CHOLESTEROL DIET-INDUCED HYPERLIPIDEMIA IMPAIRS THE CARDIOPROTECTIVE EFFECT OF POSTCONDITIONING: ROLE OF PEROXYNITRITE

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Abstract

The aim of the present study was to investigate if hyperlipidemia interferes with the infarct size limiting effect of postconditioning and to study the involvement of peroxynitrite in this phenomenon. Rats were fed a 2% cholesterol-enriched or normal diet for 12 weeks. Infarct size by triphenyltetrazolium-chloride staining was measured in hearts isolated from both groups and subjected to 30 min coronary occlusion followed by 120 min reperfusion with or without postconditioning protocol induced by 6 cycles of 10-sec coronary occlusion and 10-sec reperfusion at the onset of the reperfusion. Postconditioning significantly decreased infarct size in the normolipidemic but not in the hyperlipidemic group. Postconditioning increased cardiac 3-nitrotyrosine concentration (a marker for peroxynitrite formation) in the normal but not in the cholesterol-fed group when measured at the 5th min of reperfusion. Then we tested if postconditioning-induced acute increase in peroxynitrite is involved in the cardioprotection in normolipidemic animals in separate experiments. Postconditioning failed to decrease infarct size in the presence of the peroxynitrite decomposition catalyst FeTPPS (20 mg/L) in normolipidemic animals. We conclude that an early increase in peroxynitrite after postconditioning plays a role in cardioprotection. Furthermore, hyperlipidemia blocks the cardioprotective effect of postconditioning at least in part via deterioration of postconditioning-induced early increase in peroxynitrite formation.

Keywords: postconditioning, infarct, cholesterol, heart, peroxynitrite
Introduction

Ischemic heart disease is the leading cause of death in the industrialized world. Although ischemic heart disease in humans is a complex disorder caused by or associated with other systemic diseases and conditions, most experimental studies on cardioprotection have been undertaken in healthy juvenile animal models, in which ischemia/reperfusion is imposed in the absence of other disease processes and risk factors including hyperlipidemia, atherosclerosis, hypertension, diabetes, insulin resistance, heart failure and aging (9). In these diseases and aging, the pathological processes are associated with fundamental molecular alterations that can potentially affect the development of ischemia/reperfusion injury per se and responses to cardioprotective interventions such as ischemic preconditioning i.e. brief exposure to ischemia/reperfusion prior to sustained ischemia (21), and postconditioning i.e. brief repetitive episodes of ischemia/reperfusion at the immediate onset of reperfusion (22) (see for a recent extensive review: (9)).

Although existing data in the literature are still somewhat contradicting, the majority of the studies show that experimental hyperlipidemia independently from the development of coronary atherosclerosis interferes with the cardioprotective effect of preconditioning (see for reviews: (5; 9; 10)). The loss of preconditioning has been shown in hypercholesterolemic humans as well (32). However, very little is known about the effect of postconditioning in hyperlipidemia. Iliodromitis et al. (15) have recently shown that the infarct size limiting effect of postconditioning is lost in rabbits with experimental
hyperlipidemia and atherosclerosis, but the paper by Donato et al (4) has not confirmed this result.

The mechanism by which hyperlipidemia may interfere with cardioprotective mechanisms are still not known, however, a decrease in cardiac nitric oxide content due to altered nitrosative stress has been observed (3; 11; 14; 31). Nitrosative stress is induced by formation of peroxynitrite by the non-enzymatic reaction of nitric oxide and superoxide (6; 25). We and others have previously shown that early increase in myocardial peroxynitrite formation triggers the development of cardioprotection by preconditioning (1; 2) see for reviews: (8; 18). However, it is not known if peroxynitrite-induced nitrosative stress contributes to postconditioning and if hyperlipidemia via alteration of nitrosative stress may lead to the loss of the cardioprotective effect of postconditioning.

Therefore, here we examined if (i) experimental hyperlipidemia induced by cholesterol-enriched diet interferes with cardioprotective effect of postconditioning, (ii) peroxynitrite-induced nitrosative stress may trigger the cardioprotective effect of postconditioning, and (iii) alteration of nitrosative stress signal by experimental hyperlipidemia may contribute to the possible loss of postconditioning effect.
Materials and Methods

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (National Institutes of Health publication 85-23, revised 1996), and it was approved by a local animal ethics committee of the University of Szeged.

