Cholesterol-Fed and Casein-Fed Rabbit Models of Atherosclerosis

Part 1: Differing Lesion Area and Volume Despite Equal Plasma Cholesterol Levels

Sandra J. Daley, Ed E. Herderick, J. Fredrick Cornhill, Kem A. Rogers

Abstract

One-month-old male New Zealand White rabbits were fed either a cholesterol-free casein diet (CAS; n=10); low-level cholesterol-supplemented (0.125% to 0.5% by weight) chow (CH; n=10); or standard laboratory rabbit chow (n=3) for 24 weeks, during which total plasma cholesterol (TPC) levels were matched for the two experimental groups (TPC_CAS = 475±39 mg/dL; TPC_CH = 515±70 mg/dL). The percentage of cholesterol partitioned into each of the lipoprotein fractions except high-density lipoprotein (HDL) was significantly different for the experimental groups: casein-fed rabbits had a primarily low-density lipoprotein (LDL) hypercholesterolemia while cholesterol-fed rabbits had approximately equal levels of very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and LDL cholesterol. Despite matched TPC, lesions in CH animals covered twice the luminal surface area (as detected by oil red O staining; P<.05) and had three times the total volume compared with lesions in the CAS group (P<.05). Lesion volume was positively correlated with TPC and IDL and LDL cholesterol for the CAS group and with TPC and IDL cholesterol for the CH group. When the experimental groups were combined, TPC and VLDL and IDL cholesterol were positively correlated with the lesion volume. Probability of occurrence maps revealed, however, that both groups were virtually identical with respect to the topographic distribution of lesions in the thoracic and abdominal aortas. The data suggested that the differential partitioning of cholesterol into the lipoprotein fractions seen in CAS and CH rabbits influenced lesion area and volume but not topographic distribution. (Arterioscler Thromb. 1994;14:95-104.)

Key Words • rabbits • casein • lesion formation • hypercholesterolemia • atherosclerosis • cholesterol • aorta

Almost 90 years ago, in 1908, Ignatowski discovered that diets of milk, meat, and eggs resulted in atherosclerotic lesions in the rabbit. In 1913 Anitschkow and Chalatow demonstrated that the atherogenic component of the diet was cholesterol. Their pioneering experiment, in which dietary cholesterol was used to induce hyperlipidemia that resulted in atherosclerotic lesions, established the rabbit as an experimental model of atherosclerosis that is still used today. More recently, this animal model has been used extensively in biochemical studies of lipoprotein metabolism.

Only a few groups have characterized different rabbit models of atherosclerosis by using both extensive biochemical analyses and detailed morphological examinations of lesion formation. However, extensive morphological evaluation including lesion area, volume, and topographic distribution has not been performed. In 1978, Ross et al examined the characteristics of the casein-fed and the cholesterol-fed rabbit models to compare the effects of different lipoprotein profiles on atherosclerotic lesion formation. When animals were matched for total plasma cholesterol (TPC), the casein-fed and cholesterol-fed rabbits, although differing in lipoprotein profile, had the same extent of lesion formation. In an attempt to fully characterize the casein-fed and the cholesterol-fed rabbit models of atherosclerosis, in the present study we examined the lipoprotein profiles and the atherosclerotic lesions in the entire aorta of rabbits fed a semisynthetic diet enriched in casein and in rabbits fed a cholesterol-supplemented diet. Because the two groups were matched for TPC, we were able to examine the effect of the different lipoprotein profiles in these animals on the extent, volume, and topographic distribution of lesion formation. The relation of the lipoprotein profiles of these animals to the microscopic morphology and site specificity of lesion type is presented in the accompanying article.

