Increased Ischemia-Reperfusion Injury to the Heart Associated With Short-term, Diet-Induced Hypercholesterolemia in Rabbits

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The effects of increased dietary cholesterol content on coronary vascular hemodynamics and endothelial cell transport function were assessed in isolated rabbit hearts during 3.5 hours of reperfusion after 30 minutes of global, no-flow ischemia. In control hearts from rabbits fed normal chow, perfusion pressure, left ventricular end-diastolic pressure, maximum +dP/dt, and the rate of intravascular clearance of radiolabeled albumin remained constant during 5 hours of continuous perfusion, while the mean transit time of radiolabeled albumin increased 1.6 × baseline. In ischemic hearts from rabbits fed normal chow, perfusion pressure increased 59% during reperfusion while left ventricular end-diastolic pressure and maximum +dP/dt returned toward control levels. The rate of intravascular clearance of radiolabeled albumin decreased 36%, and the mean transit time of albumin increased ~3 × baseline. Ischemia-reperfusion injury to the cardiac vasculature and musculature was markedly increased in hearts of rabbits fed chow supplemented with 2% cholesterol for 2–3 weeks compared to rabbits fed the same diet for a longer duration (5–16 weeks) or rabbits fed normal chow. Prior to ischemia, permeation of the coronary vasculature by albumin was increased twofold in rabbits fed cholesterol for 2–3 weeks while myocyte contractile function was normal relative to chow-fed controls or the group fed cholesterol for 5–16 weeks. These effects of acute cholesterol feeding precede occlusive atherosclerotic coronary artery disease and occur at plasma cholesterol concentrations one third of those in rabbits fed cholesterol for the longer duration. These findings suggest that the altered metabolic milieu associated with an abrupt increase in cholesterol consumption and/or a rapidly increasing plasma cholesterol concentration impairs the functional integrity of the coronary vasculature and leads to increased susceptibility of the heart to ischemia-reperfusion injury. (Circulation Research 1987;60:551–559).

The association between high plasma cholesterol concentrations and atherosclerotic vascular disease is well documented, although the mechanism(s) by which hypercholesterolemia injures the vasculature and contributes to atherosclerosis remains unclear. Several investigators have suggested that hypercholesterolemia affects vascular functional integrity prior to the onset of occlusive vascular disease. Increased permeation of the aorta by a variety of tracer molecules has been observed as early as 2–3 weeks following the induction of dietary-induced hypercholesterolemia.1,2 The possibility that hypercholesterolemia may promote coronary vasospasm, independent of atherosclerosis, is suggested by observations that contractile responses of coronary vascular smooth muscle to calcium ions and to a variety of vasoactive agents are accentuated by hypercholesterolemia or even acute cholesterol exposure.3–5 Despite the fact that an extensive body of information is available on vascular reactivity and permeability changes following cholesterol feeding in a variety of animal models, few studies have assessed effects of hypercholesterolemia on myocyte contractility,6–8 and these studies are not in agreement. Since virtually no information is available on effects of hypercholesterolemia on responses of the myocardial vasculature and musculature to ischemia-reperfusion injury, the present experiments were undertaken to assess coronary vascular hemodynamics, endothelial cell transport function, and myocardial contractility prior to and after global, no-flow ischemia in isolated hearts from rabbits fed a diet supplemented with 2% cholesterol.

Materials and Methods

Perfusion Techniques

Male New Zealand rabbits were housed individually, and either normal Purina laboratory rabbit chow (Ralston Purina, Richmond, Ind.) or rabbit chow supplemented with 2% cholesterol (one group for 2–3 weeks and a second group for 5–16 weeks) was allowed ad libitum, as well as drinking water. Nonfasted rabbits were anticoagulated intravenously with 1,000 U sodium heparin 10 minutes prior to surgery. Ani-
mals were stunned by a blow to the neck; the heart was excised rapidly and immediately connected to the arterial cannula of the perfusion chamber via the aorta. Hearts were atrially paced at 180 beats/min and perfused at constant flow with nonrecirculating Krebs-Henseleit (KH) buffer containing 1% dialyzed bovine serum albumin, 0.5% dialyzed polyvinylpyrrolidone (MW 360,000), 3 mM pyruvate, 8 mM dextrose, and 100 mU/l insulin (pork, regular Iletin II). The perfusate was filtered through 0.45-μm Gelman minicapsule filters, warmed to 37° C, and oxygenated at pH 7.4 by dialysis against 95% O₂ and 5% CO₂ across medical grade silastic tubing (0.058 in. i.d. x 0.077 in. o.d.; Dow Corning, Midland, Mich.). Buffer flow-rate was adjusted during baseline to achieve a perfusion pressure of ~45 mm Hg.