Experimental design and infarct size measurements

Six-week-old male Wistar rats were fed normal (n=61) or 2% cholesterol-enriched rat chow (n=58) for 12 weeks. Wistar rats were chosen for the study since this species shows a moderate increase in serum cholesterol and triglyceride level due to a high-cholesterol diet and no substantial atherosclerosis develops, therefore, the direct myocardial effect of hyperlipidemia, independent from atherosclerosis, can be studied in this model (11; 24). At the end of 12-week diet, serum cholesterol and triglyceride were measured. In separate experiments, cholesterol (n=7-8) and free fatty acid levels (n=4 in each group) were measured in myocardial tissue samples from normolipidemic and hyperlipidemic animals.

At the end of the diet period, animals were anesthetized with diethylether and given 500 U/kg heparin. Hearts were isolated and perfused with Krebs-Henseleit buffer according to Langendorff with constant pressure as described (24). Regional ischemia was induced by 30 min coronary occlusion followed by 120 min reperfusion. Postconditioning was induced by 6 consecutive cycles of 10-sec coronary occlusion and 10-sec reperfusion immediately at the onset of...
reperfusion (Fig 1). Cardiac electrogram was monitored during the study to measure heart rate and incidence of reperfusion-induced ventricular fibrillation.

At the end of the 120 min reperfusion infarct size was determined by triphenyltetrazolium-chloride staining and evaluated by planimetry (Infarctsize™ 1.0, Pharmahungary, Szeged, Hungary). Infarct size was expressed in percentage of area at risk.

In separate experiments, hearts from control and cholesterol-fed groups were subjected to 30 min coronary occlusion followed by 5 min reperfusion with or without of postconditioning and left ventricular tissue was sampled, homogenized and used for biochemical measurements (Fig 1).

To check if peroxynitrite plays a role in the cardioprotective mechanism of postconditioning, in separate experiments normolipidemic hearts were subjected to 30 min regional ischemia followed by postconditioning protocol in the presence (n=10) or absence of FeTPPS (5,10,15,20-tetrakis-[4-sulfonatophenyl]-porphyrinato-iron[III]; 20 mg/L, n=10). The dose of 20 mg/L FeTPPS was selected according to our previous studies (7; 24). FeTPPS is a ferric porphyrin complex that catalytically isomerizes peroxynitrite to nitrate. FeTPPS does not directly react with superoxide or NO, therefore, it is considered as a selective peroxynitrite decomposition catalyst (17; 20; 29).

Krebs-Henseleit solution contained FeTPPS only during the last 20 sec of the 30 min ischemic period and during postconditioning. At the end of the 120-min reperfusion period infarct size was measured as described above.

In a separate set of experiments, to check if a stronger stimulus of postconditioning could postcondition the heart, hearts were isolated from normolipidemic and hyperlipidemic groups and subjected to 30 min coronary
occlusion and 120 min reperfusion with or without a postconditioning protocol of 
12 cycles of 10-second coronary occlusion and 10-second reperfusion. At the 
end of the 120 min reperfusion infarct size was determined as mentioned 
above.

Measurement of lipids

Serum cholesterol and triglyceride were measured by a colorimetric 
assay (Triglyceride PAP and Cholesterol PAP assay, Diagnosticum Zrt.). Tissue 
cholesterol were measured (Cholesterol/Cholesterol Ester Quantification kit, 
BioVision) from ventricular homogenates. For measurements of cardiac free 
fatty acids, ventricular homogenate were extracted with chloroform/methanol 
(18ml; 2:1; v/v) according to a modified method of Folch et al (12). All the 
extraction solvents contained 0.01% butylated hydroxytoluene as an 
antioxidant. Free fatty acids were separated from the other fraction by thin layer 
chromatography (TLC) on pre-coated silica gel 60 plates (10 x 10 cm) without a 
fluorescent indicator (1.05721, Merck) using the solvent system petroleum 
benzine (bp 40-60 C)/diethyl ether (peroxide free)/acetic acid (90:30:1, by vol) 
as previously described (33). Individual phospholipid classes were separated by 
TLC on pre-coated silica gel 60 plates (10 x 10 cm) without a fluorescent 
indicator using chloroform/petroleum benzene/methanol/acetic acid/boric acid 
(40:30:20:10:1.8 v/v/v/v/w) as solvent (13). The lipid bands containing PC, PE, 
PS, PI and SM were visualized with long wave ultraviolet light after spraying the
plates with chloroform/methanol (1:1, by vol) containing BBOT (2,5-bis-(5’-tert-
butylbenzoxazolyl-[2´])thiophene; 10 mg/100 mL; (Sigma Chemical Co.).