Methods

Animals and Diets

Twenty-three male New Zealand White rabbits (Reimans Fur Ranches, Guelph, Ontario, Canada) weighing 1.6 kg on
TABLE 1. Experimental Diets

<table>
<thead>
<tr>
<th>Diet</th>
<th>Casein diet*</th>
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<tbody>
<tr>
<td>Casein</td>
<td>270</td>
</tr>
<tr>
<td>Dextrose</td>
<td>585</td>
</tr>
<tr>
<td>Alfacel</td>
<td>50</td>
</tr>
<tr>
<td>Salt mix</td>
<td>40</td>
</tr>
<tr>
<td>Molasses (1:1 with water)</td>
<td>30</td>
</tr>
<tr>
<td>Fat (corn oil containing fat-soluble vitamins)</td>
<td>10</td>
</tr>
<tr>
<td>Water soluble vitamins</td>
<td>15</td>
</tr>
<tr>
<td>Cholesterol-supplemented chow†</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>165</td>
</tr>
<tr>
<td>Carbohydrates (nitrogen-free extract)</td>
<td>422</td>
</tr>
<tr>
<td>Fiber</td>
<td>225</td>
</tr>
<tr>
<td>Ash</td>
<td>63</td>
</tr>
<tr>
<td>Fat</td>
<td>25</td>
</tr>
<tr>
<td>Moisture</td>
<td>100</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>1.25-5</td>
</tr>
</tbody>
</table>

The compositions of the two diets are given in grams/kilogram.
*For sources of ingredients and composition of the salt and vitamin mixtures see Roberts et al.*
†Agway Prolab High Fiber Rabbit Chow.

Arrival were individually housed in a controlled environment maintained at 19°C with food and water provided ad libitum. Animals were cared for in accordance with the guidelines of the Canadian Council on Animal Care. Twenty of the animals were pair matched according to their initial TPC levels and randomly assigned to two experimental groups. All animals were fed standard laboratory rabbit chow pellets for the first 2 days and powdered rabbit chow for the next 3 days; they were then weaned onto either a semipurified diet containing casein (CAS; n=10) or cholesterol-supplemented (0.125% to 0.5% cholesterol by weight) powdered rabbit chow (CH; n=10) (Table 1). Animals were weaned onto their experimental diets over a 6-day period, with 2 days each of 25% experimental diet:75% chow, 50% experimental diet:50% chow, and 75% experimental diet:25% chow. Control animals (n=3) were fed powdered rabbit chow (Prolab Laboratories, Agway Inc, Syracuse, NY).

The level of dietary cholesterol given to each CH animal was initially adjusted on a weekly basis, and later every few weeks, to match the mean TPC concentration of the CH group with that of the CAS group over time. Animals were pair matched at each bleed, and taking into consideration the response of individual animals to previous levels of dietary cholesterol, individual cholesterol levels were adjusted to maintain the TPC level of the pair matched for that week.

Blood Sample Collection and Analyses

TPC levels (measured using the high-performance CHOD-PAP enzymatic kit; Boehringer Mannheim, Dorval, Quebec, Canada) of blood samples collected after a 16-hour fast at weeks 0, 1, 2, 3, 4, 6, 7, 10, 14, 17, 20, 22, and 24 were used to match TPC in the experimental groups over time. Briefly, samples taken from the marginal ear vein after an injection of droperidol-fentanyl (0.1 mL/kg) to dilate blood vessels were mixed with EDTA to achieve a 0.15% concentration in the sample and placed on ice until separation of plasma by low-speed centrifugation. Larger plasma samples (15 mL) taken bimonthly were obtained from the middle ear artery as described above and were further separated by sequential ultracentrifugation to obtain VLDL (d<1.006 g/mL); intermediate-density lipoprotein (IDL; d=1.006 to 1.019 g/mL); LDL (d=1.019 to 1.063 g/mL); and high-density lipoprotein (HDL; d=1.063 to 1.21 g/mL) fractions. The cholesterol concentration of each lipoprotein fraction, assayed by using the same enzymatic kit, was subsequently used to determine the cholesterol lipoprotein profiles of the two experimental groups. Total plasma triglycerides (TPTG) and lipoprotein triglycerides (TG) levels were measured with the GPO-PAP enzymatic kit (Boehringer Mannheim).

