Exercise restores coronary vascular function independent of myogenic tone or hyperglycemic status in \( db/db \) mice

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Moien-Afshari F, Ghosh S, Elmi S, Khazaei M, Rahman MM, Sallam N, Laher I. Exercise restores coronary vascular function independent of myogenic tone or hyperglycemic status in \( db/db \) mice. Am J Physiol Heart Circ Physiol 295: H1470–H1480, 2008. First published July 18, 2008; doi:10.1152/ajpheart.00016.2008.—Regulation of coronary function in diabetic hearts is an important component in preventing ischemic cardiac events but remains poorly studied. Exercise is recommended in the management of diabetes, but its effects on diabetic coronary function are relatively unknown. We investigated coronary artery myogenic tone and endothelial function, essential elements in maintaining vascular fluid dynamics in the myocardium. We hypothesized that exercise reduces pressure-induced myogenic constriction of coronary arteries while improving endothelial function in \( db/db \) mice, a model of type 2 diabetes. We used pressurized mouse coronary arteries isolated from hearts of control and \( db/db \) mice that were sedentary or exercised for 1 h/day on a motorized exercise-wheel system (set at 5.2 m/day, 5 days/wk). Exercise caused a ~10% weight loss in \( db/db \) mice and decreased whole body oxidative stress, as measured by plasma 8-isoprostane levels, but failed to improve hyperglycemia or plasma insulin levels. Exercise did not alter myogenic regulation of arterial diameter stimulated by increased transmural pressure, nor did it alter smooth muscle responses to U-46619 (a thromboxane agonist) or sodium nitroprusside (an endothelium-independent dilator). Moderate levels of exercise restored ACh-simulated, endothelium-dependent coronary artery vasodilation in \( db/db \) mice and increased expression of Mn SOD and decreased nitrotyrosine levels in hearts of \( db/db \) mice. We conclude that the vascular benefits of moderate levels of exercise were independent of changes in myogenic tone or hyperglycemic status and primarily involved increased nitric oxide bioavailability in the coronary microcirculation.

MATERIALS AND METHODS

Animals. All experimental procedures were approved by the Animal Care Committee of the University of British Columbia. Five-week-old male \( db/db \) (BKS.Cg-m\(^{-/-}\) Lepr\(^{db}\)/J) and age-matched control (BKS.Cg-m\(^{-/-}\) Lepr\(^{+/+}\)/J) mice, simply referred to as wild-type (WT) in the present study, were purchased from Jackson Laboratory. Mice were housed in standard animal facility conditions with 12:12-h light-dark cycles at 26°C and allowed free access to standard chow and water. WT and \( db/db \) mice were divided into exercised and sedentary subgroups (8–10 in each group).

Exercise protocol. Mice (5 wk old) assigned to the exercise group were trained to run on a motorized exercise-wheel system (Lafayette Instrument) for 8 wk. Exercise intensity was incrementally increased to allow for animal acclimatization during the first 2 wk of training. The initial exercise speed of 2.5 m/min for 1 h (150 m) per day was gradually increased to a target of 1 h of exercise at 5.2 m/min, which represents a daily forced exercise of 312 m. Mice were exercised daily, 5 days/wk for 8 wk, at a set time each day. Mice were housed in the animal facility between exercise sessions. Sedentary animals were placed in the nonrotating wheels for the same duration as the exercised group.

Blood and tissue samples. At 13 wk of age, mice were anesthetized by intraperitoneal injection of pentobarbital sodium (Somnotol; 30 mg/kg) and heparin sodium (50 U/kg). Blood samples were collected results in a mismatch of myocardial supply and demand, thus provoking cardiac ischemia and myocardial infarction (32, 39).