Assessment of cardiac peroxynitrite-induced nitrosative stress

Cardiac free and protein bound 3-nitrotyrosine content, as markers for
peroxynitrite-induced nitrosative stress was measured at the 5th min of
reperfusion.

Cardiac free 3-nitrotyrosine level was measured by enzyme-linked
immunosorbent assay ELISA (Cayman Chemical; Ann Arbor, MI) from control
and cholesterol-fed heart tissue samples. Briefly, supernatants of ventricular
tissue homogenate was incubated overnight with anti-nitrotyrosine rabbit IgG
specific to free 3-nitrotyrosine and nitrotyrosine acetylcholinesterase tracer in
pre-coated (mouse anti-rabbit IgG) microplates followed by development with
Ellman's reagent. Free nitrotyrosine content was normalized to protein content
of cardiac homogenate and expressed as ng/mg protein.

To measure the abundance of 3-nitrotyrosine-protein adducts, we
performed SDS-PAGE Western blot. Heart tissues were homogenized and
centrifuged. Protein concentrations of supernatants were measured by the
bicinchoninic acid assay. Equal amounts (20 µg) of proteins were separated by
10% SDS-PAGE, transferred to nitrocellulose membrane (Amersham), the blot
was blocked in Tris-buffered saline/Tween-20 supplemented with 5% nonfat dry
milk for overnight. The primary 3-nitrotyrosine antibody (MAB5404; Chemicon
International) was used at the manufacturer-recommended dilution. Membrane
was developed with an enhanced chemiluminescence kit (ECL Plus; GE
Healthcare), exposed to X-ray film, and scanned. Band density was calculated by integrating the area (in pixels X intensity, expressed in arbitrary units). By this method, we detected the degree of nitrosylation of tyrosine side chains of proteins, as low molecular weight free 3-nitrotyrosine is eliminated in the SDS-page.

Statistical Analysis

Results were expressed as mean±SEM when appropriate. Differences among means were analyzed by using one-way analysis of variance followed by Tukey post hoc test. The incidence of VF was analysed by Fisher’s exact test. Differences were considered significant at p<0.05.
Results

At the end of the 12-week diet, the body weight of the animals were 400-580 g, and there was no significant difference between groups. High cholesterol diet did not affect basal hemodynamic parameters except heart rate which was lower in the hyperlipidemic group at the end of reperfusion after 12x10^4 cycles postconditioning. The diet did not affect the area at risk (Fig 2B, Table 3). In the cholesterol-fed group, plasma cholesterol, triglyceride, heart weight and tissue DHGLA were increased significantly (see Table 1 and Table 2).

To examine if experimental hyperlipidemia interferes with the cardioprotective effect of postconditioning, infarct size and the incidence of reperfusion-induced VF was assessed in rat hearts with coronary occlusion in both normolipidemic and hyperlipidemic rats. Postconditioning with 6x10^4 cycles significantly decreased infarct size and the incidence of VF in hearts of rats with normolipidemic diet. However, in heart of cholesterol-fed animals, infarct size and incidence of VF were not changed by 6X10^4 postconditioning (Figs 2A, 3). Neither postconditioning, nor cholesterol diet affected coronary flow, or area at risk significantly (Table 1 and Table 3).

Next we studied if postconditioning altered myocardial nitrosative stress at early reperfusion in normal and cholesterol-fed animals. Therefore, in separate experiments, we measured free and protein-bound 3-nitrotyrosine, a marker for peroxynitrite-induced nitrosative stress, by ELISA and Westen blot, respectively, in left ventricular tissue samples at 5 min of reperfusion after postconditioning (Fig 4A and 4B). Postconditioning significantly increased
peroxynitrite-induced nitrotyrosine formation in normolipidemic hearts, but not in hyperlipidemic conditions (Figs 4A, 4B).