Lesion Analyses

After 24 weeks, animals were anesthetized with an injection of ketamine (100 mg/kg) and xylazine (10 mg/kg) intramuscularly and then killed with an intravenous overdose of ketamine (200 mg bolus). Animals were then perfused fixed at 100 mm Hg via the left ventricle as described previously. Briefly, aortas were perfused with Hanks' balanced salt solution with 0.15 mmol/L N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (pH 7.4) containing heparin (1 IU/mL) for 10 minutes, followed by dilute Karmowsky's fixative for 15 minutes. An additional 30 minutes was allowed for immersion fixation in the same fixative.

Lesion Area and Distribution

The heart and entire aorta were dissected free, removed from the animal as a unit, and later separated at the root of the aorta. The bulk of fat and tissue adhering to the adventitia were dissected from the aorta, and the thoracic and abdominal aortas were separated above the celiac artery and opened along their ventral surface. The thoracic aorta was then divided into the arch and the proximal and distal descending portions. Once samples had been taken from various areas for microscopic examination, both the thoracic and abdominal aortas were stained with oil red O in propylene glycol for 30 minutes. All remaining traces of adventitial fat were removed from the vessels, which were subsequently pinned flat, imersed in 0.06 mol/L phosphate buffer (pH 7.4), and photographed en face.

The percentage of the surface area stained with oil red O, which was quantified by using SIGMA SCAN software (Jandel Scientific, Calif), was used as an index of the extent of lesion formation in the aortas of both casein-fed and cholesterol-fed rabbits.

Probability of occurrence maps were used to outline regions susceptible to atherosclerotic lesion formation in the aortas of the two experimental groups. This technique involves the use of computer digitization to initially create a representative schematic template of the vessel surface that represents the average morphology of all sample vessels in the study. Digital images of oil red O–stained thoracic and abdominal aortas are then divided into triangular subsections by using anatomic landmarks (ie, ostia) to outline the vertices of those triangles; these are then linearly transformed so that each vessel is mapped onto the representative template, thus removing anatomic variation between individual vessels. For each pixel in the template, the probability of a lesion's being present is calculated for the entire sample set and represents the average morphology of all sample vessels in the study. Difference maps were generated by subtracting the probability of lesion occurrence at a particular pixel in the template in the CAS group from the probability in the CH group.

Difference maps were generated by subtracting the probability of lesion occurrence at a particular pixel in the template in the CAS group from the probability in the CH group. Positive values in the difference map indicated sites at which the CH rabbits were more likely to have lesion formation, while negative values represented regions where the CAS rabbits were more likely to have lesion formation.

Lesion Volume

Each segment of the thoracic aorta was immersed in 10% sucrose at room temperature for 1 hour and then immersed in 20% sucrose for at least 1 hour before being embedded in Tissue-Tek OCT Compound (Miles Inc, Elkhart, Ill) and...
Lesion Analyses

Lesion Area and Distribution

When stained with oil red O, the thoracic aortas of the CH rabbits had twice the luminal surface area occupied by lesions as did the CAS rabbits (Table 2), although both groups exhibited a similar range of lesion extent (2% to 92%). Lesion formation in the abdominal aorta was not as extensive as in the thoracic aorta of either group. In both groups, the abdominal aortas had about 60% as much lesion occurrence as the thoracic aortas. The CH group had 1.7 times the extent of lesion formation in the abdominal aorta as that in the CAS group. When the entire aorta was included in the analysis, the CH group had 1.9 times the surface area occupied by lesions compared with the CAS group. Control animals did not have any grossly detectable lesions.

Probability of occurrence maps created for the arch and the proximal descending thoracic aorta are shown in Fig 5A. In the region of the arch there was a greater probability of lesion formation in the greater curvature and posterior wall in both hypercholesterolemic groups. The greatest probability of lesion formation in the proximal descending thoracic aorta appeared to center around the ductus scar in the CAS group. In the CH group, this region of high probability was not limited to the ductus scar but extended around the circumference.