The cardiovascular benefits of regular exercise in patients with type 2 DM are well accepted. Exercise alters myocardial redox status and calcium handling, improves energy metabolism, and induces the formation of heat shock proteins and other cardioprotective molecules (2, 26). Exercise also reduces insulin resistance, an important cause for the elevated plasma glucose and insulin levels in humans and animals with diabetes (37). However, the mechanisms by which exercise promotes improved coronary microcirculatory function in type 2 DM hearts are incompletely understood, especially in relation to altered myogenic tone and endothelial function in coronary resistance arteries. We hypothesized that exercise reduces pressure-induced myogenic constriction of coronary arteries while simultaneously improving endothelial function in \( db/db \) mice, a frequently used animal model of type 2 DM. We found that the vascular benefits of moderate levels of exercise in our study were independent of changes in myogenic tone or hyperglycemic status and primarily involved increased nitric oxide (NO) bioavailability in the coronary microcirculation.

CARDIOVASCULAR DISEASE is the leading cause of mortality in patients with diabetes (29). Endothelium-dependent vasodilation is markedly reduced and myogenic tone of resistance arteries is increased in animal models of type 2 diabetes mellitus (DM) (3, 25, 53). These changes are likely to reduce tissue blood perfusion. Blood flow is regulated by the influence of several constrictors (e.g., increased intravascular pressure, endothelial constrictors) and dilators (e.g., reduced intravascular pressure, endothelial dilators) (35). In many cases, endothelial dysfunction precedes the onset of cardiovascular disease in type 2 DM. It is likely that loss of endothelial regulation

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Reaction mixtures consisted of 50 mM Tris (pH 8), 0.1 mM oxalo-
4°C for 5 min at 13,200 rpm. The supernatant was assayed for CS
X-100 and 0.5 mM EDTA (pH 8) using a glass homogenizer (14).

Oral glucose tolerance test. Mice underwent an oral glucose
tolerance test (OGTT) at 10 wk of age (i.e., after 5 wk of exercise).
After 6 h of fasting, mice were loaded with glucose (1.5 g/kg) by oral
gavage of a 40% glucose solution, and blood samples were taken at 0,
10, 20, 60, and 120 min. Plasma was separated by centrifugation and
stored at −76°C for later analysis of glucose. For reduction of short-term
treatment effects, animals were not exercised for 24 h before
the OGTT.

Western blot analysis. Pieces of whole hearts were ground in liquid
and homogenized in a Polytron homogenizer three times for
30 s each in ice-cold homogenization buffer (20 mM Tris-HCl, 250
mM EGTA, 200 mM EDTA, 100 mM PMSF, 100 mM NaF, 2-mer-
captoethanol, leupeptin, aprotinin, NP-40, 10% SDS, and 5% DCA).
Tissue samples were homogenized in 100 mM Tris buffer (pH 8) containing 0.1% Triton
acetate, and 0.1 mM acetyl-CoA, and 0.1 mM 5,5-dithiobis(2-nitrobenzo-
Briefly, the reaction was initiated by addition of 25 μl of muscle
extract and linking of the release of free CoA to 5,5-dithiobis(2-
by a colorimetric agent. The CS activity was moni-
tored at 412 nm using a spectrophotometer (Lambda 35 UV/VIS,
Perkin Elmer). Calculations of activity used a millimolar extinction
coefficient of 13.6 and were corrected for background acetyl-CoA
decaylase activity by determination of the rate of change in absorb-
bance at 412 nm in the absence of oxaloacetate. CS activity was
expressed as micromoles per minute per milligram protein of the
(extract measured by Bradford assay).

Resistance artery preparation. Mouse coronary septal arteries were
located through a right ventricular wall opening, dissected, and
TABLE 6


time to develop a

5% dextrose (D5W).

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Statistical analysis and calculations. Values are means ± SE. Data were analyzed using NCSS-2000 computer software. Repeated-
measures or one-way ANOVA with multiple comparisons using Bonfer-
roni’s test was performed where appropriate. GraphPad Prism (version 3.02-2000) was used for curve fit and dose-response analysis. The results of statistical tests were considered significant at $P < 0.05$.