To test if the early increase in nitrosative stress signal observed in normolipidemic animals is a necessary trigger for the development of cardioprotection by postconditioning, in separate experiments, postconditioning was induced in the presence of a peroxynitrite decomposition catalyst FeTPPS in normolipidemic animals. While FeTPPS did not affect infarct size significantly in the non-postconditioned group, it abolished the infarct size limiting effect of postconditioning (Fig 5).

Finally, to test if a more potent postconditioning stimulus could protect the hyperlipidemic heart, in a separate set of experiments, postconditioning with 12x10^3 cycles of brief ischemia/reperfusion was applied. However, this postconditioning protocol failed to reduce infarct size not only in hyperlipidemic but also in normolipidemic hearts. Therefore, no additional biochemical or pharmacological studies were performed in these groups (see Table 3).
Discussion

We have shown here that the infarct size limiting effect of postconditioning was lost in hearts of hyperlipidemic rats. Furthermore, we have shown that cardiac nitrotyrosine content was increased during early reperfusion after postconditioning, which was not seen in hyperlipidemic hearts. Finally, we have shown that postconditioning in the presence of the peroxynitrite decomposition catalyst FeTPPS failed to reduce infarct size in normal hearts. This is the first demonstration that an early increase in peroxynitrite-induced nitrosative stress after postconditioning is involved in the triggering mechanism of cardioprotection by postconditioning and that in hyperlipidemia, the absence of this mechanism may contribute to the loss of postconditioning in hyperlipidemia.

Our present results confirm that of Iliodromitis et al. (15) showing that the infarct size limiting effect of postconditioning is lost in rabbits with experimental hyperlipidemia and atherosclerosis. Another study by Zhao et al (36) showed in mini-pigs that postconditioning under normolipidemic condition can reduce the area of no-reflow and necrosis area, while postconditioning under hypercholesterolemic condition cannot. In contrast, Donato et al (4) showed that ischemic postconditioning reduces infarct size by activation of A1 receptors and K+(ATP) channels in both normal and hypercholesterolemic rabbits. The discrepancies can be attributed to differences in experimental hyperlipidemia and the presence of coronary atherosclerosis which may interfere with the severity of coronary occlusion per se. Therefore, hyperlipidemic rats that do not develop significant atherosclerosis seem to be a more suitable model to study
the direct myocardial effect of hyperlipidemia on cardioprotective mechanisms. Therefore, we conclude that similarly to the effect of preconditioning, the effect of postconditioning is also influenced by hyperlipidemia (see for review: (9)).

Our present results show that postconditioning increases formation of cardiac 3-nitrotyrosine, a marker for peroxynitrite-induced nitrosative stress, at early reperfusion, however, the increased peroxynitrite formation was not observed in hyperlipidemic hearts subjected to postconditioning. We have previously shown that hyperlipidemia itself increases nitrosative stress in the heart in baseline conditions (24), however, our present study shows that hyperlipidemia itself does not significantly modify post-ischemic 3-nitrotyrosine levels, but blocks postconditioning-induced early increase in peroxynitrite. As in hyperlipidemic animals, postconditioning was ineffective and the early increase in peroxynitrite after postconditioning was not present, we conclude that the lack of a nitrosative trigger signal may be involved in the loss of postconditioning in hyperlipidemia.

As peroxynitrite has been previously shown to be involved in the trigger mechanism of the cardioprotective effect of preconditioning (1; 2), it was plausible to speculate that peroxynitrite may be also involved in the triggering mechanism of postconditioning. To test this hypothesis, in normal animals, postconditioning was performed in the presence of a peroxynitrite decomposition catalyst FeTPPS at a dose, that significantly reduced peroxynitrite-induced myocardial effects in different models (7; 20). We have found here, that the infarct size limiting effect of postconditioning was abolished in the presence of FeTPPS, which shows for the first time that increased nitrosative stress at early reperfusion after postconditioning is necessary to
trigger its cardioprotective effect. This is another important example of the physiological regulatory role of mild nitrosative stress (6; 25). In isolated adult rat cardiac myocytes, Wang et al (34) suggested that hypoxic postconditioning is partly attributable to the reduced peroxynitrite formation following reoxygenation. The discrepancy may be due to the fact that they measured peroxynitrite late in reoxygenation and used a different model and postconditioning trigger.