Results

All animals remained healthy throughout the 24-week experimental period, aside from minimal patchiness of fur seen in a few of the CAS animals. Both the casein and cholesterol diets remained palatable to the animals during the study, and weight gain in all groups was consistent.

Biochemical Analyses

TPC levels were matched for the CAS and CH groups over the 24-week experimental period and were consistently higher than that of the control group (Fig 1). TWAs of TPC for individual animals were calculated as the area under their cholesterol concentration-time curves divided by 24 weeks. Mean TPC TWAs for the two experimental groups were similar (CAS=475±39 mg/dL and CH=515±70 mg/dL; Fig 1).

The levels of VLDL-C and HDL-C over time were not significantly different for the CH and CAS groups (Fig 2A and 2B), but the LDL-C levels were higher in the CAS group for VLDL, LDL, and HDL fractions. In both experimental groups the percentage of cholesterol carried in the HDL fraction was considerably less than that carried in the HDL fraction of the control animals, whereas the percentage of cholesterol carried in the VLDL and IDL fractions was considerably greater in the experimental groups compared with controls. A change in the percentage of cholesterol carried in the LDL fraction with the induction of hypercholesterolemia was only evident in the CAS group.

In all groups TPTG dropped considerably within the first 2 months of the experimental period. TPTG levels were matched over time for the CH and control groups but were significantly higher in the CAS group (Fig 4). This was reflected in significantly higher TG levels in the CAS group for VLDL, IDL, LDL, and HDL (data not shown).

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Statistical Analyses

Group comparisons of TPC, lipoprotein cholesterol (VLDL-C, IDL-C, LDL-C, and HDL-C), TPTG, and lipoprotein TG levels over time were made by using ANOVAs with repeated measures on the time factor. Group comparisons of time-weighted averages (TWAs) for TPC, lipoprotein cholesterol, TPTG, lipoprotein TG, and of derived measures of percent distribution for cholesterol and TG into the lipoprotein fractions between the CAS and CH groups were performed by using ANOVA. Because of the small number of animals in the control group, no attempt was made to statistically compare their values with those for the experimental groups; control values are shown for reference only. Two-tailed correlations were made on TPC TWAs, VLDL-C TWAs, IDL-C TWAs, LDL-C TWAs, and HDL-C TWAs with the extent of lesion formation in the thoracic aorta and the entire aorta and with lesion volume for each of the experimental groups and for the entire population. All statistical analyses were done with spss/PC+ software (Chicago, Ill); P<.05 was used as the cutoff point for significance.

Lesion Analyses

Lesion Area and Distribution

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Fig 2. Line graphs showing absolute concentrations of cholesterol in the lipoprotein fractions of casein-fed, cholesterol-fed, and control rabbits. Time-weighted averages for the groups are shown in parentheses. Values are mean±SEM. Significance was assessed by ANOVA with repeated measures on the time factor (P<.05 for low-density lipoprotein [LDL] cholesterol for casein vs cholesterol). VLDL indicates very-low-density lipoprotein; IDL, intermediate-density lipoprotein; and HDL, high-density lipoprotein.

Fig 3. Bar graph showing percentage distribution of cholesterol into the very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) lipoprotein fractions. Values are mean±SEM. Significance was assessed by ANOVA (*P<.05).

of the vessel at this level, becoming confluent with the high-probability region of the greater curvature and extending down toward, but not reaching, the first pair of intercostal ostia. The difference map shows blue and dark blue areas in which there are equal or greater probabilities of lesion formation in the CH group compared with the CAS group.

In probability of occurrence maps of the distal descending thoracic aorta (Fig 5B), the highest probability of lesion formation occurred distal and lateral to the intercostal ostia in both groups. In the CH group, the extent of the aortic surface spared from lesion formation just proximal to each intercostal ostium increased in length beginning at the first pair of intercostal ostia and moving distally. Most areas of the distal descending thoracic aorta in which the CAS group had at least a 40% probability of lesion formation were duplicated in the CH group with areas of equal or greater probability, as indicated in the difference map.

