Different Effects of a High-Cholesterol Diet on Ischemic Cardiac Dysfunction and Remodeling Induced by Coronary Stenosis and Coronary Occlusion

Hiroyuki Yaoita, MD, Kazuyuki Yoshinari, MD, Kazuhira Maehara, MD, Masahito Sando, MD, Kenichi Watanabe, MD, Yukio Maruyama, MD
Fukushima, Japan

OBJECTIVES
The aim of the study was to assess whether and how the high-cholesterol diet (HCD)-related worsening of heart failure differs between coronary stenosis (CS)-induced myocardial ischemia and coronary occlusion-induced myocardial infarction (MI).

BACKGROUND
An HCD, a risk factor for coronary artery disease, also worsens ischemic heart failure. Although accelerated coronary plaque formation may be a cause of this, other mechanism(s), such as its effects through the coronary microcirculation, remain to be clarified.

METHODS
In rats fed a normal chow diet or HCD, CS or MI was created surgically, and we assessed left ventricular (LV) function by echocardiography and myocardial inflammation by histopathology. In the CS groups, CS severity by histopathology, myocardial perfusion by microspheres, myocardial protein kinase C (PKC) translocation by Western blotting, and myocardial endothelial nitric oxide (NO) function were also investigated by the in vitro myocardial oxygen consumption method.

RESULTS
Coronary stenosis impaired myocardial endothelial NO function and reduced coronary flow reserve, evoking myocardial ischemia, as shown by PKC-ε activation, myocardial inflammation, fibrosis, cardiac dysfunction, and remodeling. By itself, HCD greatly augmented such CS-induced myocardial abnormalities without modulating the CS severity. Such detrimental effects of HCD were ameliorated by supplying a cofactor of endothelial NO synthase—tetrahydrobiopterin. In contrast, MI-induced heart failure was not aggravated by HCD.

CONCLUSIONS
The CS-induced ischemic myocardium seems to be more susceptible to the pro-inflammatory effect of HCD than infarcted myocardium, leading to aggravation of LV dysfunction and remodeling via modification of the coronary circulation downstream of the epicardial CS site, partly through impairment of endothelial NO. (J Am Coll Cardiol 2005;45:2078–87) © 2005 by the American College of Cardiology Foundation

Dietary cholesterol in excess promotes coronary plaque formation, which increases the susceptibility to myocardial ischemia and aggravates ischemic heart disease (1–3). Moreover, a cholesterol burden closely related to a continuous high-cholesterol diet (HCD) impairs coronary endothelial nitric oxide synthase (eNOS) activity in small vessels, as well as in conduit vessels through formation of complexes of eNOS protein and inhibitory caveolin (4). Coronary microvascular eNOS dysfunction impairs the regulation of coronary flow and mitochondrial respiration (5) and promotes inflammatory cell infiltration (6,7). Although acute myocardial ischemia-reperfusion injury is aggravated by cholesterol burden (6), it remains to be clarified whether and how cholesterol burden aggravates cardiac function in the infarcted heart and in the ischemic heart with chronic coronary stenosis (CS) without modulating the severity of CS. Myocardial damage induced by HCD, if any, may not be the same in the two ischemic conditions—that is, infarcted or ischemic but viable myocardium. Therefore, in the animal models of these conditions, we assessed how different kinds of myocardial ischemia due to CS or myocardial infarction (MI) are involved in the worsening of heart failure by HCD and also how HCD affects endothelial NO dysfunction.

METHODS
This investigation conformed to the Guideline on Animal Experiments of Fukushima Medical University, the Japanese Government Animal Protection and Management Law (no. 115), and the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH publication no. 85–23, revised 1996).

Diets. Male Sprague-Dawley rats were divided into two groups: the normal chow diet (NCD) group (n = 252) and the 2% HCD (8) group (n = 268), which was started at seven weeks of age. Either CS or MI was created at 10 weeks of age in rats on NCD and at nine weeks of age in rats on HCD to minimize differences in body and heart weights between the two groups.