On the other hand, the role of reactive oxygen species (ROS) including peroxynitrite in cardioprotection including postconditioning is still not clear, furthermore, still little is known on the balance between the detrimental and protective effects of peroxynitrite [see for reviews (6; 8; 19; 25; 26)]. It has been shown that oxidative/nitrosative stress is involved in myocardial ischemia/reperfusion injury (35) and that peroxynitrite decomposition catalysts provide significant cardioprotection against myocardial/reperfusion injury (16). However, recent studies suggest that some ROS species at low concentrations could protect ischemic hearts (27). ROS scavengers N-acetyl-L-cysteine (NAC) or mercaptopropionyl glycine (MPG) given at the beginning of reperfusion abolished postconditioning-induced protection (30). Nossuli et al showed that in a feline model of coronary occlusion/reperfusion, intraventricular infusion of authentic peroxynitrite (1 μmol/L) 10 min before reperfusion was associated with a reduction in infarct size (23; 30). Furthermore, it has been shown that peroxynitrite regulates mitogen-activated protein kinases (MAPKs) which are also involved in the mechanism of postconditioning (19; 28).

As we have shown here that in hyperlipidemia, postconditioning with 6 brief cycles of ischemia/reperfusion was unable to induce cardioprotection, it
was feasible to speculate that this might be due to an increased threshold for postconditioning triggers in hyperlipidemia. Therefore, we tested if an increased postconditioning stimulus of 12 brief cycles of coronary occlusion/reperfusion could postcondition the hyperlipidemic heart, but found that this protocol was unable to induce postconditioning in both hyperlipidemic and normolipidemic animals. This suggests that the loss of postconditioning in hyperlipidemia is not due to an increased threshold for triggers of postconditioning, but possibly due to the disruption of the cardioprotective cellular pathways (9).

Our current findings should be interpreted within the constraints of potential limitations. Although FeTPPS is thought to be specific to peroxynitrite, it cannot be excluded that it reacts with non-peroxynitrite species as well. Due to technical limitations to measure local concentrations and cellular sources peroxynitrite (6), nitrosative stress in the coronary endothelium, endocardial endothelium, cardiac nerves, fibroblasts, and cardiac myocytes could all contribute to changes in nitrotyrosine levels and to the cardioprotective effect of postconditioning. Furthermore, we can not exclude that nitrotyrosine can be formed by peroxynitrite-independent pathways as well, for example, via the actions of peroxidases in the presence of nitrite.

In conclusion, this is the first demonstration that nitrosative stress is involved in the triggering mechanism of postconditioning and that hyperlipidemia leads to the loss of the cardioprotective effect of postconditioning at least in part via deterioration of the nitrosative trigger. Furthermore, we emphasize the importance of preclinical studies that examine cardioprotective mechanisms specifically in relation to complicating disease states such as hyperlipidemia in order to maximize the likelihood of identifying rational
approaches to therapeutic protection of the ischemic heart in the presence of risk factors.

**Acknowledgement**

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Figure legends

Figure 1
Experimental protocol. Control and 2% cholesterol-enriched chow-fed animals were subjected to 30 min coronary occlusion and 120 min reperfusion. Postconditioning was induced by 6x10 s cycles of coronary occlusion and reperfusion. Infarct size was measured at the 120th min of reperfusion. In separate experiments, hearts were frozen in liquid nitrogen at the 5th min of reperfusion for 3-nitrotyrosine measurements. In separate groups of normolipidemic hearts, a peroxynitrite decomposition catalyst FeTPPS was infused into the perfusion line at 20 mg/L final concentration during postconditioning and corresponding time period.

Figure 2
Myocardial injury after 30 min of regional ischemia and 120 min of reperfusion: myocardial area at risk (AAR; panel B) and infarct size (IS) relative to AAR (IS/AAR; panel A). Infarct size measured at the end of 120 min reperfusion with (Control Post) or without 6x10 s cycles of postconditioning (Control) in control chow-fed rats as well as cholesterol-fed animals (Chol Post and Chol). *p<0.05 vs. Control; n=7-10 in each group. ○ Individual values; ● average values.

