesterol. The \( k \) values are converted to fractional synthesis rate (FSR) by the following equation:

\[
\text{FSR} \,(\%\,/\text{d}) = k \times 24 \times 100
\]

**Tissue collection.** Animals were killed by an overdose of ketamine/acepromazine administered intramuscularly, injection followed by exsanguination. Their
ages ranged from 58 to 73 d (mean 65 ± 4.2) arranged according to a schedule that resulted in no statistically significant difference in the mean age at time of killing among the four groups. The liver was removed immediately. A section of the right lobe (~20 g of tissue) was resected and placed in a beaker of 154 mmol/L NaCl on ice. Liver, spleen, kidneys, and brain were quickly removed and weighed. The heart and aorta were removed intact down to the iliac bifurcation, weighed immediately fixed in 10% buffered formalin, and later sectioned at 5 µm, stained with hematoxylin and eosin, Oil-Red-O, Alcian Blue and PAS, and treated with monoclonal antibody to muscle actin (ENZO Diagnostics, New York, NY).

**Hepatic hydroxymethylglutaryl (HMG)-CoA reductase activity assay.** Liver tissue was transported from necropsy room to laboratory in 154 mmol/L NaCl on ice for immediate processing. Tissue was scissors-minced and pushed through a tissue press. Two grams of the pressed liver was suspended in 18 mL ice-cold homogenization buffer (0.3 mol/L sucrose, 50 mmol/L NaCl, 10 mmol/L NaEDTA, 10 mmol/L dithiothreitol, pH 7.4 with NaOH). The samples were homogenized in a motor-driven Potter Elvehjem homogenizer at ~55 rpm. After seven strokes, the homogenate was centrifuged at 8500 × g for 15 min at 4°C. The supernatant fraction was centrifuged at 100,000 × g for 60 min at 4°C. The microsomal pellets from each 2-g sample of liver were resuspended in 7 mL homogenization buffer and kept on ice until assay.

The microsomal fraction was assayed for 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity using minor modifications of the method described by Brown et al. (1979). The assay for porcine hepatic HMG-CoA reductase has been optimized in this laboratory regarding the length of preincubation, the composition of homogenation and assay media, and the saturating substrate concentration. The assay is within the linear range for microsomal protein and time (V. McWhinney, personal communication). Ninety microliters of microsomal suspension (~400 µg protein) was preincubated at 37°C for 60 min. After preincubation, the following were added to yield a final concentration of 0.2 mol/L KH₂PO₄, 40 mmol/L glucose-6-phosphate, 5 mmol/L NADP, 20 mmol/L NaEDTA and 10 mmol/L dithiothreitol adjusted to pH 7.4 with KOH at 21°C. In addition, there was 1 unit glucose-6-phosphate dehydrogenase (Type XII from Torula Yeast, Sigma Chemical, St. Louis, MO), 25 µL 176 µmol/L HMG-CoA (Sigma) and 3.7 kBq [¹⁴C] HMG-CoA (New England Nuclear, Boston, MA) in a volume of 210 µL. The reaction, in triplicate, was allowed to proceed for 15 min at 37°C. The reaction was stopped by the addition of 25 µL 6 mol/L HCl and then incubated at 37°C for 30 min to promote the conversion of mevalonolactone to mevalonolactone.

The derivative of the reaction product, [¹⁴C]mevalonolactone, was separated from [¹⁴C]HMG-CoA by TLC. Denatured protein was removed by centrifugation, and deproteinized samples (66 µL) plus unlabeled mevalonolactone (20 µL of a 0.15 mmol/L solution) to act as a carrier were spotted onto the loading zone of a 5-mm lane on a silica gel G TLC plate (Analtech 250 µm) that had been activated at 110°C for 1 h prior to use. The TLC plates were developed in toluene:acetone 1:1 (v/v) for 15 cm. The area from 0.3 to 0.7 Rf was scraped into a scintillation vial. [¹⁴C]Mevalonolactone was quantified by counting in a liquid scintillation counter. The protein concentration in each assay was determined using the Sigma Diagnostics Protein Assay Kit (Sigma Diagnostics, St. Louis, MO) after precipitation of the protein with trichloroacetic acid. The HMG-CoA reductase activity was expressed as pmol of mevalonolactone·mg microsomal protein⁻¹·min⁻¹.