Coronary stenosis and MI. Coronary stenosis was created in rats (Fig. 1), according to our previous reports (9,10), with the modification using 325 μm diameter thread instead of 275 μm (9,10). Briefly, rats were anesthetized by intraperitoneal administration of 45 mg/kg sodium pentobarbital and, under artificial ventilation, the left chest was opened. Then, the proximal portion of the left coronary

From the First Department of Internal Medicine, Fukushima Medical University, Fukushima, Japan.
Manuscript received June 3, 2004; revised manuscript received February 28, 2005, accepted March 10, 2005.

Journal of the American College of Cardiology
© 2005 by the American College of Cardiology Foundation
Published by Elsevier Inc. doi:10.1016/j.jacc.2005.03.037
artery, 1 to 2 mm below its origin from the aorta, was occluded together with the thread using the surgical strings followed by thread removal. Transient coronary occlusion and recanalization were confirmed by the elevation of the ST-segment exceeding an R-wave amplitude on an electrocardiographic limb lead II and its reversal, respectively. Then, the left chest was closed, and the rats were allowed to recover.

Myocardial infarction (Fig. 1) was made by ligation at the equivalent site of the left coronary artery, which was confirmed by persistent ST-segment elevation.

Thirty-five cases with either persistent ST-segment elevation after thread removal or fatal arrhythmia just after creating CS or MI were excluded from the study.

Rats that recovered from the surgery (n = 236 in the NCD group, n = 249 in the HCD group) were returned to their cages and allowed free access to food and water until they were sacrificed at 1 day or 1, 4, or 12 weeks later.

**Blood pressure and heart rate in the awake state.** Systolic blood pressure and heart rate in the awake state were measured by the tail-cuff method before and 4, 8, and 12 weeks after surgery.

**Echocardiography.** Using the echocardiographic equipment (a 10-MHz probe and a Hewlett Packard Sonos 100), LV end-diastolic and end-systolic diameters and LV ejection fraction were obtained by the Pombo method (9,10) before and 4, 8, and 12 weeks after surgery.

**In vitro myocardial oxygen consumption (MVO$_2$) measurement.** We measured the functional activity of myocardial at NO 24 h after surgery in the sham and CS groups (n = 7 each; Fig. 1). Under anesthesia, the risk area was determined by infusing Evans blue into the ascending aorta at a perfusion pressure of 100 mm Hg after reoccluding the previous CS site. As described previously (5,9), myocardial tissue of the risk area, weighing about 30 mg, was bathed in Krebs' solution; then, oxygen uptake by myocardial specimens (MVO$_2$) was measured in the chamber polarographi-
cally (5,9). The principle of this method is that the mitochondrial respiratory chain and cytochrome c oxidase activity are suppressed by NO in a concentration-dependent manner (5). Myocardial oxygen demand was measured for 5 min (i.e., decrease of oxygen content in the buffer/min/wet g) and calculated as a rate (%) of decrease in MVO₂ of the buffer. Then, after 10⁻⁴ mol/l bradykinin (to assess bradykinin receptor-mediated endothelial NO effect) or 10⁻⁴ mol/l sodium nitroprusside was added to the bath. We also assessed the effect of pretreating the specimens with 10⁻⁴ mol/l N-ω-nitro-L-arginine methyl ester (L-NAME) on MVO₂ changes to confirm involvement of NO in the effect of bradykinin.

**Cardiac catheterization.** Four weeks (n = 10 each in the four NCD and HCD CS groups with and without tetrahydrobiopterin supplementation, as described later) or 12 weeks (total n = 24 to 34 in the six NCD and HCD groups with sham, CS, or MI) after surgery, LV hemodynamics were measured in the anesthetized state by cardiac catheterization (Fig. 1).

**Myocardial perfusion.** One or 12 weeks after surgery, myocardial blood flow (ml/min/wet g) and coronary flow reserve (ml/min/wet g; maximal myocardial blood flow by myocardial blood flow (ml/min/wet g) and coronary flow (ml/min/wet g)) were measured in the risk area were measured by the colored reserve (ml/min/wet g; maximal myocardial blood flow by myocardial blood flow (ml/min/wet g) and coronary flow (ml/min/wet g)) and calculated as a rate (%) of decrease in MVO₂ of the buffer. Then, after 10⁻⁴ mol/l bradykinin (to assess bradykinin receptor-mediated endothelial NO effect) or 10⁻⁴ mol/l sodium nitroprusside was added to the bath. We also assessed the effect of pretreating the specimens with 10⁻⁴ mol/l N-ω-nitro-L-arginine methyl ester (L-NAME) on MVO₂ changes to confirm involvement of NO in the effect of bradykinin.

