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Cardiovascular Research 58 (2003) 663–670

Cardiovascular Research

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Hyperlipidemia induced by a cholesterol-rich diet leads to enhanced peroxynitrite formation in rat hearts

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Received 17 February 2003; accepted 20 February 2003

Abstract

Objective: We investigated the influence of experimental hyperlipidemia on the formation of cardiac NO, superoxide, and peroxynitrite ($ONOO^-$) in rat hearts. **Methods:** Wistar rats were fed 2% cholesterol-enriched diet or normal diet for 8 weeks. Separate groups of normal and hyperlipidemic rats were injected twice intraperitoneally with 2×20 μmol/kg FeTPPS (5,10,15,20-tetrakis-[4-sulfonatophenyl]-porphyrinato-iron[III]), a $ONOO^-$ decomposition catalyst, 24 h and 1 h before isolation of the hearts. **Results:** A cholesterol diet significantly decreased myocardial NO content, however, myocardial Ca^{2+}-dependent and Ca^{2+}-independent NO synthase activity and NO synthase protein level did not change. Myocardial superoxide formation and xanthine oxidase activity were significantly increased; however, cardiac superoxide dismutase activity did not change in the cholesterol-fed group. Dityrosine in the perfusate, a marker of cardiac $ONOO^-$ formation, and plasma nitrotyrosine, a marker for systemic $ONOO^-$ formation, were both elevated in hyperlipidemic rats. In cholesterol-fed rats, left ventricular end-diastolic pressure (LVEDP) was significantly elevated as compared to controls. Administration of FeTPPS normalized LVEDP in the cholesterol-fed group. **Conclusion:** We conclude that cholesterol-enriched diet-induced hyperlipidemia leads to an increase in cardiac $ONOO^-$ formation and a decrease in the bioavailability of NO which contributes to the deterioration of cardiac performance and may lead to further cardiac pathologies.

Keywords: Cholesterol; Contractile function; Enzyme; Free radicals; Nitric oxide

1. Introduction

High-cholesterol diet is regarded as an important factor in the development of cardiac diseases since it leads to development of hyperlipidemia, atherosclerosis, and ischemic heart disease. The heart of hyperlipidemic/atherosclerotic patients adapts poorly to oxidative or other kinds of stress, suggesting that the endogenous adaptive mechanisms against myocardial stress are impaired [1]. The focus of research so far has been mainly on the coronary effects of cholesterol, i.e. coronary sclerosis, and the possible direct effect of hypercholesterolemia on the heart was neglected. Very few studies looked at the cellular effects of cholesterol-enriched diet on the myocardium; however, intracellular lipid accumulation in cardiomyocytes and several alterations in the structural and functional properties of the myocardium have been observed [2,3]. Furthermore, we have previously shown that cholesterol-enriched diet-induced hyperlipidemia attenuates the cardioprotective effect of ischemic preconditioning via a mechanism independent from the vascular effects of hyperlipidemia ([4,5], see Ref. [6] for review).

Increasing evidence accumulated in recent years showing that high-cholesterol diet impairs NO-cGMP signaling in both endothelial and nonendothelial cells [4,7–9]. In the normal heart, nitric oxide (NO) is synthesized by Ca^{2+}-dependent NO synthases in cardiac myocytes, vascular and endocardial endothelium (NO synthase III) as well as in specific cardiac neurons (NO synthase I) and plays an important role in the regulation of coronary circulation and cardiac contractile function [10]. Atherosclerosis is a well known 'NO deficient state' in the vasculature which leads

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Time for primary review 28 days.

0008-6363/03/$ – see front matter
doi:10.1016/S0008-6363(03)00330-4

to sustained arterial hypertension (see Ref. [11] for review) and reduced cardiovascular tolerance to stress (see Ref. [6] for review). Lefer and Ma [7] observed reduced NO release from rabbit aorta in hypercholesterolemia, and Deliconstantinos et al. [9] showed that incorporation of high concentrations of cholesterol into endothelial cell membranes caused downregulation of NO synthase. Reduced vascular NO release in hyperlipidemia has been also shown as a consequence of increased formation of superoxide, which then reacts with NO to form peroxynitrite ($ONOO^-$) [12,13]. However, the effect of hyperlipidemia on the formation of NO, superoxide, and $ONOO^-$ in the heart is not known.