**Pathology.** The heart with the left ventricle opened longitudinally and the aorta to the bifurcation of the iliac arteries were fixed in a 10% buffered formalin. Slices were excised transversely of the left and right coronary arteries, and the ascending branch of the left coronary artery, and longitudinally of the origin of the aorta with the aortic valve. The aorta was opened longitudinally and inspected for plaques. The size of the longitudinal plaques ranged from 2 mm × 1 mm to 20 mm × 7 mm. Grades were assigned to each aorta according to plaque size: 0 = absent; 1 = mild; 2 = moderate; 3 = severe, to the lowest, middle and highest one third of plaque size, respectively. Sections were obtained transversely 5 and 18 cm distal to the aortic valve and transversely through any plaques. The pathologist was unaware of the treatment group of each animal.

The slices were embedded in paraffin, sectioned at 4 µm and stained with hematoxylin and eosin (H&E). Selected sections of grossly visible plaques were stained with PAS and Alcian Blue. Frozen sections at 5 µm were stained with Oil-Red-O. Five-micrometer sections of paraffin-embedded tissues were deparaffinized, washed and treated to destroy endogenous peroxidase. A monoclonal antibody to muscle actin (ENZO Diagnostics) was applied at 1:20 dilution for 2 h at 37°C. After washing, localization of antibody binding was determined with biotinylated anti-mouse immunoglobulin and streptavidin peroxidase conjugate. The chromogen was diaminobenzidine. In negative control sections, ascites fluid replaced the primary antibody.

**Statistics.** Statistical analysis was performed using ANOVA with repeated measures (BMDP 1990) for body weight, total protein, albumin, triglyceride, TC, HDL-C and LDL-C + VLDL-C. Differences with respect to genetic group, dietary cholesterol consumption and time were tested for main effects as well as for interaction. Fisher’s LSD (least significant difference) test for multiple comparisons was utilized in the presence of significant time-by-diet or time-by-genetic group interaction to detect differences at individual time points (Milliken and Johnson 1987). There were...
There was no genetic or dietary effect on the weights of liver or brain when corrected for body weight (Table 2). The weights per kilogram body weight of the intact heart and aorta and the spleen were higher in the lean strain (P < 0.01) regardless of diet. Independent effects of diet and genetic group were noted with respect to relative kidney weights in that the lean pigs had larger kidneys than the obese (P < 0.04), and pigs fed the no-cholesterol diet in each genetic group had larger kidneys than littersmates fed 1% cholesterol (P < 0.02).

**Serum total protein and albumin.** Total protein and albumin in serum were measured on d 1, 15, 35 and 55. There were no differences with respect to serum total protein or albumin concentrations between pigs fed no cholesterol and those fed 1% cholesterol. Lean pigs had a higher total protein level [NC = 55 ± 4 g/L; HC = 52 ± 4 g/L] than obese pigs [NC = 49 ± 7 g/L; HC = 51 ± 5 g/L] (P < 0.05). Lean pigs also had a higher mean serum albumin level [NC = 33 ± 12 g/L; HC = 29 ± 11 g/L] compared with the obese pigs [NC = 28 ± 13 g/L; HC = 28 ± 13 g/L] (P < 0.01).

**Serum lipid profile.** Serum triglyceride, total cholesterol and high density lipoprotein cholesterol were measured and LDL-C + VLDL-C was calculated on d 1, 15, 35, and 55. Serum TG decreased with time on study (Fig. 2A). The lean pigs had higher TG levels than obese pigs at 1 d of age after all pigs had received colostrum (P < 0.05). At 15 d, however, the obese pigs had higher TG levels than the lean pigs regardless of diet (P < 0.05). The mean triglyceride concentration over the course of the experiment was not affected by diet or genetic group (Table 3); however, LDL-C + VLDL-C concentration increased with time (Fig. 2C) in both lean and obese pigs fed dietary cholesterol (P < 0.001).