**Semiquantitative histopathology.** One (n = 7 each), four (n = 10 each), and 12 weeks (n = 10 to 13) after surgery, following perfusion fixation with 4% paraformaldehyde at a pressure of 100 mm Hg, we assessed CS severity (%) by the cross-sectional areas of the coronary inner lumen (areas at the CS site/areas at the reference site) (9,10), using light microscopy and the following procedure. In 5-μm-thick paraffin-embedded sections stained with elastica Van Gieson, cross-sectional areas of left coronary arterial inner lumen were measured by the point counting method of Weibel (11) by light microscopy at ×200 magnification. The areas at the stenotic site (identified by the minimal areas) divided by the reference cross-sectional areas located 50 sections proximal to the sections of the stenotic area multiplied by 100 was considered to be the degree (%) of CS (9,10).

One day (n = 7 each, following the in vitro MVO₂ study) and one week (n = 7 each) or 12 weeks (n = 10 to 13) after CS and one day (n = 7 each) or 12 weeks (n = 12 or 13) after MI, myocardial specimens were divided into three short-axial myocardial slices. From the middle slice, 5-μm-thick paraffin-embedded sections were stained with anti-monocyte monoclonal antibodies (ED-1, Chemicon International, Temecula, California) or anti-monocyte chemoattractant protein (MCP-1) antibodies (Santa Cruz Biotechnology Inc., Santa Cruz, California) (7). The LV free wall was divided into five radial segments (12). The following examinations were done in the central three segments (within the risk area). Because tissue MCP-1 positivity was observed, especially in coronary small vessels (<300 μm in diameter) of the risk area, we counted MCP-1-positivity (number of MCP-1-positive vessels [<300 μm in diameter] per 1,000-point count area of the three segments) in the endocardial layers (where coronary vessels appear in their short axes) of the risk areas using the point counting method (11). We also assessed the grade of myocardial fibrosis (point counts for fibrotic area/point counts for the three segment areas [%]), ED-1 positivity (ED-1–positive cell number per 1,000-point count area of the three segment areas), and TUNEL positivity of cardiomyocytes (point counts for TUNEL-positive cardiomyocytes/point counts for the three segment areas [%]) (12).

**Western blotting for myocardial protein kinase C (PKC).** In six subgroups of CS and shams (n = 6 each; Fig. 1), we assessed whether CS transmitted intracellular signals, which are provoked by ischemia in this species (13). The ischemic preconditioning (IPC: two episodes of 5-min coronary occlusion followed by 5-min coronary reperfusion) was used as a positive control (n = 7 each in the NCD and HCD sham plus IPC groups). One week after surgery, using the homogenates of the anterior wall (the risk area) and the posterior one-third portion of the interventricular septum (the non-risk area) in the middle LV, Western blotting for myocardial PKC-ε isoform was performed and then quantified by densitometry. The PKC-ε translocation ratio was expressed as PKC-ε density in the membrane fraction divided by its density in the cytosolic fraction, and we considered it an approximate index of its activation. The data in each group are presented as the ratio (%) to that of the NCD sham one week after surgery.

**Intervention with tetrahydrobiopterin.** To assess the effects of ameliorating eNOS on CS-induced heart failure, we created CS under general anesthesia in the NCD (n = 40) and HCD (n = 41) groups. Six hours after creating CS when surviving animals (n = 37 each) were awake, we started to administer 10 mg/kg/day tetrahydrobiopterin, a co-factor of eNOS (NCD or HCD CS plus tetrahydrobiopterin, dissolved in water; Fig. 1) through a cannula gently inserted into the mouth every morning for one day, one week, or four weeks after CS. The doses administered were determined according to the previous report (14). Randomly selected groups of 27 rats each in the NCD and HCD groups were used one day (n = 7 each) for in vitro...
MVO\textsubscript{2} and histopathologic studies, or one week (n = 20 each) for the PKC, microsphere, and histopathologic studies after CS, and the other 10 rats in each group were used for echocardiography (the data were compared with 10 rats each of the NCD and HCD groups sacrificed four weeks after CS), catheterization, and histopathologic studies four weeks after CS.