The majority of areas having more than 30% lesion probability in the abdominal aortas of the CAS animals were matched by areas in the CH animals having a 50% or more probability of lesion formation, indicated as blue and dark blue in the difference map (Fig 6). The only exception was seen in the region proximal to the ostium of the superior mesenteric artery, where the CAS animals had a 10% to 15% greater probability of
Lesion Volume

Abdominal lesion averages for the groups are shown in parentheses. Values were consistently found in the region of the lumbar ostium of the superior mesenteric artery. Lesions were 3.4 times greater in the CH group than in the CAS group. There was 1.8 times greater in the CH group than in the CAS group. Lesions were found not only for LDL-C and lesion volume but also for TPC and IDL-C with lesion volume. In addition, Schwenke and Carew\cite{30} report that with TPC levels of 250 to 600 mg/dL, LDL carries an increasing percentage of the cholesterol. In our CAS rabbits the majority of cholesterol in the plasma (more than 50%) was carried in the LDL fraction. The mechanism by which hypercholesterolemia is induced in the casein-fed rabbit involves a reduction in bile acid and fecal steroid excretion.\cite{8,28} The liver responds with an increased secretion of \( \beta \)-VLDL, delayed uptake of VLDL remnants (due to downregulation of hepatic LDL receptors), enhanced conversion of VLDL to LDL to LDL, and an increase in the direct synthesis of LDL by the liver.\cite{8,13,28} The marked LDL hypercholesterolemia seen in our CAS rabbits is consistent with other studies, including those done by Ross et al\cite{18} and Bauer and Covert.\cite{29}

Hypercholesterolemia in the cholesterol-fed rabbit is the result of an accumulation of cholesterol of exogenous origin. These rabbits are incapable of increasing sterol excretion; rather, the liver secretes lipoproteins packed with an unusually large amount of cholesterol ester, and these lipoproteins have an increased residence time in the plasma as a result of downregulation of LDL receptors.\cite{5,6,8} Our CH rabbits carried their plasma cholesterol equally in the VLDL, IDL, and LDL fractions. Although this result appears to conflict with a number of studies reporting a primarily \( \beta \)-VLDL hypercholesterolemia in the cholesterol-fed rabbit,\cite{9} Brattsand\cite{30} has shown that VLDL is the major carrier of cholesterol in cholesterol-fed rabbits only when TPC levels are greater than 800 mg/dL. Moreover, with increasing TPC levels (up to 1400 mg/dL), VLDL carries an increasing percentage of the cholesterol. In addition, Schwenke and Carew\cite{30} report that with TPC levels of 250 to 600 mg/dL, LDL in cholesterol-fed rabbits carries 28% of the TPC. Given that our TPC falls within this range, our value of 29% for LDL-C is in close agreement with both of these reports.\cite{8,28}

Many groups have assessed lesion extent in the cholesterol-fed rabbit by using methods such as visual grading,\cite{12} xerography,\cite{34} and mechanical or computerized planimetry.\cite{34,35} The few groups that have assessed the extent of lesion formation in the casein-fed rabbit model have done so by using visual grading.\cite{12,18,36} We used computerized planimetric methods to determine more accurately the extent of luminal surface area involved in lesion formation. Unlike Ross et al.,\cite{18} who also matched TPC in cholesterol-fed and casein-fed rabbits exhibiting different lipoprotein profiles but found no difference in lesion extent, we found a marked difference in the extent of lesion formation between the two groups. Our CH group had twice the lesion area in the thoracic aorta and 1.7 times the lesion area in the abdominal aorta compared with the CAS group.

Discussion

Despite matched TPC levels, both the oil red \( \text{O} \)-stained surface area and the volume of lesion formation were greater in CH rabbits than in CAS rabbits.