Total cholesterol (Fig. 2B) and HDL-C (Fig. 2D) also rose in both genetic groups of pigs fed 1% cholesterol and remained nearly constant in those fed no-cholesterol diets (P < 0.01). There was a genetic influence on the rise in TC and HDL-C as well, with the obese strain demonstrating a greater concentration in both variables at d 35 (P < 0.01). By d 55, the TC levels of the two genetic groups of pigs fed 1% cholesterol were comparable. The HDL-C levels remained significantly higher

### RESULTS

**Growth.** Body weight measurements on d 1 through d 6, then at 7- to 10-d intervals during the experiment, showed no significant difference between animals fed 1% cholesterol and those fed no-cholesterol diets. All four groups of pigs followed parallel growth curves (Fig. 1). Lean pigs were larger than obese pigs beyond 32 d of age (P < 0.01). Organ weights are shown in Table 2.

![FIGURE 1 Body weights of lean and obese pigs fed no-cholesterol or 1% cholesterol diets. Values are means ± SD. Final mean values are at the time of necropsy (mean age 65 ± 4.2 d). Lean pigs were heavier than obese pigs (P < 0.01).](https://academic.oup.com/jn/article-abstract/126/2/564/4724621)

### TABLE 2

Relative weights of major organs at necropsy in lean and obese pigs fed either the no-cholesterol (NC) or 1% cholesterol (HC) diet

<table>
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</thead>
<tbody>
<tr>
<td>Liver, g/kg body wt</td>
<td>24.00 ± 0.30</td>
<td>24.00 ± 6.00</td>
<td>25.00 ± 3.00</td>
<td>28.00 ± 4.00</td>
<td>0.555</td>
</tr>
<tr>
<td>Heart and aorta, g/kg body wt</td>
<td>5.20 ± 0.10</td>
<td>5.40 ± 0.04</td>
<td>4.60 ± 0.30</td>
<td>4.30 ± 0.20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Brain, g/kg body wt</td>
<td>2.30 ± 0.30</td>
<td>2.20 ± 0.30</td>
<td>2.40 ± 0.10</td>
<td>2.30 ± 0.30</td>
<td>0.384</td>
</tr>
<tr>
<td>Spleen, g/kg body wt</td>
<td>2.30 ± 0.50</td>
<td>2.30 ± 0.30</td>
<td>1.00 ± 0.06</td>
<td>1.30 ± 0.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Kidneys, g/kg body wt</td>
<td>6.80 ± 0.60</td>
<td>5.50 ± 0.80</td>
<td>5.60 ± 0.60</td>
<td>5.00 ± 0.60</td>
<td>0.038</td>
</tr>
</tbody>
</table>

1 Values are means ± SD. GG, genetic group.
in the obese pigs at d 55 ($P < 0.01$). The TC/HDL-C ratio tended ($P < 0.1$) to be greater in the lean pigs at 35 d and at 55 d compared with the obese pigs (Fig. 3). Although serum LDL-C + VLDL-C concentrations were higher in the obese pigs fed cholesterol than in the lean pigs fed cholesterol at d 35, the overall difference between obese and lean pigs also was not statistically significant (Fig. 2C; $P > 0.05$).

**Fractional cholesterol synthesis rates.** There was a significantly lower FSR of cholesterol in both the lean and obese pigs fed the 1% cholesterol diet in comparison with their littermates fed the no-cholesterol diet.

### TABLE 3

<table>
<thead>
<tr>
<th>Mean levels d 1–55</th>
<th>Lean NC ($n = 3$)</th>
<th>Lean HC ($n = 4$)</th>
<th>Obese NC ($n = 3$)</th>
<th>Obese HC ($n = 4$)</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>$2.61 \pm 0.21$</td>
<td>$8.15 \pm 0.67$</td>
<td>$3.03 \pm 0.21$</td>
<td>$10.03 \pm 3.46$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>$0.93 \pm 10.04$</td>
<td>$1.24 \pm 0.16$</td>
<td>$1.14 \pm 0.15$</td>
<td>$1.81 \pm 0.31$</td>
<td>$0.00$</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>$0.56 \pm 0.05$</td>
<td>$0.56 \pm 0.14$</td>
<td>$0.59 \pm 0.17$</td>
<td>$0.70 \pm 0.15$</td>
<td>$0.31$</td>
</tr>
<tr>
<td>Total/HDL cholesterol, mol/mol</td>
<td>$2.83 \pm 0.07$</td>
<td>$6.20 \pm 1.18$</td>
<td>$2.75 \pm 0.12$</td>
<td>$4.82 \pm 0.42$</td>
<td>$0.08$</td>
</tr>
</tbody>
</table>