Myogenic tone was calculated as percentage of arterial constriction at each pressure step as follows: $%$ constriction = $100 \times (D_{Ca \text{ free},P} - D_{Ca \text{ free},P})$, where $D_{Ca \text{ free},P}$ is arterial diameter at pressure P in calcium-free PSS and $D_P$ is diameter in PSS at pressure P. Percentage of relaxation/dilation of the arteries in response to ACh and SNP was calculated as follows: $100 \times (D_{Ca \text{ free},P} - D_{Ca \text{ free},P})$, where $D_{Ca \text{ free},P}$ is arterial diameter at a particular $P$ in the presence of U-46619, and $D_{Ca \text{ free},P}$ is passive diameter in calcium-free PSS at 20 mmHg pressure. Passive vascular distensibility was calculated as $100 \times$ passive $D_P/D_{Ca \text{ free},P}$, where passive $D_P$ is vascular diameter in Ca-free PSS at pressure P.

**RESULTS**

Exercise, body weight, heart weight, and plasma parameters. Moderate-intensity exercise decreased body weight of db/db mice (~10%) compared with their sedentary littermates, whereas exercise did not alter body weight of WT mice. Heart weight appeared lower in db/db than in WT mice; however, the difference was not significant (158 ± 6 vs. 177 ± 6 mg). Exercise did not increase heart weight significantly in either group (170 ± 7 and 184 ± 6 mg; respectively; Table 1). Plasma glucose and plasma insulin levels were significantly higher in db/db than in age-matched lean WT mice. Eight weeks of moderate-intensity exercise did not significantly alter plasma glucose and insulin levels in db/db mice (Table 1). As an indicator of the physiological effectiveness of our exercise protocol, CS activity was significantly increased in exercised WT and db/db mice (Table 1). An OGTT was performed after 5 wk of exercise in db/db and WT mice (Table 2). Exercise did not alter plasma glucose levels in db/db or WT mice within 120 min after an oral glucose load (Table 2). There was a significant difference between db/db and WT groups at all time points.

Exercise decreases whole body and tissue oxidative stress. Whole body oxidative stress was estimated by measurement of plasma 8-isoprostane, a lipid peroxidation by-product (36). Plasma levels of 8-isoprostane were elevated in db/db mice and were significantly reduced by exercise (Table 1). Estimation of antioxidant protein expression in the whole heart by Western blotting revealed that Mn SOD was significantly decreased and nitrotyrosine was significantly increased in db/db mice compared with their age-matched lean WT mice. Exercise significantly increased Mn SOD (Fig. 1A) and extracellular SOD (Fig. 1F) and lowered nitrotyrosine levels in db/db hearts (Fig. 1D). However, exercise did not alter the protein expression levels of Cu,Zn SOD and catalase in db/db or WT mouse hearts (Fig. 1, B and C). Nitrotyrosine levels increased in the absence of changes in cardiac eNOS levels, which were unaffected by diabetes or exercise (Fig. 1E). To ensure that the increases in SOD were localized to the coronary arteries, we utilized an immunofluorescence approach (Fig. 2).

Exercise and coronary arteriolar tone. Pressure-constriction curves (10–120 mmHg transmural pressure) were not statistically different in sedentary and exercised db/db and WT mice (Fig. 3, Table 3). Bosentan did not have a significant effect on myogenic tone in coronary arteries of sedentary and exercised WT or db/db mice (Fig. 3, C–F). We found no significant difference in arteriolar distensibility or passive diameter at 80 mmHg between db/db and WT mice (Fig. 3B, Table 3). Agonist-induced constriction by U-46619 (10−6 M) was also the same in WT and db/db coronary arteries, and exercise did not cause a significant change in this response (Table 3).

Exercise and endothelium-dependent arteriolar relaxation. Endothelium-dependent coronary vasodilation induced by cumulative concentrations of ACh was significantly attenuated in coronary arteries of db/db compared with WT mice (Figs. 4 and 5). EC50 values for the ACh response were similar in coronary arteries of db/db and WT mice in sedentary (−log $EC_{50} = 7.12 \pm 0.08$ and 7.26 ± 0.08, respectively) and exercised (−log $EC_{50} = 7.18 \pm 0.11$ and 7.28 ± 0.09, respectively) groups. The maximal relaxation in response to ACh ($E_{max}$) was significantly lower in db/db than in lean WT mice (51.3 ± 1.7 vs. 91.3 ± 2.7%). Exercise significantly improved $E_{max}$ of the ACh response in db/db mice (85.3 ± 3.5%; Figs. 4 and 5). The endothelium-independent coronary vasodilatory response to an exogenous NO donor (SNP) was not different in WT and db/db mice (Figs. 4 and 5). There were no statistical differences in