Figure 3
The effect of 6x10" cycles of postconditioning on the incidence of reperfusion-induced ventricular fibrillation (VF) during the first 30 min of reperfusion in
control (Control and Control Post) and cholesterol-fed (Chol and Chol Post) 
groups. *p<0.05 vs. Control; n=7-9 in each groups.

Figure 4
Cardiac 3-nitrotyrosine content, a marker of endogenous peroxynitrite-induced 
nitrosative stress. Free 3-nitrotyrosine assayed by ELISA (panel A) and protein-
bound 3-nitrotyrosine by Western blot (panel B) in control (Control and Control 
Post) and cholesterol-fed (Chol and Chol Post) groups. *p<0.05 vs. Control; 
n=10-13 in each groups.

Figure 5
Myocardial injury after 30 min of regional ischemia and 120 min of reperfusion: 
myocardial area at risk (AAR; B) and infarct size (IS) relative to AAR (IS/AAR; 
A). Infarct size measured at the end of 120 min reperfusion with (Control Post) 
or without 6x10" postconditioning (Control) in the presence or absence of 20 
mg/L FeTPPS, a peroxynitrite decomposition catalyst. *p<0.05 vs. Control; n=6-
10 in each group. ○ Individual values; ● average values.
**Fig 1**

- **Normolipidemic**
  - Ischemic control (Control)
  - Postconditioned (Control Post)

- **Cholesterol-fed Hyperlipidemic**
  - Postconditioned (Chol Control)
  - Postconditioned (Chol Post)

**FeTPPS (20mg/L)**

- **Normolipidemic**
  - Postconditioning + FeTPPS (Post+FeTPPS)

- **FeTPPS perfusion**

- **Regional ischemia**
  - 10’
  - 30’
  - 5’

- **Cardiac nitrotyrosine measurement**

- **Infarct size measurement**
Fig 2

A

NORMOLIPIDEMIC

HYPERLIPIDEMIC

Infarct size/area at risk (%)

Control Post Chol Chol Post

B

NORMOLIPIDEMIC

HYPERLIPIDEMIC

Area at risk (%)

Control Post Chol Chol Post
Fig 3

Incid
ence of VF (%)

Control Control Post Chol Chol Post

Normolipidemic Hyperlipidemic
Fig 4

**A**

Cardiac free 3-nitrotyrosine (ng/mg protein)

Control    Control    Post    Chol    Chol    Post

**B**

Cardiac protein-bound 3-nitrotyrosine (arbitrary units)

Control    Control    Post    Chol    Chol    Post
Table 1, Cardiac functional parameters in normal and hyperlipidemic rat hearts

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<td>Control</td>
<td>Post(6x10&quot;)</td>
<td>Control</td>
<td>Post(6x10&quot;)</td>
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<tr>
<td><strong>Before ischemia</strong></td>
<td></td>
<td></td>
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<tr>
<td>Coronary flow (ml/min)</td>
<td>20.5 ± 1.5</td>
<td>17.7 ± 1.2</td>
<td>19.4 ± 1.1</td>
<td>17.0 ± 0.9</td>
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<td>Heart rate (beat per min)</td>
<td>382 ± 49</td>
<td>332 ± 30</td>
<td>352 ± 31</td>
<td>251 ± 17</td>
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<tr>
<td>Heart wet weight (g)</td>
<td>1.90 ± 0.15</td>
<td>1.78 ± 0.07</td>
<td>2.11 ± 0.06*</td>
<td>2.13 ± 0.11*</td>
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<td>Body weight (g)</td>
<td>527 ± 17</td>
<td>435 ± 11</td>
<td>500 ± 23</td>
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<td>Heart weight/body weight (%)</td>
<td>0.38 ± 0.02</td>
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<td><strong>End of ischemia</strong></td>
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<td>Coronary flow (ml/min)</td>
<td>10.3 ± 1.3</td>
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<td>Heart rate (bpm)</td>
<td>336 ± 59</td>
<td>311 ± 22</td>
<td>287 ± 15</td>
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<tr>
<td><strong>End of reperfusion</strong></td>
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<tr>
<td>Coronary flow (ml/min)</td>
<td>17.9 ± 1.7</td>
<td>19.0 ± 0.9</td>
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<td>19.4 ± 0.9</td>
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<td>Heart rate (bpm)</td>
<td>277 ± 44</td>
<td>316 ± 40</td>
<td>258 ± 20</td>
<td>258 ± 76</td>
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Values are mean±SEM; *P<0.05 vs normolipidemic;
Post: postconditioning induced by 6x10" cycles of ischemia and reperfusion
Table 2, Influence of cholesterol-enriched diet on lipid profile in rats