We have previously shown that cardiac NO level is significantly decreased in hearts of cholesterol-fed rats [4]. However, the mechanism of reduced NO level in the heart is not known. Here we hypothesized, that the decrease in cardiac NO level is secondary to increased production of superoxide and formation of $ONOO^-$. Therefore, in the present study we systematically analyzed if hyperlipidemia influences formation of cardiac NO, superoxide, and $ONOO^-$. Here we measured myocardial levels of NO, superoxide, and their reaction product $ONOO^-$. We also determined activities of major enzymatic sources for NO and superoxide, i.e. NO synthases and xanthine oxidoreductase, the major antioxidant enzyme superoxide dismutase (SOD) as well as several parameters of myocardial contractile function.

2. Methods

The investigation conforms with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and were approved by the ethics committee of the University of Szeged.

2.1. Experimental groups, induction of hyperlipidemia

Male Wistar rats (18-weeks-old), housed in a room maintained at 12 h light–dark cycles and a constant temperature of 22±2 °C, were fed laboratory chow enriched with 2% cholesterol or standard chow for 8 weeks. At the end of the diet period, hearts were isolated for measurement of cardiac function and biochemical parameters. Separate groups of normal and hyperlipidemic rats were injected twice intraperitoneally with 2×20 μmol/kg FeTPPS (5,10,15,20-tetrakis-[4-sulfonatophenyl]-porphyrinato-iron[III]), a $ONOO^-$ decomposition catalyst, 24 h and 1 h before the isolation of the hearts to allow sufficient time for repair mechanisms and de novo protein synthesis to recover $ONOO^-$-induced cellular injury. At the end of the 8-week diet period, body weights of the animals were 350–400 g, and there were no significant difference between groups; plasma cholesterol and triglyceride level increased by 20% and 300%, respectively, which was consistent with our previous findings [14].

2.2. Measurement of cardiac function in isolated rat hearts

At the end of the diet and FeTPPS treatment, rats were anesthetized with diethyl ether and injected intravenously with 500 U/kg heparin. After 30 s, hearts were excised and placed in perfusion fluid of 4 °C until contractions ceased. Each heart was then cannulated through the aorta and the left atrium and prepared for working heart perfused at 37 °C with Krebs–Henseleit bicarbonate buffer containing (in mM) NaCl 118.4, KCl 4.1, $CaCl_2$ 2.5, $NaHCO_3$ 25, KH_2PO_4 1.17, $MgCl_2$ 1.46 and glucose 11.1; gassed with 95% O_2 and 5% CO_2 and supplemented with 0.3 mmol/l L-tyrosine [15]. Preload (1.7 kPa) and afterload (9.8 kPa) were kept constant throughout the experiments. After a 10 min normoxic, normothermic perfusion, cardiac mechanical functional and hemodynamic parameters including heart rate (HR), coronary flow (CF), aortic flow (AF), left ventricular developed pressure (LVDP) and its first derivatives ($+dP/dt_{max}$, $-dP/dt_{max}$), and left ventricular end-diastolic pressure (LVEDP) were monitored as described [15,16]. Coronary effluent and myocardial tissue were sampled and frozen in liquid nitrogen for further biochemical measurements.

2.3. Measurement of cardiac NO and superoxide

In separate experiments, NO content of ventricular tissue was measured using electron spin resonance spectroscopy after loading the heart with the NO-specific spin trap Fe^{2+}-*N*-methyl-D-glucosamine-dithiocarbamate (MGD) as described [15,16]. The spin-trap for NO was prepared freshly before each experiment. MGD (175 mg) and $FeSO_4$ (50 mg) dissolved in distilled water (pH 7.4, volume 6 ml) was infused into the aortic cannula under Langendorff perfusion (constant pressure at 9.8 kPa) for 5 min at a rate of 1 ml/min in order to measure basal myocardial NO content. Tissue samples from the apex of the heart (approximately 150 mg) were collected at the end of the infusion of $Fe^{2+}(MGD)_2$ and placed into quartz ESR tubes and frozen in liquid nitrogen. Electron spin resonance spectra of $NO–Fe^{2+}(MGD)_2$ adducts were recorded with a Bruker ECS106 spectrometer (Rheinstetten, Germany; ESR parameters: X band, 100 kHz modulation frequency, 160 K temperature, 10 mW microwave power, 2.85 G modulation amplitude, 3356 G central field) and analyzed for NO signal intensity as described [16].