**Blood samples and liver weight.** In 12-week survivors, we measured serum lipid levels without starvation by spectrophotometry, norepinephrine, and vitamin C by high-performance liquid chromatography, as well as ascorbyl free radicals, a marker of endogenous free radicals, by the electron spin trap resonance method (10).

**Statistics.** Data are expressed as the mean value ± SE. Multiple comparisons were performed by one-way analysis of variance in Figures 2 through 7. If the F test results were significant, post hoc comparisons were made using Bonferroni’s test.

**Figure 2.** Effects of NCD and HCD on left ventricular echocardiographic findings in rats with coronary stenosis (left) and MI (right). n = 24 to 34; for statistical analysis, n = 33 each (CS groups vs. sham, NCD vs. HCD) and 24 each (MI groups) for Bonferroni’s post-hoc comparisons. *p < 0.01 vs. corresponding sham; †p < 0.01 NCD-CS. (n) indicates survivors for 12 weeks.

**Figure 3.** Effects of tetrahydrobiopterin (n = 10 each) on left ventricular echocardiographic findings four weeks after CS. The data on NCD- and HCD-sham (n = 33 each) are the same as those in Figure 2 (for statistical analysis, n = 10 each in other four groups for Bonferroni’s post-hoc comparisons). Open circles = NCD; closed circles = HCD. N = 33 each in NCD- and HCD-sham. N = 10 each in NCD- and HCD-CS. N = 10 each in NCD- and HCD-CS with tetrahydrobiopterin. *p < 0.01, †p < 0.05 vs. corresponding sham; ‡p < 0.01, §p < 0.05 vs. corresponding CS; ||p < 0.01, ¶p < 0.05 vs. corresponding NCD. LV = left ventricular; other abbreviations as in Figure 1.
RESULTS

Survival rates at 12 weeks. Survival rates at 12 weeks (Table 1, Fig. 1) tended to be lower (p = NS) in the HCD groups than in the NCD groups, as well as in the MI groups than in the CS groups (74%, 64%, 67%, and 55% in the NCD-CS, HCD-CS, NCD-MI, and HCD-MI groups, respectively, excluding rats sacrificed).

Body weights. Body weights (g) 12 weeks after surgery were higher (*p < 0.05) or tended to be higher in the HCD groups than in each corresponding NCD group (375 ± 8, 365 ± 12, and 359 ± 28 in the NCD-sham, -CS, and -MI groups, respectively, and 395 ± 7, 381 ± 11, and 366 ± 31 in the HCD-sham, -CS and -MI groups, respectively). Twelve weeks after surgery, liver weight (Table 1) was larger in the HCD group and related to obesity.

Echocardiography. The LV end-diastolic and end-systolic diameters increased, and LV ejection fraction decreased in the NCD-CS and -MI groups compared with the NCD-sham group, although LV end-diastolic and end-systolic diameters were less in the NCD-CS group than the NCD-MI group (p < 0.05 at four weeks, p < 0.01 at eight and 12 weeks, respectively; asterisks not shown in Fig. 2). Compared with the NCD-CS group, the HCD-CS group had increased LV diameters and decreased LV ejection fraction (p < 0.01 each). In contrast, HCD did not affect LV diameters and LV ejection fraction in the MI groups (Fig. 2).

Four weeks after CS with tetrahydrobiopterin supplementation, LV end-diastolic and end-systolic diameters were smaller and LV ejection fraction larger than in the corresponding NCD-CS (p < 0.05 each) and HCD-CS groups (p < 0.01 each) (Fig. 3).

Hemodynamics in the anesthetized state. Twelve weeks after CS or MI, compared with the NCD- or HCD-sham groups, LV end-diastolic pressure was greater, /dP/dt was lower, and plasma norepinephrine was higher in the NCD- and HCD-CS and -MI groups (Table 1).