1 Values are means ± SD. GG, genetic group.
and degrees are depicted in Figure 5. Unpaired t test comparison of the mean values of obese and lean pigs fed the high cholesterol diet showed that lean pigs had more severe atherogenesis than obese pigs \(P < 0.05\). Plaques were most common 3–5 cm from the origin of the aorta but occurred up to 18 cm distally. The plaques were solitary or multiple, but there were never more than four in any given animal. They were slightly elevated with a rough surface of the same color as the surrounding aorta.

Microscopically, the plaques developed in the tunica intima. The endothelium was intact. The intima was up to 0.5 mm thick and consisted of degenerate fibers often with preserved nuclei. The cytoplasm sometimes showed large vacuoles with hematoxylin and eosin

**FIGURE 5** The incidence and degree of aortic atherosclerosis plaques in obese and lean pigs fed no-cholesterol or 1% cholesterol diet. Lean pigs fed high cholesterol had more severe atherosclerosis than obese pigs \(P < 0.05\). \(0 = \text{absent}, 1 = \text{mild}, 2 = \text{moderate}, 3 = \text{severe}\). X, individual pigs; ■, ○ are means ± SD; NC, no-cholesterol; HC, high cholesterol; atherosclerosis was more extensive in pigs fed HC vs. NC diet \(** P < 0.01\). \(P = 0.08\) for lean vs. obese genetic groups.
stain (H&E). Oil-Red-O revealed a moderate amount of fine fat granules but there were never accumulations of fat globules to explain the vacuoles in the H&E sections. The vacuoles were negative with Alcian Blue (no mucin) and with PAS. The elastica interna was poorly defined and frequently disrupted. The origin of the fibers of the tunica intima could not be defined with certainty with conventional stains. Immunochemistry for muscle actin was therefore performed.

Arterioles unaffected by plaque formation provided internal controls for the immunochemistry (Fig. 6, left). In the normal vessels, cytoplasmic processes of cells of the tunica media expressed muscle actin, whereas cells of the tunica intima and adventitia were negative as expected. In aortic plaques, slender fusiform cells within the expanded tunica intima expressed the myogenic antigen (Fig. 6, right).

**DISCUSSION**

Although cholesterol may play an important role in infant nutrition, there were no differences in the growth rates or serum protein concentrations of pigs fed a diet containing 1% cholesterol as compared with those deprived of cholesterol during the first 2 mo of life. The genetic differences in weight between these lean and obese pigs have been previously described (Mersmann 1991). Despite the greater body weight of lean pigs, the percentage of body fat is higher in obese pigs from birth. The only major organ that demonstrated a dietary effect with respect to its weight at necropsy was the kidney, which was heavier in pigs fed the NC diet ($P < 0.02$). This difference could not be explained by more extensive renal artery atherosclerosis in pigs fed the HC diet. Nutritional variables (serum total protein and albumin) showed only genetic differences, indicating adequate nutrition with or without cholesterol in the diet. This was not unexpected, because there is no evidence to suggest that infants fed low cholesterol formulas are malnourished.

As in previous experiments with these lean and obese pigs (Patterson et al. 1992), the serum TC and HDL-C concentrations of the obese pigs were higher
than those of the lean pigs at ~1 mo of age in response to the HC diet. Although LDL-C and VLDL-C levels were not measured directly, the calculated sum of these potentially atherogenic particles also tended to be higher in the obese pigs fed the HC diet. By 2 mo of age, the levels of both TC and LDL-C + VLDL-C concentrations were nearly equal in the lean and obese pigs, but the HDL-C levels remained higher in the obese pigs. This resulted in a tendency for a lower TC/HDL-C ratio in the obese (4.82 ± 0.42) compared with the lean (6.20 ± 1.18) pigs. There is apparently a genetic difference with respect to the adaptive mechanisms in these two strains of pigs when challenged with a high cholesterol diet in infancy.