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<td>Serum cholesterol (mmol/L)</td>
<td>2.57 ± 0.20</td>
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<tr>
<td>Serum triglyceride (mmol/L)</td>
<td>1.47 ± 0.10</td>
<td>2.15 ± 0.20*</td>
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<td>Myocardial tissue cholesterol (μg/mg)</td>
<td>0.88 ± 0.02</td>
<td>0.93 ± 0.10</td>
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<td>Myocardial free fatty acid content:</td>
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<td>DHGLA (μg/g)</td>
<td>4.13 ± 2.0</td>
<td>16.3 ± 4.1*</td>
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<td>Palmitic acid (μg/g)</td>
<td>178 ± 50</td>
<td>316 ± 50</td>
</tr>
<tr>
<td>Stearic acid (μg/g)</td>
<td>162 ± 30</td>
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Values are mean±SEM; *P<0.05 vs normolipidemic
DHGLA: dihomo-gamma-linolenic acid
Table 3, Cardiac functional parameters in normal and hyperlipidemic rat hearts

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<td>Control</td>
<td>Post(12x10&quot;)</td>
<td>Control</td>
<td>Post(12x10&quot;)</td>
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<tr>
<td>Coronary flow (ml/min)</td>
<td>20.4 ± 0.9</td>
<td>23.9 ± 2.1</td>
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</tr>
<tr>
<td>Heart rate (beat per min)</td>
<td>375 ± 36</td>
<td>345 ± 31</td>
<td>328 ± 36</td>
<td>313 ± 29</td>
</tr>
<tr>
<td>Heart wet weight (g)</td>
<td>1.79 ± 0.08</td>
<td>2.11 ± 0.16</td>
<td>1.94 ± 0.09</td>
<td>1.89 ± 0.09</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>556 ± 14</td>
<td>572 ± 26</td>
<td>556 ± 24</td>
<td>515 ± 22</td>
</tr>
<tr>
<td>Heart weight/body weight(%)</td>
<td>0.32 ± 0.01</td>
<td>0.37 ± 0.02</td>
<td>0.36 ± 0.01</td>
<td>0.35 ± 0.01</td>
</tr>
<tr>
<td><strong>End of ischemia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary flow (ml/min)</td>
<td>11.0 ± 0.9</td>
<td>12.9 ± 1.5</td>
<td>12.4 ± 0.8</td>
<td>10.4 ± 0.6</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>308 ± 27</td>
<td>353 ± 32</td>
<td>342 ± 22</td>
<td>289 ± 14</td>
</tr>
<tr>
<td><strong>End of reperfusion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary flow (ml/min)</td>
<td>16.8 ± 1.8</td>
<td>16.0 ± 1.5</td>
<td>17.3 ± 1.5</td>
<td>14.3 ± 1.0</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>367 ± 33</td>
<td>345 ± 32</td>
<td>335 ± 14</td>
<td>256 ± 16*#</td>
</tr>
<tr>
<td>Infarct size (%)</td>
<td>31.3 ± 4.7</td>
<td>31.9 ± 3.4</td>
<td>35.2 ± 5.7</td>
<td>36.5 ± 4.6</td>
</tr>
<tr>
<td>Area at risk (%)</td>
<td>40.0 ± 3.9</td>
<td>40.4 ± 3.5</td>
<td>33.9 ± 2.1</td>
<td>35.8 ± 3.9</td>
</tr>
</tbody>
</table>

Values are mean±SEM; *P<0.05 vs normolipidemic; #P<0.05 vs hyperlipidemic
Post: postconditioning induced by 12x10" cycles of ischemia and reperfusion