Four weeks after CS, in the NCD and HCD groups with tetrahydrobiopterin supplementation, compared with the corresponding groups without supplementation, LV systolic pressure did not change (e.g., 126 ± 5 mm Hg in the NCD group and 123 ± 4 mm Hg in the HCD group with tetrahydrobiopterin supplementation), but LV end-diastolic pressure (3 ± 1 mm Hg* vs. 8 ± 1 mm Hg in the NCD group and 4 ± 1 mm Hg† vs. 11 ± 2 mm Hg in the HCD group).
group, respectively) was lower, whereas \( \frac{dP}{dt} \) was larger (i.e., \( 6,301 \pm 458 \) mm Hg/s vs. \( 4,432 \pm 265 \) mm Hg/s in the NCD group and \( 5,921 \pm 363 \) mm Hg/s vs. \( 3,858 \pm 180 \) mm Hg/s in the HCD group, respectively; \( \ast p < 0.05, \ast \ast p < 0.01 \)).

**Myocardial perfusion.** Myocardial blood flow decreased in the HCD-CS group compared with the NCD-CS group, but coronary flow reserve decreased comparably in the NCD-CS and HCD-CS groups, compared with the corresponding sham group both one and 12 weeks after surgery. Moreover, HCD itself (HCD-sham) decreased coronary flow reserve at both 1 and 12 weeks after surgery (Fig. 4).

**In vitro MVO\(_2\).** Bradykinin decreased in vitro MVO\(_2\) 24 h after surgery in the NCD-sham group, which was reversed by pretreatment with L-NAME, whereas bradykinin did not affect the HCD-sham group, regardless of pretreatment with L-NAME (Fig. 5, left panel). Moreover, bradykinin decreased MVO\(_2\) in the NCD-CS group but not the HCD-CS group (Fig. 5, right panel). In the NCD-CS group, the bradykinin-induced MVO\(_2\) decrease was smaller than that in the NCD-sham group. Sodium nitroprusside

![Figure 5. Changes in the in vitro MVO\(_2\) by bradykinin and sodium nitroprusside 24 h after sham surgery or CS. (Left) The effect of 10\(^{-4}\) mol/l L-NAME on the in vitro MVO\(_2\) changes by bradykinin and sodium nitroprusside in the sham group with NCD and HCD. (Right) The effect of BH4 on those in the CS groups with NCD and HCD (n = 7 in each group for Bonferroni’s post-hoc comparisons). \( \ast p < 0.01 \) vs. baseline; \( \ast \ast p < 0.01 \) vs. NCD-sham; \#p < 0.05 HCD-CS vs. NCD-CS. P < 0.05 NCD-CS + BH\(_4\) vs. NCD-CS; p < 0.01 HCD-CS + BH\(_4\) vs. HCD-CS (asterisks not shown on right panel). BK = bradykinin; SNP = sodium nitroprusside; other abbreviations as in Figures 1 and 4.](image)

**PKC translocation ratios 1 week after surgery (% of NCD-sham)**

![Figure 6. Protein kinase C (PKC)-\(\alpha\) translocation ratios in myocardium at risk one week after CS or sham surgery (n = 6 each for Bonferroni’s post-hoc comparisons). Ischemic preconditioning (IPC) (n = 7 each) was used as a positive control for PKC-\(\alpha\) activation by ischemia. n = 6 each group except n = 7 each in the NCD-sham + IPC and HCD-sham + IPC groups. \( \ast p < 0.01 \) vs. corresponding sham; \( \ast \ast p < 0.01 \), \( \ast \ast \ast p < 0.05 \) vs. corresponding CS; \#p < 0.05 vs. NCD-CS. Abbreviations as in Figure 1.](image)
similarly decreased MVO₂ in the NCD- and HCD-sham and CS groups.

In the CS groups with tetrahydrobiopterin supplementation, the responses of in vitro MVO₂ to bradykinin became greater (Fig. 5, right panel, asterisks not shown) than in the corresponding NCD- and HCD-CS groups without supplementation.