Superoxide production in freshly minced ventricles was assessed by lucigenin-enhanced chemiluminescence [15]. Approximately 100 mg of the apex of the heart was placed in 1 ml air-equilibrated Krebs–Henseleit solution containing 10 mmol/l HEPES–NaOH (pH 7.4) and 5 μmol/l

lucigenin. Chemiluminescence was measured at room temperature in a liquid scintillation counter using a single active photomultiplier positioned in out-of-coincidence mode in the presence or absence of the superoxide scavenger nitroblue tetrazolium (NBT, 200 μmol/l). NBT-inhibitable chemiluminescence was considered an index of myocardial superoxide generation. It should be noted that NBT, like other superoxide scavengers, is not entirely specific for superoxide.

2.4. Measurement of cardiac NO synthase, xanthine oxidoreductase, and SOD activities

To estimate endogenous enzymatic NO production, Ca^{2+}-dependent and Ca^{2+}-independent NO synthase activities in ventricular homogenates were measured by the conversion of L-[^{14}C]arginine to L-[^{14}C]citrulline as we previously described [15]. Powdered frozen ventricular tissue was placed in four volumes of ice-cold homogenization buffer (composition given in Ref. [17]) and homogenized with an Ultra-Turrex disperser. The homogenate was centrifuged (1000×*g* for 10 min) at 4 °C and the supernatant was kept on ice for immediate assay of enzyme activities. The protein concentration was measured from the supernatant using a Lowry–Folin method. Samples were incubated for 25 min at 37 °C in the presence or absence of either EGTA (1 mM) or EGTA plus N^G-monomethyl-L-arginine (1 mM) to determine the level of Ca^{2+}-dependent and Ca^{2+}-independent NO synthase activities, respectively.

Activity of xanthine oxidoreductase (xanthine oxidase and xanthine dehydrogenase), one of the dominant sources of superoxide in rat hearts, was determined from ventricular homogenates by a fluorometric kinetic assay based on the conversion of pterine to isoxanthopterine in the presence (total xanthine oxidoreductase activity) and absence (xanthine oxidase activity) of the electron acceptor methylene blue as described [15,18]. Ventricular homogenates were prepared as for the measurement of NO synthase activity.

Total activity of SOD was measured by a spectrophotometric assay using a kit (Randox Laboratories Ltd, UK). Approximately 100 mg ventricular tissue was homogenized in 10 volumes of ice-cold phosphate buffer (0.01 M, pH 7.0). Total SOD activity in homogenates was determined by the inhibition of formazan dye formation due to superoxide generated by xanthine and xanthine oxidase.

2.5. Measurement of cardiac NO synthase III (Western blot)

Ventricular homogenates used for NO synthase activity assays were further diluted in the homogenization buffer to allow loading of 25 μg of total protein in each lane of 8% polyacrylamide gel. Electrophoresis was conducted at 200 V, 8 mA for 1.5 h, and proteins were transferred on to nitrocellulose membrane (25 V, 200 mA, 4 °C, 1.5 h) by Western blotting. Membranes were then incubated at room temperature for 1 h with monoclonal anti-NO synthase III antibody (Transduction Laboratories Lexington, KY, USA) at 1:200 dilution, and then with horseradish peroxidase-conjugated polyclonal goat anti-mouse IgG (Transduction Laboratories) at 1:500 dilution for 2 h. The membranes were then developed with an enhanced chemiluminescence kit (NEN Boston, MA, USA). NO synthase III level was assessed by densitometry.

2.6. Measurement of markers of $ONOO^-$

We measured both dityrosine by spectrofluorometry and free nitrotyrosine by enyme-linked immunosorbent assay (ELISA; Cayman Chemical, Ann Arbor, MI, USA) in the perfusate as markers of cardiac $ONOO^-$ formation [15]. $ONOO^-$ promotes nitration of phenolic compounds such as tyrosine, the nitration of which leads to the formation of stable products, dityrosine and 3-nitrotyrosine. Therefore, to measure cardiac $ONOO^-$ generation, Krebs–Henseleit buffer was supplemented with L-tyrosine and dityrosine and nitrotyrosine formation was detected in the coronary effluent as described [15]. Dityrosine and nitrotyrosine formation was normalized to coronary flow and wet weight of the hearts and expressed as pmoles/min/mg protein.

We also measured plasma nitrotyrosine as a marker of systemic $ONOO^-$ generation as described [13]. Plasma $ONOO^-$ concentration was expressed as nmoles/l.