In our CAS rabbits the majority of cholesterol in the plasma (more than 50%) was carried in the LDL fraction. The mechanism by which hypercholesterolemia is induced in the casein-fed rabbit involves a reduction in bile acid and fecal steroid excretion.\cite{8,28} The liver responds with an increased secretion of \( \beta \)-VLDL, delayed uptake of VLDL remnants (due to downregulation of hepatic LDL receptors), enhanced conversion of VLDL to LDL to LDL, and an increase in the direct synthesis of LDL by the liver.\cite{8,13,28} The marked LDL hypercholesterolemia seen in our CAS rabbits is consistent with other studies, including those done by Ross et al.\cite{18} and Bauer and Covert.\cite{29}

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The majority of studies either have not reported actual

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Fig 4. Line graph showing total plasma triglyceride levels of casein-fed, cholesterol-fed, and control rabbits. Time-weighted averages for the groups are shown in parentheses. Values are mean±SEM. Significance was assessed by ANOVA with repeated measures on the time factor (\( P<.01 \) for cassein vs cholesterol).

TABLE 2. Percentage of Surface Area Covered With Lesion in the Casein- and Cholesterol-Fed Groups

<table>
<thead>
<tr>
<th>Aorta</th>
<th>CAS</th>
<th>CH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thoracic</td>
<td>34±10</td>
<td>68±10*</td>
</tr>
<tr>
<td>Abdominal</td>
<td>23±8</td>
<td>39±8</td>
</tr>
<tr>
<td>Entire</td>
<td>30±9</td>
<td>58±9*</td>
</tr>
</tbody>
</table>

CAS Indicates casein-fed rabbits and CH, cholesterol-fed rabbits. Values are percent aortic surface area covered and represent mean±SEM.

*\( P<.05. \)
values or have reported percentages of surface area involvement of the abdominal aorta much less than the 39% that we saw in our low-level cholesterol-fed rabbits. Recent studies in our laboratory on lesion formation in Yucatan miniature swine fed a diet containing 1.5% cholesterol and 15% beef tallow (an animal model reported to have a lesion distribution similar to that seen in humans) have shown a similar result, with the extent of lesion formation in the abdominal aorta being 60% of that in the thoracic aorta (authors' unpublished data, 1992).

Attempts have been made to determine the magnitude of lesion formation by using measures including thickness or grading of histological sections, added...
scores of aortic intimal thickness, cholesterol content, volume estimates based on derived plaque thickness indices, or point-counting volume ratios. We determined the mean absolute volume of lesions in the thoracic aorta by serial sectioning of the entire vessel. Unlike other morphological techniques assessing lesion volume, ours is a direct method of measurement. In addition, differences in the relative ratios of cholesterol to cellular and extracellular constituents of the lesion do not affect the direct measure of lesion volume, whereas the determination of total cholesterol is directly affected by the morphology of the lesion; ie, fatty streaks and fibrous lesions of equal volume would not be expected to contain the same amount of cholesterol. By using both the surface area and volume measurements, we were also able to determine the average lesion thickness. With this method we found that not only the extent (percent surface area involvement) but also the volume and thickness of lesions in the CH group were significantly greater than in the CAS group. Thus, over the time period examined, lesions of the CH group expanded not only in length and width but in thickness as well. We are not aware of any other studies that have made a three-dimensional assessment of lesion extent in any other rabbit model of atherosclerosis.

Others have carried out experiments in which TPC levels were matched between two groups of rabbits made hypercholesterolemic by differing means. Ross et al compared casein-fed with cholesterol-fed rabbits.

<table>
<thead>
<tr>
<th>TABLE 3. Lesion Volume, Area, and Thickness in the Thoracic Aorta of the Casein- and Cholesterol-Fed Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td>Lesion volume, mm³</td>
</tr>
<tr>
<td>Lesion area, cm²</td>
</tr>
<tr>
<td>Average lesion thickness, µm</td>
</tr>
</tbody>
</table>

CAS indicates casein-fed rabbits and CH, cholesterol-fed rabbits. Values represent mean±SEM. *P<.05.