Left ventricular end-diastolic pressure (LVEDP) was recorded continuously from an isovolumic balloon placed in the left ventricle and secured at the level of the mitral valve; for baseline values, the pressure was adjusted to ~5 mm Hg by filling the balloon with KH buffer. The maximum rate of left ventricular pressure development, + dP/dt, was obtained by differentiation of the left ventricle pressure signal. Hearts were perfused apex-up and were rinsed continuously over the surface and through the right atrium and ventricle with KH buffer to ensure that radiolabelled tracer exiting the coronary vasculature was removed very rapidly from the detector. Hearts were held in the apex-up position by a suture connected to the balloon in the left ventricle; this prevented herniation of the balloon toward the atria with increasing left ventricle contracture and also provided a drainage site for KH buffer entering the left ventricle by aortic regurgitation or from the thebesian veins.

Experimental Protocol

Nonischemic control hearts were perfused continuously for 5 hours under constant flow conditions; ischemic hearts were perfused for 1 hour prior to 30 minutes of global, no-flow ischemia, then reperfused at the same baseline flow rate for 3.5 hours. Every 30 minutes during the perfusion, a 25-μl bolus of 125I-BSA in KH buffer was injected into the aortic cannula near the coronary ostia, and the passage of this tracer to the coronary vasculature was monitored with a lead-shielded and collimated NaI detector (Bicron Corp., Newbury, Ohio) positioned to view the entire heart. At the termination of each experiment, hearts were trimmed, blotted dry, and weighed, and transmural left ventricle sections were dried to constant weight at 70° C for determination of water content and total dry weight of the heart. Nonfasting total plasma cholesterol content was determined at the time of sacrifice.

Preparation of Radiolabelled Bovine Serum Albumin (BSA)

Twenty milligrams of purified monomer albumin (Sigma Chemical Co., St. Louis, Mo.) in phosphate-buffered saline were iodinated with 1 mCi 125I at 37° C, pH 7.0, by the lactoperoxidase method as described previously. Prior to use, 125I-BSA was dialyzed extensively against KH buffer (pH 7.0, 4° C), filtered through 0.2-μm Acrodisc filters (Gelman Sciences), and vacuum concentrated with the use of collodion bags having a 25,000 MW cut-off (Schleicher and Schuell).

Mathematical Model for Assessment of Radiolabelled Albumin Transport

We have interpreted the uptake and clearance of a bolus injection of 125I-BSA with a compartmental model describing temporal changes in the distribution of tracer due to transport of label within the vasculature and between the vascular and extravascular spaces. Components of the model and complete mathematical derivations have been published previously and will not be described in detail. Conventional requirements are assumed to hold for the validity of tracer stimulus-response methods concerning stationarity and linearity. Although some of the model features describing albumin transmembrane mass-transport conductivities change continuously during reperfusion after ischemia, the stationarity requirement is satisfied reasonably well because the rates at which these features change over the interval during which a tracer response curve is monitored are small enough that the preparation is effectively stationary during that period. Tracer movements are interpreted in terms of hypothesized diffusive and convective transport mechanisms operating simultaneously across a barrier separating albumin-accessible vascular and extravascular spaces. No morphological or functional details concerning this barrier need to be specified in our model for it to account adequately for the observed movements of the tracer. On the other hand, we do not hesitate to identify the capillary endothelium as the seat of the observed resistances to tracer transport nor, therefore, to infer alterations in endothelial cell integrity from observed changes in transbarrier resistances. The model parameters and their numerical estimates from the experimental data represent global averages. Spatial uniformity of detection efficiency is assumed.