**Protein kinase C.** Compared with the sham group, the translocation ratios of PKC-ε one week after CS did not change in the non-risk area (105 ± 8% of the NCD-sham group). In contrast, they increased in the risk areas (p < 0.01 each; Fig. 6) and were higher (p < 0.05) in the HCD-CS group than the NCD-CS group, and those of the HCD-CS group were comparable to the IPC group. The increases in PKC-ε levels by CS were reversed by tetrahydrobiopterin supplementation (p < 0.05 in NCD-CS, p < 0.01 in HCD-CS).

**Histopathology.** The severity (%) of CS was not different in the NCD and HCD groups at 1 and 4 weeks (1.6 ± 0.1% and 2.0 ± 0.1% [1 week] and 2.1 ± 0.3% and 2.0 ± 0.2% [4 weeks] in NCD- and HCD-sham groups, respectively; and 56.3 ± 8.9% and 57.1 ± 9.2% [1 week] and 58.3 ± 9.6% and 59.8 ± 10.4% [4 weeks] in NCD-CS and HCD-CS groups, respectively; *p < 0.01 vs. corresponding sham) and 12 weeks after CS and sham surgery (Table 1) and in the two tetrahydrobiopterin groups four weeks after CS (59.2 ± 9.2% and 58.4 ± 10.1%*, respectively; *p < 0.01 vs. corresponding sham). There were no indications of intimal and medial thickening, thrombi, or plaque formation in any group at the CS site (data not shown).

Twelve weeks after surgery, the myocardial fibrosis was similar in the MI groups, whereas it was higher (p < 0.01) in the HCD-CS group than in the NCD-CS group (Table 1). Myocardial fibrosis at four weeks after CS with supplementation was lower (p < 0.01 each), not only in the NCD-CS group (20.7 ± 4.1% vs. 7.2 ± 2.8%), but also in the HCD-CS group (55.1 ± 9.9% vs. 15.2 ± 2.8%), compared with the CS groups without supplementation.

There were no MCP-1 immunoreactivities one day after CS, but there were at one and 12 weeks after CS (Figs. 7A and
Table 1. Cardiac Catheterization and Postmortem Examination 12 Weeks After Coronary Stenosis or Myocardial Infarction

<table>
<thead>
<tr>
<th>Groups</th>
<th>Coronary Stenosis Severity (%)</th>
<th>Heart Rate (beats/min)</th>
<th>LVSP/EDP (mm Hg)</th>
<th>Ascorbyl Free Radical (Relative Intensities)</th>
<th>Non-epinephrine (ng/ml)</th>
<th>Cholesterol (mg/dl)</th>
<th>Liver Weight (g)</th>
<th>Liver Fibrosis of Risk Area (%)</th>
<th>Liver Cholesterol (pmol/l)</th>
<th>Fibrosis of Liver (Relative Intensities)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCD-sham (33)</td>
<td>21.4 ± 0.1</td>
<td>125 ± 5.2</td>
<td>128 ± 83</td>
<td>0.7 ± 0.2</td>
<td>39.7 ± 6.1</td>
<td>6,308</td>
<td>17.5 ± 0.7</td>
<td>5.6 ± 0.5</td>
<td>23 125</td>
<td>4.1 ± 0.6</td>
</tr>
<tr>
<td>HCD-sham (33)</td>
<td>21.4 ± 0.1</td>
<td>125 ± 5.1</td>
<td>128 ± 83</td>
<td>0.7 ± 0.1</td>
<td>37.7 ± 0.1</td>
<td>6,158</td>
<td>17.1 ± 0.1</td>
<td>4.0 ± 0.1</td>
<td>23 128</td>
<td>4.0 ± 0.1</td>
</tr>
<tr>
<td>NCD-coronary stenosis (34)</td>
<td>21.4 ± 0.1</td>
<td>125 ± 5.2</td>
<td>128 ± 83</td>
<td>0.7 ± 0.2</td>
<td>39.7 ± 6.1</td>
<td>6,158</td>
<td>17.1 ± 0.1</td>
<td>4.0 ± 0.1</td>
<td>23 128</td>
<td>4.0 ± 0.1</td>
</tr>
<tr>
<td>HCD-coronary stenosis (34)</td>
<td>21.4 ± 0.1</td>
<td>125 ± 5.1</td>
<td>128 ± 83</td>
<td>0.7 ± 0.1</td>
<td>37.7 ± 0.1</td>
<td>6,158</td>
<td>17.1 ± 0.1</td>
<td>4.0 ± 0.1</td>
<td>23 128</td>
<td>4.0 ± 0.1</td>
</tr>
<tr>
<td>NCD-myocardial infarction (26)</td>
<td>21.4 ± 0.1</td>
<td>125 ± 5.2</td>
<td>128 ± 83</td>
<td>0.7 ± 0.2</td>
<td>39.7 ± 6.1</td>
<td>6,158</td>
<td>17.1 ± 0.1</td>
<td>4.0 ± 0.1</td>
<td>23 128</td>
<td>4.0 ± 0.1</td>
</tr>
<tr>
<td>HCD-myocardial infarction (24)</td>
<td>21.4 ± 0.1</td>
<td>125 ± 5.1</td>
<td>128 ± 83</td>
<td>0.7 ± 0.1</td>
<td>37.7 ± 0.1</td>
<td>6,158</td>
<td>17.1 ± 0.1</td>
<td>4.0 ± 0.1</td>
<td>23 128</td>
<td>4.0 ± 0.1</td>
</tr>
</tbody>
</table>