2.7. Measurement of plasma malondialdehyde (MDA)

MDA is a marker of lipid peroxidation that reacts with thiobarbituric acid (TBA). As the reaction is not entirely specific for MDA, the assay is called the thiobarbituric acid-reactive substance (TBARS) assay [19]. Plasma samples were mixed thoroughly with 1.2 volumes of a stock solution of 15% w/v trichloroacetic acid, 0.375% w/v TBA and 0.25 N HCl and heated for 30 min at 95 °C. After cooling and centrifugation at 1000×*g* for 10 min, the supernatant containing TBARS were extracted in butanol and assayed spectrophotometrically at 535 nm. Freshly diluted tetramethoxypropane which yields MDA was used as the external standard.

2.8. Statistical analysis

Data were expressed as means±S.E.M. and analyzed with unpaired *t*-test or ANOVA followed by Tukey's test as appropriate. $P<0.05$ was accepted as a statistically significant difference.

3. Results

3.1. Cardiac NO content and NO synthase

Myocardial NO content was significantly decreased in the hyperlipidemic group as measured by electron spin resonance spectroscopy after ex vivo spin trapping of NO in isolated hearts (Fig. 1A).

To test whether a decrease in cardiac NO in hyperlipidemia is a consequence of diminished enzymatic synthesis, we measured cardiac activities of NO synthases. Endogenous enzymatic sources of NO, Ca^{2+}-dependent and Ca^{2+}-independent NO synthase activities (Fig. 1B) were not changed in the myocardium due to cholesterol diet. As Ca^{2+}-independent NO synthase activity was negligible in both groups, we have measured only NO synthase III protein content. NO synthase III did not change in hyperlipidemic hearts when compared to controls (Fig. 1C).

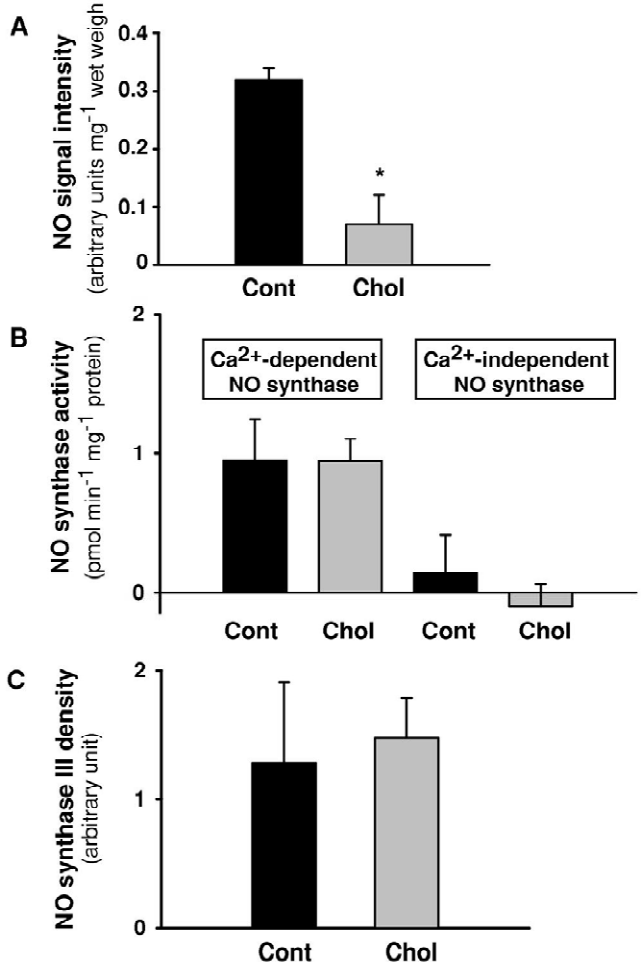

Fig. 1. Myocardial NO content (A), myocardial Ca^{2+}-dependent and Ca^{2+}-independent NO synthase activity (B), and NO synthase III protein content (C) in the control (Cont) and cholesterol-fed (Chol) groups. Results are means±S.E.M. ($n=7$ in both groups). *$P<0.05$ vs. control.

3.2. Cardiac superoxide, xanthine oxidase and superoxide dismutase

To test if cholesterol-enriched diet increases cardiac superoxide generation, we performed a lucigenin-enhanced chemiluminescence assay in freshly minced cardiac tissue. Cardiac superoxide generation was significantly increased due to the high-cholesterol diet as compared to controls (Fig. 2A). To test possible changes in the enzymatic synthesis of superoxide, we measured the activity of xanthine oxidoreductase enzyme complex, one of the major enzymatic sources of superoxide in rat hearts. Xanthine oxidase activity was significantly increased in the hyperlipidemic group (Fig. 2B). We also assayed total activity of SOD in the myocardium, the major enzyme