Table 4. Correlations of Plasma and Lipoprotein Cholesterol With Thoracic Lesions

<table>
<thead>
<tr>
<th>Cholesterol</th>
<th>All Animals</th>
<th>CAS</th>
<th>CH</th>
</tr>
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<tbody>
<tr>
<td>Area</td>
<td>TPC_TWA</td>
<td>.47</td>
<td>...</td>
</tr>
<tr>
<td>VLDL-C_TWA</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>
| IDL-C_TWA   | .53         | .67 | ...
| LDL-C_TWA   | ...         | ... | ...|
| HDL-C_TWA   | ...         | ... | ...|

<table>
<thead>
<tr>
<th>Volume</th>
<th>TPC_TWA</th>
<th>.65</th>
<th>.72</th>
<th>.70</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL-C_TWA</td>
<td>.65</td>
<td>...</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>IDL-C_TWA</td>
<td>.72</td>
<td>.71</td>
<td>.65</td>
<td></td>
</tr>
<tr>
<td>LDL-C_TWA</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>HDL-C_TWA</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td></td>
</tr>
</tbody>
</table>

CAS indicates casein-fed rabbits; CH, cholesterol-fed rabbits; TPC, total plasma cholesterol; TWA, time-weighted average; VLDL-C, very-low-density lipoprotein cholesterol; IDL-C, intermediate-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; and HDL-C, high-density lipoprotein cholesterol. Correlation coefficients shown for P<.05.
and found that the distribution of cholesterol into the various lipoprotein fractions was similar to that reported in our study. However, they reported that the extent of lesion formation, as determined by visual grading, was similar for the two groups. The discrepancy between their results and ours may be related to their use of a rather subjective method of lesion assessment and/or to a lack of statistical power (only four animals on each diet). Rosenfeld et al.\textsuperscript{14,15} compared Watanabe heritable hyperlipidemic (WHHL) to New Zealand White rabbits fed a high-fat, high-cholesterol diet containing 20% casein. Lipoprotein cholesterol distribution was again different between groups, with LDL the primary carrier in the WHHL rabbits and VLDL the primary carrier in the cholesterol-fed rabbits. The focus of their study was arterial cellular responses, which were qualitatively similar between groups. The concentrations of free and esterified cholesterol deposited in the abdominal aorta tended to be higher (by 45% to 100% at 8 months) in their cholesterol-fed animals, although this difference was not significant. The comparison between our results and those of Rosenfeld et al.\textsuperscript{14,15} is problematic, since our data represent the total volume of atherosclerotic intimal expansion, and the closest comparative parameter would be the total mass of lipid deposited (concentration multiplied by wet weight), which was not reported in their article.

Topographic analysis of the distribution of lesions in the CAS and CH groups confirmed results obtained by using polar-coordinate mapping, which has shown that lesions in hypercholesterolemic rabbits occur laterally and distally to most branch orifices,\textsuperscript{37,44} and has revealed no differences in the location of lesions despite different methods of induction of hypercholesterolemia.\textsuperscript{48} It also supports our view that the abdominal aorta is involved in substantial atherosclerotic lesion formation in the low-level cholesterol-fed rabbit. Despite the fact that lesion extent was reduced in the abdominal aorta compared with the thoracic aorta, there is still a greater than 40% probability of lesion formation in the proximal two thirds of the abdominal aorta. Although it has been noted that the topographic distribution of lesions seen in rabbits is unlike that seen in humans and Sinclair minipigs,\textsuperscript{26,49} it is conceivable that those differences in system geometry and hemodynamic characteristics between species that could play a role in localization of atherosclerotic lesion development may well account for the differences seen in lesion pattern formation, such that regions of high and low wall shear stress may occur at different sites in different species.\textsuperscript{30,51}