Using a parameter-estimation technique, the biexponential function

\[ r(t) = A_1 e^{-α_1 t} + A_2 e^{-α_2 t} \]  

is fitted to the observed residue count-rate data. Here, \( t \) represents time elapsed after the instant the maximum count rate is observed in the heart following a bolus injection of 125I-BSA. Parameter estimation is accomplished by using an algorithm based on maximum-likelihood estimation procedures for Poisson-distributed data; this provides estimates of \( A_1, A_2, α_1, \) and \( α_2 \) of the biexponential intensity function, which are related to the compartmental turnover-rate constants of the model according to
\[
k_{01} = \alpha_1 - (\alpha_1 - \alpha_2)A_2/r_0 \tag{2a}
\]
\[
= \alpha_2 + (\alpha_1 - \alpha_2)A_1/r_0 \tag{2b}
\]
\[
k_{12} = \alpha_2/\kappa_{01} \tag{3}
\]

and
\[
k_{21} = (\alpha_1 + \alpha_2) - (\kappa_{01} + \kappa_{12}) \tag{4}
\]

where \( r_0 = r(t = 0) \) is the peak count rate, whose instant of occurrence defines zero time. The \( \kappa_j \) in equations 2–4 represent the fraction of the mass of tracer in compartment \( j \) that is transported, per unit time, into compartment \( i \); here, subscripts 1 and 2 denote, respectively, the vascular space and extravascular space accessible to albumin, and the subscript 0 denotes surroundings external to the heart and detector field of view.

Although the assumptions underlying compartmental modeling of time-activity curves are valid for a barrier-limited tracer such as albumin under the experimental conditions, the perfusion defect (described below) that occurred during reflow after ischemia in hearts from rabbits fed the 2% cholesterol diet for 2–3 weeks was so great that the analysis of the \(^{125}\text{I}-\text{BSA} \) time-activity curves with a two-compartment model was not reliable. In particular, the assumptions related to using compartmental models — including the assumption that the contents of the two compartments are uniform and well mixed — may no longer be valid. For this reason, we have reported only baseline estimates of the vascular into extravascular space turnover-rate constant, \( \kappa_{21} \), for all hearts. We have shown all data for \( \kappa_{01} \) (for interpretation, see Figure 5, discussed in the "Results" section); estimates of \( \kappa_{01} \) obtained from the two-compartment model that are subject to error are shown as a dotted line and are included only to emphasize the general trend.

The mean of the distribution of radioalbumin transit times through the heart, \( t_{\text{BSA}} \), is calculated from Zierler’s moment theorem\(^6\) as
\[
t_{\text{BSA}} = \int_0^\infty r(t)dt/r_0, \tag{5}
\]

and is independent of the assumptions used in compartmental modeling.

**Statistical Analysis**

Means and standard deviations (standard errors of the mean are used for data presented graphically) were calculated for each parameter assessed for normal diet and cholesterol diet groups at each perfusion time interval. A repeated-measures analysis of variance (ANOVA) test based on data obtained during the last 2 hours of perfusion was employed to assess the statistical significance of differences between groups of hearts. Student’s \( t \) test was used to assess differences in plasma cholesterol concentration, total water content, and baseline \( \kappa_{21} \) and \( t_{\text{BSA}} \).

**Results**

**Plasma Cholesterol Concentrations**

Plasma cholesterol concentrations from rabbits fed normal chow were 50 ± 17 mg/dl and 45 ± 20 mg/dl for nonischemic and ischemic control groups, respectively (Table 1). Plasma cholesterol concentrations were increased ~15-fold in rabbits fed a 2% cholesterol-enriched chow for 2–3 weeks (\( t = 5.27, p < 0.001 \)) and were increased ~45-fold in rabbits fed cholesterol for 5–16 weeks (\( t = 7.96, p < 0.001 \) vs. nonischemic controls; \( t = 4.53, p < 0.001 \) vs. 2–3 week cholesterol-fed group).