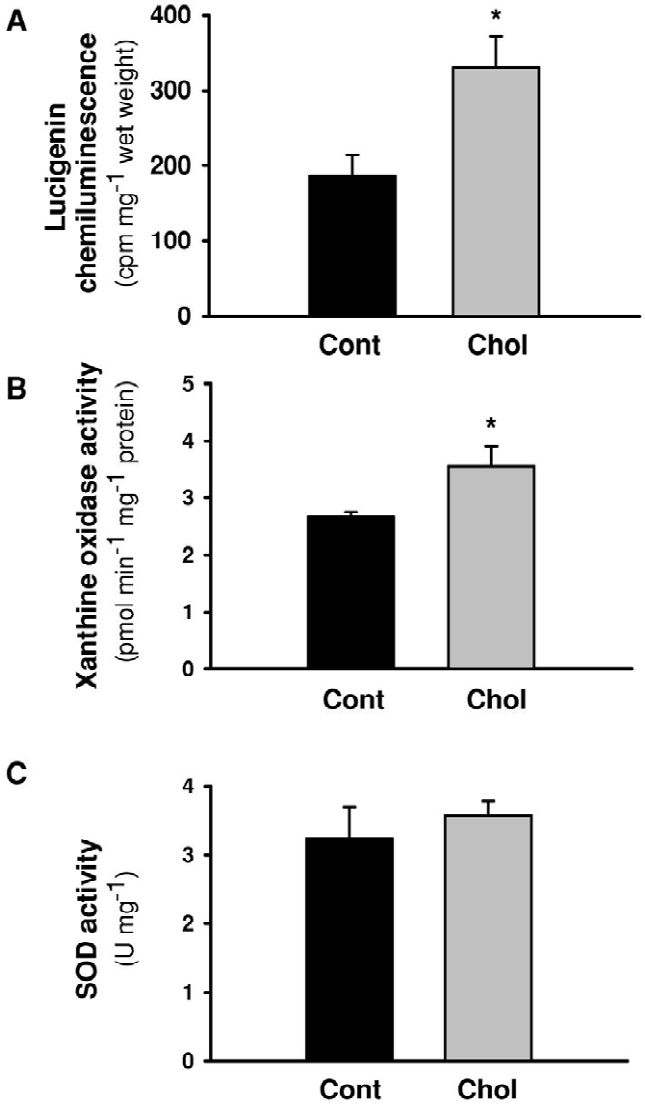

Fig. 2. Cardiac superoxide production (A), myocardial xanthine oxide activity (B), and myocardial SOD activity (C) in the control (Cont) and cholesterol-fed (Chol) groups. Results are means±S.E.M. ($n=7$ in both groups). *$P<0.05$ vs. control.