Atherosclerotic lesions developed in the thoracic and abdominal aorta of all but one of the pigs fed the HC diet, and in none of those fed the NC diet. The thickened tunica intima and migration of muscle cells from tunica media to tunica intima are pathologic changes similar to those seen in human atherosclerosis. The fact that lesions were less extensive in obese pigs fed high cholesterol as compared with lean pigs fed high cholesterol (P < 0.05, t = 2.558 with 6 df), despite a tendency for higher serum cholesterol and LDL-C + VLDL-C concentrations in the obese pigs, suggests the possibility of a genetic resistance to atherosclerosis in the obese pigs, in support of previous work (Pond et al. 1985). The most likely mechanism for this difference is the higher HDL-C and the tendency for a lower TC/HDL-C ratio in the obese pigs.

Contrary to data in children (Berenson et al. 1992), obesity was not a risk factor for atherosclerosis in these pigs. However, the propensity for lean men to develop more extensive coronary artery disease than their obese peers in response to a high cholesterol diet has been reported, by Goff et al. (1992) in the Chicago Western Electric Study. Although LDL-C and HDL-C measurements could not be made in this large retrospective study, the authors speculated that the increased coronary mortality in the lean men was due to an increase in the ratio of LDL-C to HDL-C, as seen in the lean pigs in this experiment. Although the distribution of cholesterol among its lipoprotein particles seems to be more important, an association between atherosclerosis and obesity cannot be ruled out due to the young age of these pigs at the time of necropsy.

Gender was not rigorously evaluated as a potential risk factor for atherosclerosis in this study due to the small number of animals and their prepubertal status. As expected, there was no apparent trend toward higher lipid levels or more extensive atherosclerosis in the male pigs at this young age.

Cholesterol synthesis, measured in vivo using deuterium enrichment of red blood cell cholesterol and at termination by assaying the activity of hepatic HMG Co-A reductase, was suppressed by the HC diet in both lean and obese pigs. A recent study of in vivo cholesterol synthesis in human infants has also demonstrated suppression of cholesterol synthesis by dietary cholesterol (Wong et al. 1993). The differences between these two strains of pigs with respect to their cholesterol levels and their propensity to develop atherosclerosis must therefore be due to absorption, elimination or transport of cholesterol.

There is general agreement that dietary fat and cholesterol should be reduced in children after 2 y of age (National Cholesterol Education Program 1992). The role of cholesterol in early infant nutrition remains unclear. Breast milk contains 0.2–0.5% cholesterol and is high in saturated fat. Infant formulas contain 0.2–0.05% cholesterol and are high in polyunsaturated fat. Breast-fed infants have higher cholesterol levels than formula-fed infants at the time of weaning (Freedman et al. 1987), but a recent study by Fall et al. (1992) of over 5,000 men showed that men who were breast-fed as infants and weaned by 1 y of age had lower cholesterol levels and mortality rates from ischemic heart disease than men who were bottle-fed in infancy. A similar trend, although not statistically significant, was detected in the Bogalusa Heart Study, in children who had been fed high cholesterol cow’s milk as infants had lower cholesterol and triglyceride levels at 7 y of age than children who had been fed low cholesterol formulas in infancy (Freedman et al. 1987).

Although the extremely high cholesterol diet used in this experiment induced atherosclerosis in both strains, perhaps a moderately high cholesterol diet would be beneficial to the obese pigs because it raises their HDL-C and harms the lean pigs because their TC and LDL-C + VLDL-C increase more than their HDL-C, resulting in a higher TC/HDL-C ratio. Similar genetic differences in the human population would make the effects of dietary cholesterol in infancy difficult to assess. The ability to induce atherosclerosis at a very young age in these two strains of pigs and the differences in their responses to a HC diet make them a good model to investigate the effects of dietary cholesterol in infancy on cholesterol metabolism and atherosclerosis in adulthood.

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