**Heart Weight, Flow Rate, and Water Content**

Heart weight (~1.1 g dry weight) and baseline perfusate flow rate (~22.0 ml/min/g dry weight) did not differ for any of the experimental groups (Table 1). Hearts from normal-diet rabbits reperfused for 3.5 hours following 30 minutes of ischemia contained 22% more water than continuously perfused hearts (\( t = 8.48, p < 0.001 \)). Although the water content of ischemia-reperfused hearts from cholesterol-fed rabbits was increased slightly in the 2–3 week group and decreased slightly in the 5–16 week group relative to the ischemic, normal diet group, these differences were not statistically significant. However, the myo-

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Table 1. Plasma Cholesterol, Buffer Flow Rate, and Gravimetric Data From Nonischemic Controls and Hearts Subjected to 30 Minutes of Global No-flow Injury

<table>
<thead>
<tr>
<th>Experiment</th>
<th>( n )</th>
<th>Plasma cholesterol (mg %)</th>
<th>Flow rate (ml/min/g dry wt)</th>
<th>Dry weight (g)</th>
<th>( H_2O ) content (ml/g dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonischemic control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal diet</td>
<td>15</td>
<td>50 ± 17*</td>
<td>22.1 ± 2.5</td>
<td>1.05 ± 0.22</td>
<td>5.26 ± 0.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30 minute no-flow ischemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal diet</td>
<td>14</td>
<td>45 ± 20</td>
<td>22.3 ± 1.9</td>
<td>1.07 ± 0.20</td>
<td>6.40 ± 0.41†</td>
</tr>
<tr>
<td>2% Cholesterol diet</td>
<td>15</td>
<td>720 ± 492†</td>
<td>21.8 ± 2.6</td>
<td>1.07 ± 0.18</td>
<td>6.66 ± 0.63†</td>
</tr>
<tr>
<td>2% Cholesterol diet</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2–3 Week duration</td>
<td>15</td>
<td>2040 ± 976†</td>
<td>21.8 ± 2.8</td>
<td>1.21 ± 0.22</td>
<td>6.06 ± 0.42†§</td>
</tr>
<tr>
<td>5–16 Week duration</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean values ± SD; \( n \) = number of rabbit hearts evaluated.

Student’s \( t \) test (compared to nonischemic controls): \( *p < 0.001 \); Student’s \( t \) test (compared to 2–3 week cholesterol-fed rabbits): \( \dagger p < 0.001 \); \( \ddagger p < 0.05 \).
cardiac water content of the 2–3 week cholesterol-fed rabbits was significantly greater than that of the 5–16 week group (t = 2.28, p<0.05).

Physiological Pressure Data

Increases in aortic perfusion pressure (Figure 1) and LVEDP (Figure 2) during reperfusion after ischemia were similar in the normal-diet and 5–16 week cholesterol-fed rabbits and were significantly greater than in nonischemic controls (ANOVA test; p<0.0001). In contrast, increases in perfusion pressure and LVEDP in hearts from 2–3 week cholesterol-fed rabbits were much greater than in the other ischemic groups (ANOVA test; p<0.0001). In both normal-diet and 5–16 week cholesterol-fed rabbits, +dP/dt returned to baseline values after 30 minutes of reperfusion (Figure 3); -dP/dt in hearts from rabbits fed cholesterol for 2–3 weeks remained significantly depressed (by ~30–40%; ANOVA test, p<0.0001) for the entire 3 hours of perfusion.