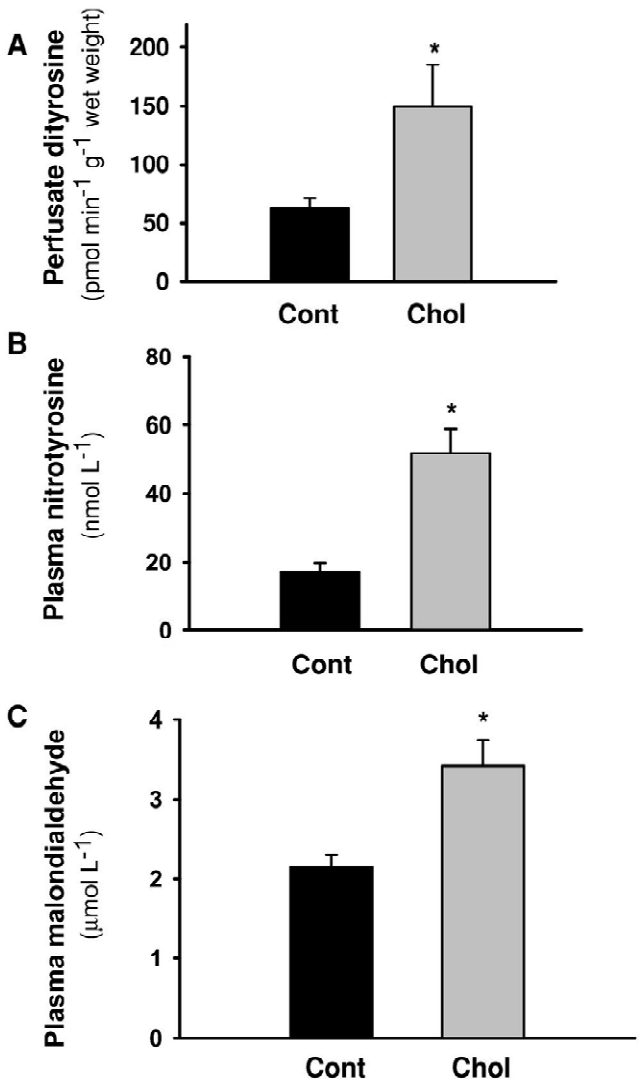

Fig. 3. Dityrosine (A) formation in the perfusate, a marker for cardiac peroxynitrite generation; plasma nitrotyrosine (B) concentration, a marker for systemic peroxynitrite generation; and plasma malondialdehyde (C) concentration, a marker for systemic lipid peroxidation in the control (Cont) and cholesterol-fed (Chol) groups. Results are means±S.E.M. (n=7 in both groups). *P<0.05 vs. control.

responsible for detoxification of superoxide. SOD activity was not changed in hyperlipidemic hearts when compared to controls (Fig. 2C).

3.3. Cardiac $ONOO^-$

To test formation of $ONOO^-$ in the heart, isolated hearts obtained from cholesterol-fed and control groups were perfused with a buffer supplemented with 0.3 mmol/l L-tyrosine. Markers of cardiac $ONOO^-$ generation, dityrosine (Fig. 3A) in the coronary effluent, were increased in the cholesterol-fed group as compared to controls. The formation of the other cardiac peroxynitrite marker, nitrotyrosine, in the coronary effluent, was not statistically significantly increased in the cholesterol-fed group (control 10.48±2.27; cholesterol-fed 14.19±4.56).

3.4. Systemic $ONOO^-$

We also studied if high-cholesterol diet increased systemic formation of $ONOO^-$. Therefore, plasma free nitrotyrosine concentration was measured in control and cholesterol-fed groups as a marker for systemic $ONOO^-$ formation. Plasma free nitrotyrosine was increased approximately two-fold in cholesterol-fed rats as compared to controls (Fig. 3B).

3.5. Plasma malondialdehyde

We also studied if high-cholesterol diet increased systemic lipid peroxidation due to oxidative stress. Therefore, plasma malondialdehyde concentration was measured in control and cholesterol-fed groups as a marker for systemic lipid peroxidation. Plasma malondialdehyde was significantly increased in cholesterol-fed rats as compared to controls (Fig. 3C).

3.6. Cardiac function

To test if an increase in cardiac $ONOO^-$ formation leads to alterations in cardiac performance, cardiac contractile parameters were measured in isolated working hearts. LVEDP was significantly increased in the hyperlipidemic group as compared to controls. Other parameters of cardiac performance such as heart rate, aortic flow, coronary flow, left ventricular developed pressure, $+dP/dt_{max}$, $-dP/dt_{max}$ were not affected significantly by cholesterol-diet when compared to the control group (Table 1). To further test if hyperlipidemia-induced elevation of LVEDP was due to enhanced $ONOO^-$ formation, hyperlipidemic and normal

Table 1
Cardiac functional parameters in the control and cholesterol-fed groups

	HR	CF	AF	CO	LVDP	$+dP/dt_{max}$	$-dP/dt_{min}$	LVEDP
Control	271.0±7.5	22.9±0.5	43.4±2.0	66.3±2.5	18.2±0.4	839.5±45.2	460.0±34.2	0.52±0.05
Cholesterol-fed	270.3±9.4	22.1±0.5	45.3±1.2	67.4±1.6	18.9±0.4	945.0±40.2	483.8±41.6	0.85±0.05*

Heart rate (HR, beats/min); coronary flow (CF, ml/min); aortic flow (AF, ml); cardiac output (CO, ml/min); left ventricular developed pressure (LVDP, kPa); left ventricular end-diastolic pressure (LVEDP, kPa); $+dP/dt_{max}$ (kPa/s); $-dP/dt_{max}$ (kPa/s). Values are means±S.E.M. (n=8 in each group). *P<0.05 shows significant difference compared to control.