Few, if any, studies of cholesterol- or casein-fed rabbits have been able to show a correlation of the extent of lesion with TPC or lipoprotein cholesterol levels. Some studies have suggested\textsuperscript{36,52} that there is a general correlation of aortic atherosclerotic scores with plasma cholesterol concentrations, but they have not statistically outlined the significance of such a relation. Others have shown correlations of cholesterol in the plasma with aortic cholesterol content\textsuperscript{16,53} and aortic cholesterol concentration with aortic atherosclerotic score.\textsuperscript{15} We have shown positive correlations of TPC, VLDL-C, and IDL-C with lesion volume for all of our hypercholesterolemic rabbits; correlations of TPC, IDL-C, and LDL-C with lesion volume in the CAS group; and correlations of TPC and IDL-C with lesion volume in the CH group.

The correlation between plasma cholesterol and the extent of coronary artery disease has been well established in humans.\textsuperscript{54,55} Here, we have identified similar correlations in two different diet-induced rabbit models of atherosclerosis. This may not have been possible previously because of the lack of an accurate means of measuring lesion volume in these animals. Our data suggest that while planimetric measures of lesion extent do afford some measure of accuracy, the measurement of the total volume of atherosclerotic intimal expansion as performed in this study represents a substantial advance in the assessment of disease severity.

The matched TPC levels for our two experimental groups, their different lipoprotein profiles, and the fact that lesion extent was twice as great and lesion volume three times as great in the CH group suggest that the amount of cholesterol carried in the VLDL and IDL fractions may be as important for lesion formation and expansion as the amount of cholesterol carried in the LDL fraction. The fact that others have found IDL-C to be a better predictor of aortic atherosclerosis than LDL-C\textsuperscript{56} and that arterial influx of VLDL, IDL, and LDL is linearly correlated with their respective concentrations in the plasma\textsuperscript{57} in the St Thomas's Hospital rabbit strain suggest that VLDL and IDL may potentially be as atherogenic as LDL in this model.

Our results suggest that cholesterol carried in VLDL and IDL particles is potentially more atherogenic than cholesterol carried in LDL particles. This may be partially explained by in vitro data demonstrating that although $\beta$-VLDL and VLDL remnants are directly recognized by intimal macrophages,\textsuperscript{58,59} LDL must be modified before uptake by these cells, thus reducing the potentiality for cholesterol carried in LDL to induce foam cell formation leading to the initiation of atherosclerotic lesion formation.\textsuperscript{60} In addition, Nordestgaard et al.\textsuperscript{61} suggest that in the St Thomas's Hospital rabbit strain, cholesterol influx into the arterial wall from IDL and VLDL may exceed that from LDL. Thus, the more rapid development of atherosclerotic lesions in the CH group seen in this study may be directly related to the preferential distribution of cholesterol into $\beta$-VLDL and IDL.

The associations of IDL-C and VLDL-C ($\beta$-VLDL) with atherogenesis in hypercholesterolemic rabbits demonstrated here are of particular interest because similar correlations have been observed with atherogenesis in humans. Both lipoproteins are elevated in type III hyperlipoproteinemia, which is a rare, highly atherogenic condition.\textsuperscript{61} In patients with a heterozygous deficiency of the LDL receptor (familial hypercholesterolemia), the risk for developing premature atherosclerosis is greater than it is for comparably hypercholesterolemic patients without this defect. Since hepatic LDL receptors are responsible for clearing IDL from the circulation, increased plasma IDL may play a role in the propensity of these patients to develop atherosclerosis.\textsuperscript{62} Finally, a study correlating specific lipoprotein plasma levels to results of clinical coronary angiography suggests that male patients with higher IDL levels have worse coronary disease and that cholesterol-rich VLDL may contribute to atherosclerosis as well.\textsuperscript{60}
In summary, it is clear that the focus on LDL particles as predictors of atherogenesis needs to be broadened to include both VLDL and IDL. Further studies using the low-level cholesterol-fed rabbit should expand our understanding of the role that these specific lipoproteins play in atherosclerotic lesion formation.

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