125I-BSA Time-Activity Curves

Figure 4 illustrates typical time-activity curves generated following a bolus injection of 125I-BSA into the proximal aorta after 60 (a), 120 (b), and 270 (c) minutes of continuous perfusion in controls and during baseline (a), 30 (b), and 180 (c) minutes of reperfusion in hearts subjected to 30 minutes of no-flow ischemia. Only 1 animal whose values most closely reflected the mean values for the entire group was selected for use in each panel. The data are normalized to the peak count rate, r°, and are plotted for the period beginning at the peak count rate to the time at which the count rate decreases to ~0.1% of the peak value. The curves generated during the baseline period (a) exhibited a rapid increase in peak activity as 125I-BSA entered the coronary circulation (and the detector field of view) and a rapid decrease in activity as the tracer was cleared from the intravascular compartment. The terminal portion of each curve exhibited monoeponential disappearance of activity to background levels over 15–20 minutes. In nonischemic hearts (Figure 4A), the y intercepts (A2) of the tails of the washout curves increased gradually with increasing duration of perfusion from ~0.009 for curve a to ~0.02 for curve c, while the slopes (α2) of the same portion of the curves remained relatively constant. In ischemic hearts from control rabbits (Figure 4B) and in hearts from rabbits fed cholesterol for 5–16 weeks (Figure 4D), the y intercepts of the tails of the washout curves increased progressively during reperfusion after ischemia from ~0.007–0.009 for curve a to ~0.08–0.09 for curve c, and the slopes became much steeper following ischemia (curves b and c) relative to baseline and controls. The slopes of the initial portion of the washout curves, α1, decreased during reperfusion in both groups of hearts. During baseline in hearts from rabbits fed cholesterol for 2–3 weeks (Figure 4C), the y intercepts of the tails of the washout curves (a) were increased approximately twofold relative to the other groups of

Figure 1. Aortic perfusion pressure for nonischemic (○; n = 15) and ischemic (●; n = 14) control hearts and ischemic hearts from rabbits fed 2% cholesterol for 2–3 weeks (△; n = 15) or 5–16 weeks (▲; n = 11). Values plotted represent mean ± SEM. Following a baseline stabilization period of 1 hour, ischemic hearts were subjected to 30 minutes of global no flow, followed by 3.5 hours of reperfusion at baseline flow rates. Nonischemic control hearts were perfused continuously for 5 hours.

Figure 2. Left ventricular end-diastolic pressure (LVEDP) for nonischemic (○; n = 15) and ischemic (●; n = 14) hearts from rabbits fed a normal diet and ischemic hearts from rabbits fed 2% cholesterol for 2–3 weeks (△; n = 15) or 5–16 weeks (▲; n = 11). Values plotted represent mean ± SEM. LVEDP was adjusted during baseline to ~5 mm Hg by filling the left ventricle balloon with KH buffer.
hearts (0.02 vs. <0.007–0.009), and during reperfusion, the biexponential character of the washout curves (b and c) was lost.

**125I-BSA Turnover Rate Constants and Mean Transit Time**

Baseline estimates of the 125I-BSA turnover-rate constant k0 (from vascular into extravascular space, Table 2) were about twice as high in the 2–3 week cholesterol-fed group as in all other groups. On the other hand, the rate at which nonextracted 125I-BSA was cleared from the coronary vasculature (k0) was similar for all groups of hearts during baseline (Figure 5). While k0 remained relatively constant in nonischemic controls, it decreased by ~33% during 3 hours of reperfusion after ischemia in hearts from normal diet controls (ANOVA test; p<0.0001 vs. nonischemic controls). In contrast, k0 of hearts from rabbits fed cholesterol for 5–16 weeks was midway between that of the nonischemic and ischemic controls after 3 hours of reperfusion and was not significantly different from either group. In hearts from rabbits fed a 2% cholesterol diet for 2–3 weeks, estimates of k0 did not differ from the other groups of hearts at the beginning of reflow but did decrease significantly during the first hour of reperfusion. During the last 2 hours of reperfusion, reliable estimates of k0 could not be obtained from the two-compartment model since 2 exponential terms no longer adequately described the experimental data. Rough estimates for the group (obtained from the model and plotted as a dotted line during the last 2 hours of reperfusion) indicated that k0 continued to decrease throughout reperfusion.