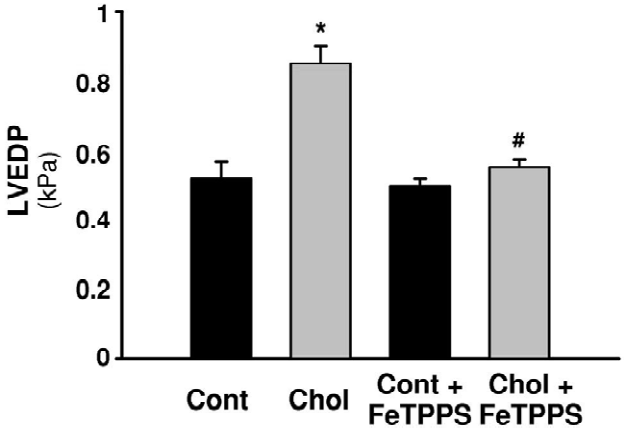

Fig. 4. Left ventricular end-diastolic pressure (LVEDP) in control (Cont), cholesterol-fed (Chol), and cholesterol-fed+FeTPPS-treated (Chol+FeTPPS) groups. Results are means±S.E.M. ($n=8$ in each group). *$P<0.05$ vs. control, #$P<0.05$ vs. cholesterol-fed.

rats were treated with FeTPPS, a $ONOO^-$ decomposition catalyst. In the hyperlipidemic group, LVEDP was recovered to control values after FeTPPS treatment, however, FeTPPS did not change LVEDP in the normal group (Fig. 4).

4. Discussion

The present results show that cholesterol-enriched diet for 8 weeks markedly reduces cardiac NO level, enhances cardiac formation of superoxide and their reaction product $ONOO^-$, thereby leading to an increase in LVEDP, which can be prevented by pretreatment with a $ONOO^-$ decomposition catalyst, FeTPPS. This is the first demonstration that high-cholesterol diet leads to enhanced $ONOO^-$ formation in the heart which results in a deterioration of cardiac function.

In accordance with our previous studies [4] we have found in the present study that high-cholesterol diet leads to a decrease in cardiac NO level. Here we further tested if a decrease in cardiac NO is due to diminished NO biosynthesis. Therefore, we measured the activity and the protein content of endogenous enzymatic sources of NO, Ca^{2+}-dependent and Ca^{2+}-independent NO synthases. We have shown here that high-cholesterol diet does not affect the activity of NO synthases and NO synthase III protein content in the myocardium. This finding shows that high-cholesterol diet-induced decrease in cardiac NO is not a consequence of diminished enzymatic synthesis. It was therefore plausible to speculate that cholesterol diet leads to an enhanced elimination of NO.

It is well known that NO rapidly reacts with superoxide to form the cytotoxic species $ONOO^-$ [20]. Hyperlipidemia has been shown to increase production of reactive oxygen species including $ONOO^-$ in the vasculature [13,20–22]. Although it is not known if hyperlipidemia leads to increased formation of reactive oxygen species in the heart, it is plausible to speculate that this mechanism is involved in the enhanced breakdown of NO in the myocardium in hyperlipidemia. Therefore, we measured cardiac superoxide and $ONOO^-$ production. Myocardial superoxide level was significantly increased due to cholesterol diet in this study. One of the major sources of superoxide in the rat heart is the xanthine oxidoreductase (XOR) enzyme [23], which comprises both xanthine dehydrogenase (XDH) and xanthine oxidase (XO). Our results show that hyperlipidemia increased myocardial activity of XO. We also assessed if a diminished elimination of superoxide also plays a role in cholesterol-rich diet-induced enhanced superoxide level, however, SOD activity in the myocardium was not changed in the cholesterol-fed group. These results show that the increase in cardiac superoxide in the hyperlipidemic group is a consequence of increased superoxide synthesis at least in part by enhanced XO activity. NAD(P)H oxidase activity, another important source of cardiac superoxide has not been measured in this study.

In addition to increased myocardial superoxide formation, we have found here that high-cholesterol diet increases formation of a potential marker of cardiac $ONOO^-$, dityrosine in the perfusate. This is the first demonstration that hyperlipidemia increases $ONOO^-$ formation in the heart. In contrast to dityrosine, perfusate nitrotyrosine was not statistically significantly increased in our present study. This can be explained by recent results showing that at relatively low level of $ONOO^-$, nitrotyrosine formation is suppressed in favor of dityrosine [24]. The source of $ONOO^-$ release in the heart was not determined in the present study. Coronary and endocardial endothelial cells, specific cardiac nerves, and cardiac myocytes may all potentially contribute to $ONOO^-$ formation in the heart, since all of these cells are able to synthesize NO and superoxide. We have also found that hyperlipidemia increases the plasma nitrotyrosine level, a marker for systemic $ONOO^-$ generation. This is in accordance with our previous study showing an increase in serum nitrotyrosine in rabbits with high-cholesterol diet [13]. The reason why nitrotyrosine level was increased in the plasma but not in the coronary effluent is not clear. However, an increased systemic $ONOO^-$ formation has a greater chance to increase nitrotyrosine level in the circulating plasma in vivo, whereas cardiac $ONOO^-$ formation has less chance to increase nitrotyrosine significantly in the perfusate as the perfusion buffer passes through the coronary circulation only once in the Langendorff preparation. It should be noted that dityrosine and nitrotyrosine have been criticised as being specific for $ONOO^-$, e.g. myeloperoxidase activity in the presence of nitrite may also lead to nitrotyrosine formation [24,25]. However, both myeloperoxidase activity and nitrite concentration are very low in granulocyte-free, Krebs-perfused hearts. This suggests that the myeloperoxidase pathway does not substantially contribute