Compared with the corresponding sham, the ED-1 positivity was increased in the NCD- and HCD-CS groups at one and 12 weeks after surgery, although it showed a prominent increase in the HCD-CS group (p < 0.01 vs. NCD; Fig. 7C). One week after CS, ED-1 positivity was lower (p < 0.01) in the NCD- and HCD-CS groups with tetrahydrobiopterin supplementation than in the corresponding groups without it. In contrast, in the MI groups, there was no significant difference in ED-1 positivity between NCD and HCD (i.e., ED-1 positivity was 101 ± 19% [NCD] and 118 ± 21% [HCD]) one day after MI, and 22 ± 7% [NCD] and 28 ± 4% [HCD] 12 weeks after MI; *p < 0.01 vs. sham, †p < 0.05 vs. sham, ‡p < 0.01 vs. one day after MI).

The extent of the risk area in short-axial sections did not differ between the NCD and HCD groups 12 weeks after surgery (80 ± 10% [NCD] and 84 ± 11% [HCD] in the MI groups).

Cause of death. The postmortem examination of the spontaneously dead rats revealed pleural effusion, suggesting the worsening of congestive heart failure. Histopathology revealed no positive findings, suggesting acute MI by hematoxylin–eosin (coagulation necrosis), elastica Masson (contraction band necrosis), and TUNEL (suggestive of apoptosis) stainings.

Serum lipid levels and liver weight. With HCD, serum cholesterol increased only in the sham group, and liver weight increased (fatty liver histologically) in the HCD groups (Table 1). Ascorbyl free radical levels were higher in the CS and MI groups compared with the sham, irrespective of NCD or HCD.

DISCUSSION
The results of this study are summarized as follows. First, HCD did not modify LV dysfunction and remodeling caused by MI. Second, CS impaired myocardial endothelial NO function and evoked myocardial ischemia (shown by PKC-ε translocation) and inflammation. Although HCD alone did not evoke myocardial inflammation in the sham group, HCD aggravated these processes caused by CS-
induced ischemia without modifying CS severity. As far as we know, this is the first study to show the different effects of HCD-induced reactions on ischemic LV dysfunction and remodeling, as well as myocardial inflammation due to CS and MI.