Estimates of tBSA averaged 6.7 seconds during baseline in nonischemic controls (Figure 6) and increased gradually during the course of perfusion by ~60% after 5 hours (t = 8.49, p < 0.001; compared to baseline). During reperfusion after ischemia, tBSA increased rapidly in ischemic hearts from normal diet controls and during the last 2 hours of reperfusion was about twice that of nonischemic controls (ANOVA test, p < 0.0001). In hearts from rabbits fed cholesterol for 2–3 weeks, tBSA was higher than in the other groups during baseline (t = 2.51; p < 0.025) and increased even more during reflow (ANOVA test; p < 0.0001 vs. all other groups of ischemic hearts). In hearts from rabbits fed cholesterol for 5–16 weeks, tBSA was the same as controls during baseline and, during reflow, remained lower than in ischemic controls (ANOVA test; p < 0.0001).

**Discussion**

The important implication of these observations is that the initial phase of cholesterol feeding alters the permeability characteristics of coronary vascular endothelium (manifested by increased baseline albumin permeation, i.e., increased k0) and increases the susceptibility of the heart to ischemia-reperfusion injury (manifested by the significantly prolonged washout of 125I-BSA and the breakdown of the biexponential character of the washout curve). The observation that plasma cholesterol concentrations were threefold higher in the 5–16 week cholesterol-fed rabbits than in the 2–3 week group suggests that these phenomena are not the consequence of hypercholesterolemia per se but, rather, are somehow linked to the altered metabolic milieu created by cholesterol feeding and/or to the rate of increase in plasma cholesterol concentration. The observation that these phenomena are absent in rabbits fed the same diet for longer periods (and with plasma cholesterol concentrations tripled) suggests that the vasculature of these rabbits has adapted to the altered metabolic milieu associated with increased cholesterol consumption and is able to reestablish normal permeability characteristics and susceptibility to ischemia-reperfusion injury or that some of the metabolic alterations (responsible for vascular injury) associated with increased cholesterol ingestion are of a transient nature.

Although several parameters assessed in ischemic hearts from 5–16 week cholesterol-fed rabbits were midway between those of ischemic and nonischemic controls, the magnitude of these differences (suggesting supranormal resistance to ischemia-reperfusion injury) was relatively small and of doubtful physiological significance. While the apparent normalization of some of the parameters measured in the 5–16 week cholesterol-fed rabbits might be attributed to occlusive intimal thickening of arteries by atherosclerotic plaques, this possibility would appear to be unlikely in view of the absence of any corresponding loss of myocyte contractility (+dP/dt) either during baseline or reperfusion after ischemia (which should occur if significant narrowing of coronary arteries is present) rela-
tive to ischemic hearts from rabbits fed a normal diet. Evaluation of oil red, O-stained frozen sections of left ventricular myocardium revealed the presence of small lipid droplets in vascular smooth muscle of the 2–3 week cholesterol-fed rabbits (unpublished observations). In the 5–16 week cholesterol-fed group, there was evidence of patchy intimal thickening (which contained numerous lipid droplets) in addition to small lipid droplets in vascular smooth muscle but no discernable narrowing of vessel lumens. No evidence of lipid deposition was observed in any of the nonischemic and ischemic hearts from rabbits fed a normal chow.

While the mechanism(s) by which the initial phase of hypercholesterolemia increases vascular permeability and susceptibility of the heart to ischemic injury remains to be determined, it is of interest that acute increases in membrane cholesterol content have been reported to 1) increase the internal viscosity and alter the permeability of natural and synthetic phospholipid membranes, 2) accentuate contractile responses of vascular smooth muscle to various stimuli, and 3) decrease Na⁺, K⁺-ATPase activity. If the effects of acute cholesterol feeding in the present experiments are due to an increase in cholesterol content of cell membranes, then the transient nature of these effects may reflect an adaptive response of cells to the presence of elevated cholesterol levels.