to nitrotyrosine formation in our present study. Furthermore, biochemical data suggesting that $ONOO^-$ does not cause tyrosine nitration [26] have been recently refuted [27]. Nevertheless, $NO_2^{\cdot}$ radical may also contribute to nitrotyrosine formation [24].

The cytotoxic effects of $ONOO^-$ include lipid peroxidation, nitration of tyrosine residues, oxidation of sulfhydryl groups, DNA-strand breakage [28], and inhibition of mitochondrial respiration [20], leading to tissue injury, which manifests itself, e.g., as a depression in myocardial contractile function [29]. Many studies show that enhanced formation of $ONOO^-$ in the myocardium is cytotoxic to the heart and contributes to ischemia/reperfusion injury both in vitro and in vivo, the spontaneous loss of cardiac function, as well as cytokine-induced myocardial contractile failure in isolated rat hearts and in dogs in vivo [30–32]. Many studies show a correlation between $ONOO^-$ formation and deterioration of cardiac function [31]. Therefore, here we tested if increased $ONOO^-$ in hearts of cholesterol-fed rats leads to a deterioration of cardiac function. We have found a significant increase in LVEDP in the cholesterol-fed group. LVEDP elevation is the most sensitive parameter of cardiac dysfunction showing that the capability of the heart to relax is deteriorated. This finding is in accordance with a study by Schwemmer et al. who reported a substantial decline in myocardial contractile and relaxation parameters in hypercholesterolemic guinea-pig hearts [33].

To further test if an increase in LVEDP was due to $ONOO^-$ formation, we examined the effect of FeTPPS, a $ONOO^-$ decomposition catalyst, on cardiac performance in cholesterol-fed and control groups. FeTPPS catalyzes the isomerization of $ONOO^-$ to nitrate anion and thereby decreases its decomposition to highly reactive intermediates such as nitrogen dioxide and hydroxyl radical [34]. Our results show that pretreatment with FeTPPS normalizes LVEDP in the cholesterol-fed group, but it does not change LVEDP in the control group. This finding further suggests that hyperlipidemia induces $ONOO^-$ formation in the rat heart which leads to an increase in LVEDP. As the biochemical measurements were not repeated in the FeTPPS groups, some unspecific effects of FeTPPS on cardiac NO and superoxide formation may also account for the effect of FeTPPS, which is a limitation of this study.

Our present results do not clarify the exact cellular mechanisms by which cholesterol diet leads to an increased formation of superoxide and therefore $ONOO^-$. We used an isolated, crystalloid-perfused rat heart model in our present study. In this model, the direct effect of plasma lipids and the effect of atherosclerosis can be excluded, since Wistar rats show moderate increase in serum cholesterol level and no substantial functional atherosclerosis develops due to cholesterol diet [4,35,36]. Therefore, enhancement of $ONOO^-$ is most likely due to the accumulation of tissue/membrane cholesterol [2] rather than the direct acute effects of high serum lipids itself. However, we have also found that plasma MDA level, a marker for lipid peroxidation, was significantly increased in the cholesterol-fed group. Although the source of plasma MDA was not determined, one may speculate the role of oxidized lipoproteins. Recent evidence shows that oxidized low density lipoprotein (oxLDL) is deposited in the myocardium which leads to expression of oxLDL receptor (LOX-1) and thereby induces apoptosis and cardiac dysfunction [37,38].

In summary, hyperlipidemia stimulates $ONOO^-$ generation in the heart which leads to myocardial dysfunction. Targeting $ONOO^-$ with pharmacological tools may be an exciting new strategy to protect the heart and the vasculature in hyperlipidemia.

Acknowledgements

This work was supported by grants from the Hungarian Scientific Research Found (OTKA T029843), Hungarian Ministry of Education (FKFP-0340/2000 and FKFP-0057/2001) and Ministry of Health (ETT 51/2000), and the Hungarian National R&D Program (NFKP-1/040).

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