**LV dysfunction and remodeling caused by ischemia and aggravated by HCD.** The myocardial NO dysfunction occurred soon after creating CS (Fig. 5). Shear stress activates eNOS in coronary microvasculatures (15,16). Coronary stenosis promoted free radical formation (17) in ischemic myocardium, expression of MCP-1 in coronary microvessels at risk and in some of the infiltrating cells (Fig. 7), inflammatory cell infiltration, necrosis, and fibrosis. In addition, HCD also impaired myocardial NO function, even in the sham group, which is consistent with previous reports that cholesterol burden accelerates the formation of eNOS protein and inhibitory caveolin complexes (4,18) and displacement of eNOS protein from caveolae, cell surface plasma membrane invaginations (19). Impairment of myocardial NO function (Fig. 5), myocardial inflammatory cell infiltration (Fig. 7), and LV dysfunction and remodeling (Table 1, Fig. 2), which were induced by CS, were augmented by HCD and ameliorated by tetrahydrobiopterin supplementation. Impairment of NO function induced myocardial inflammation via coronary microvascular MCP-1 expression and ED-1–positive cell infiltration (7). Overexpression of MCP-1 alone does not cause chemotaxis of inflammatory cells, whereas it increases the sensitivity to other inflammatory stimuli, functioning as an enhancer of inflammation (20). An HCD alone does not cause vascular inflammation. However, MCP-1, synergistically with HCD, causes monocyte infiltration (21). Taking into account these issues, HCD may have augmented inflammation in ischemic but viable myocardium in our chronic CS model through the processes following eNOS dysfunction.

The contrasting effects of HCD on myocardial inflammation and/or function between CS and MI would suggest that HCD has synergistic actions in ischemic but viable myocardium, which is susceptible to endothelial disintegration and myocardial injury.

**Clinical implications.** Hypercholesterolemia increases the risk of cardiac events in coronary artery disease (1–3). Statins lower cardiac events in ischemic heart disease (1–3,22). The 4S study also revealed that the incidence of hospitalization due to congestive heart failure was reduced by statin therapy (1). Such increased cardiac risk from cholesterol burden may be due mainly to acceleration of coronary plaque formation. However, it was documented that amelioration of nonischemic heart failure is partly due to free radical scavenging by statins (23). Thus, impaired coronary circulation downstream of epicardial CS and subsequent myocardial inflammation, especially those of the HCD-CS group, may also be therapeutic targets for future studies on the amelioration of heart failure.

**HCD in rats.** Rats are resistant to increased serum lipid levels from HCD (8,24). Therefore, it is not surprising that the increase in serum cholesterol level was slight or not significant in the HCD groups. Conversely, our results suggest that HCD is a risk factor for aggravating ischemic heart failure, even if the serum cholesterol level is not very high.

**Study limitations.** Our study has admitted limitations. First, there is inter-animal variation of the myocardial mass rendered ischemic in rats. Second, our experiment utilized a single protocol for cholesterol loading, and the effects of different grades and periods of cholesterol feeding were not assessed. Third, data on myocardial perfusion, inflammation, and injury were not obtained at multiple time points of LV dysfunction and remodeling. Fourth, by the methods utilized in the present study, it is difficult to totally rule out the possibility that HCD may have triggered MI in this CS model. Fifth, despite comparable amelioration by tetrahydrobiopterin of myocardial NO dysfunction (Fig. 5), myocardial perfusion (Fig. 4), PKC signals (Fig. 6), and myocardial inflammation (Fig. 7) in the NCD- and HCD-CS groups, there was still some difference in LV diameters and ejection fractions between the two groups (Fig. 3). Possible other mechanisms, including HCD-related substances for producing cardiac dysfunction, need to be examined in a future study. Sixth, it remains to be clarified that BH₄ may affect heart failure in our model, aside from the restoration of eNOS, as this material has several actions, including an anti-oxidative property.

**Conclusions.** Although HCD did not affect epicardial CS severity, it augmented CS-induced impairment of myocardial perfusion, including eNOS function, myocardial ischemia, inflammation, and LV dysfunction and remodeling. In contrast, MI-induced heart failure was not affected by HCD. These results suggest that HCD is a risk factor for aggravating heart failure caused by CS without modifying CS severity.

**Reprint requests and correspondence:** Dr. Yukio Maruyama, Professor and Chairman, First Department of Internal Medicine, Fukushima Medical University, Hikarigaoka 1, Fukushima 960-1295, Japan. E-mail: maruyama@fmc.ac.jp.

**REFERENCES**

5. Xie YW, Shen W, Zhao G, et al. Role of endothelium-derived nitric oxide in the modulation of canine myocardial mitochondrial respira-