Since the cholesterol content of cell membranes is

<table>
<thead>
<tr>
<th>Experiment</th>
<th>n</th>
<th>$k_{21}$ (1/sec)</th>
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</thead>
<tbody>
<tr>
<td>Baseline data for control hearts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal diet</td>
<td>15</td>
<td>0.0035 ± 0.0010*</td>
</tr>
<tr>
<td>Baseline data for ischemic hearts</td>
<td>14</td>
<td>0.0035 ± 0.0010</td>
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<tr>
<td>2% Cholesterol diet</td>
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<td></td>
</tr>
<tr>
<td>2–3 Week duration</td>
<td>15</td>
<td>0.0062 ± 0.0029†</td>
</tr>
<tr>
<td>5–16 Week duration</td>
<td>11</td>
<td>0.0037 ± 0.0014</td>
</tr>
</tbody>
</table>

*Mean values ± SD; $n$ = number of rabbit hearts evaluated.
†Student’s $t$ test (compared to all other groups): $p<0.025.$

was evidence of patchy intimal thickening (which contained numerous lipid droplets) in addition to small lipid droplets in vascular smooth muscle but no discernable narrowing of vessel lumens. No evidence of lipid deposition was observed in any of the nonischemic and ischemic hearts from rabbits fed a normal chow.

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Since the cholesterol content of cell membranes is
modulated by plasma lipoprotein composition and concentration, the increased susceptibility of the heart to ischemic injury also may reflect changes in plasma lipoproteins. It is well known that cholesterol feeding results in elevated concentration, increased size, and marked compositional changes in lipoproteins21,22 (d < 1.006 g/ml) as well as decreases in low (LDL) and high (HDL) density lipoproteins in rabbits.22 Daugherty et al (unpublished observations) demonstrated that the structural heterogeneity of cholesterol ester-rich lipoproteins (d < 1.006 g/ml) in rabbits is dependent on the duration of cholesterol feeding. During the first 4 days of cholesterol feeding, intestinally-derived particles are the major cholesterol-carrying particles in lipoproteins (d < 1.006 g/ml), and as the duration of cholesterol feeding increases, hepatic-derived particles become increasingly prominent with greater than 94% of cholesterol being present in this fraction after 1 month of cholesterol feeding. Rapid changes in lipoprotein composition and/or substances other than cholesterol also may account for the findings in the 2–3 week cholesterol-fed group. It is important to note that rabbits fed 2% cholesterol tolerated the diet well and gained weight (in view of the increased heart size for the 5–16 week cholesterol-fed group shown in Table 1) and that no animals died during the study period.

The twofold increase in baseline albumin permeation (reflected in increased values of kγ) of hearts from 2–3 week cholesterol-fed rabbits indicates that structural and/or functional integrity of vascular endothelium is compromised and suggests that short-term cholesterol feeding results in injury to vascular endothelium in the absence of ischemia. While the elevated baseline estimates of IBSA are consistent with this interpretation, they also could be accounted for by an increased extracellular fluid volume (not determined during baseline), which does not affect kγ.

The most likely explanation for the gradual increase in IBSA, in nonischemic rabbits fed a normal diet is an expanding extracellular space accessible to albumin. This interpretation is based on previous studies in our laboratory that indicate that the increased water content of these hearts (relative to nonperfused hearts or hearts perfused with erythrocyte-enriched perfusates) is restricted to the cardiac interstitium23 and may simply be a consequence of a downstream shift in pressure gradients across vessel walls (due to the low perfusate viscosity) resulting in an increase in convective transport of water. The observations that maximum +dP/dt and perfusion pressure remained constant for the entire duration of perfusion suggest that the extracellular edema did not compromise myocardial contractility or vascular resistance. The much greater increase in IBSA during the course of reperfusion of acutely hypercholesterolemic hearts could be due to 1) additional ischemia-reperfusion-induced damage to vascular endothelium, 2) increased albumin ultrafiltration in response to the higher perfusion pressures, 3) increased flow heterogeneity (see below), and/or 4) a larger volume of distribution for 125I-BSA. The latter possibility could include both an increase in the interstitial fluid space as well as the intracellular spaces of lethally injured myocytes.

The likelihood that the increased vascular resistance observed in these experiments is due, in part, to ischemic contracture of vessels is suggested by observations that in normal-diet–fed rabbits, increased vascular resistance develops during reperfusion after ischemia despite normal myocyte contractile function and can be blocked completely with the calcium antagonist diltiazem.24 The observation in these experiments that the decrease in left ventricular end-diastolic pressure during reperfusion was not accompanied by a decrease in perfusion pressure implies that tissue pressure was not completely responsible for the vascular resistance changes and further suggests that vasoconstriction during reperfusion after ischemia increases vascular resistance. The higher perfusion pressure during reflow in hearts from acutely hypercholesterolemic rabbits (vs. ischemic controls and 5–16 week cholesterol-fed rabbits) is consistent with 1) a direct effect of cholesterol or metabolic alterations associated with increased cholesterol ingestion on vascular smooth muscle, enhancing its sensitivity to a variety of vasoconstricting stimuli present within ischemic myocardium,25–29 and 2) deprivation of flow to significant portions of the myo-
cardium (i.e., areas of "no reflow" or perfusion defects). Damage to vascular endothelium also may influence the contractile state of smooth muscle through a reduction in endothelium-derived relaxing factors and/or prostacyclin production, and it is of interest, in this regard, that prostacyclin generation from exogenous arachidonic acid is impaired in the coronary vasculature of hypercholesterolemic rabbits. That acute hypercholesterolemia also contributed to impaired myocyte contractile function in response to ischemia is attested to by the marked decrease in +dP/dt and increase in LVEDP during reperfusion. Both of these observations are consistent with the development of irreversible myocardial contracture, which, by definition, is a rise in resting tension of the left ventricular wall at constant lumenal volume. The contracture itself may be the consequence of ATP depletion, calcium redistribution, and/or formation of rigor complexes within myocytes. The possibility that myocyte cell death could account for some of the changes seen during posts ischemic reperfusion is a distinct possibility, since changes observed during reflow after 30 minutes of no-flow ischemia in the 2–3 week cholesterol-fed rabbits are virtually identical to changes observed during reflow after 60 minutes of no-flow ischemia in hearts from rabbits fed a normal diet. An increase in cholesterol membrane content could contribute to the ischemia-induced contracture of both vascular smooth muscle and myocytes (as well as the increased baseline albumin permeation) by inhibiting Na\(^+\), K\(^+\)-ATPase activity with a resultant increase in intracellular Ca\(^{2+}\).

It has been suggested that ischemic myocardial contracture can lead to impaired vascular perfusion during reflow after ischemia; however, the pathophysiological mechanisms responsible for such hemodynamic changes are unclear and may involve other factors such as vasospasm, vascular compression resulting from endothelial cell and/or myocyte edema, and alterations in blood viscosity and/or thrombosis in addition to myocyte contracture. The observation that \( k_{in} \) (clearance of intravascular tracer from the heart) was not decreased early during reflow (relative to baseline) in the face of a markedly elevated LVEDP indicates that myocyte contracture preceded the perfusion defect but does not prove a causal relation between the two.

We elected to use asanguineous Krebs-Henseleit buffer to perfuse isolated rabbit hearts to avoid difficulties associated with aggregation of cellular elements and/or hemolysis during 5 hours of perfusion. Although the oxygen-carrying capacity of blood-free medium is much less than that of erythrocyte-enriched perfusates, the perfusate viscosity also is decreased, and coronary flow can be increased to levels sufficient to prevent hypoxia and to maintain myocardial contractile function. Isolated rabbit hearts perfused with Krebs-Henseleit buffer have been shown to have the capacity to rapidly synthesize, release, and inactivate prostaglandins as well as several other potent vasoactive substances that can modulate coronary resistance and cardiac performance. While the perfusion pressure used in this study is considerably below in vivo arterial pressure, it provides a high flow rate for sufficient tissue oxygenation because of the relatively low viscosity of the perfusate. It is noteworthy that maximum +dP/dt remained constant for the entire duration of perfusion and was comparable to that reported in previously published studies using erythrocyte-enriched perfusates.

In conclusion, the transient nature of the heart’s increased susceptibility to ischemic injury in rabbits fed cholesterol-enriched chow shows that the altered metabolic milieu and/or the rapidly increasing plasma cholesterol concentration associated with short-term cholesterol feeding are significant risk factors for ischemic injury to the heart independent of occlusive coronary artery disease produced by chronic hypercholesterolemia.

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Increased ischemia-reperfusion injury to the heart associated with short-term, diet-induced hypercholesterolemia in rabbits.

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