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### Table 1. General characteristics of the animals

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>WT</th>
<th>WT exe</th>
<th>db/db</th>
<th>db/db exe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart wt, mg</td>
<td>177±6</td>
<td>184.6</td>
<td>158.6</td>
<td>170.7</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>27.01±0.39</td>
<td>26.5±0.54</td>
<td>49.68±0.36</td>
<td>46.20±1.18*</td>
</tr>
<tr>
<td>Plasma glucose, mmol/l</td>
<td>6.45±0.31</td>
<td>5.74±0.24</td>
<td>47.53±3.90†</td>
<td>48.29±4.11†</td>
</tr>
<tr>
<td>Plasma insulin, µg/l</td>
<td>1.51±0.15</td>
<td>1.16±0.16</td>
<td>3.64±0.55†</td>
<td>3.73±0.73†</td>
</tr>
<tr>
<td>CS activity, µmol·min⁻¹·mg protein⁻¹</td>
<td>52.46±3.14</td>
<td>68.43±2.93†</td>
<td>51.41±2.39</td>
<td>67.12±1.77*</td>
</tr>
<tr>
<td>Plasma 8-isoprostane, pg/ml</td>
<td>8.86±5.11</td>
<td>7.9±4.11</td>
<td>43.49±3.21†</td>
<td>16.58±3.52*</td>
</tr>
</tbody>
</table>

Values are means ± SE for 8–10 mice in each group. WT, wild-type; exe, exercise; CS, citrate synthase. Parameters were measured at the time the animals were killed (13 wk of age). *P < 0.01 vs. db/db, †P < 0.05 vs. WT.

**Table 2. Summary of OGTT results from mice that were exercised for 5 wk**

<table>
<thead>
<tr>
<th>Time, min</th>
<th>WT</th>
<th>WT exe</th>
<th>db/db</th>
<th>db/db exe</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.78±0.63</td>
<td>6.64±0.60</td>
<td>37.97±1.91*</td>
<td>35.03±2.77*</td>
</tr>
<tr>
<td>10</td>
<td>14.12±1.03</td>
<td>15.65±0.41</td>
<td>42.70±5.28*</td>
<td>49.84±2.73*</td>
</tr>
<tr>
<td>20</td>
<td>13.04±1.37</td>
<td>14.16±0.92</td>
<td>48.16±2.43*</td>
<td>49.56±3.96*</td>
</tr>
<tr>
<td>60</td>
<td>9.15±1.04</td>
<td>8.76±0.46</td>
<td>40.61±2.58*</td>
<td>41.75±3.05*</td>
</tr>
<tr>
<td>120</td>
<td>6.95±0.60</td>
<td>6.72±0.57</td>
<td>32.84±1.00*</td>
<td>28.20±1.59*</td>
</tr>
</tbody>
</table>

Values are means ± SE for 9–10 mice in each group. Mice were fasted for 6h and loaded with glucose at 1.5 g/kg body wt. OGTT, oral glucose tolerance test. *P < 0.01, db/db and db/db exe vs. WT and WT exe (repeated-measures ANOVA).

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Emax and EC50 of the responses to SNP within the sedentary (Emax = 86.9 ± 3.4 and 82.7 ± 4.9% for WT and db/db, respectively; −log EC50 = 7.2 ± 0.1 and 7.0 ± 0.2 for WT and db/db, respectively) and exercised (Emax = 85.8 ± 2.9 and 85.1 ± 3.6% for WT and db/db, respectively; −log EC50 = 7.0 ± 0.1 and 6.9 ± 0.1 for WT and db/db, respectively) groups.

**Exercise and endothelial NO bioavailability.** We repeated ACh concentration-response curves in the presence of l-Arg (an eNOS substrate, 10^{-3} M) + BH4 (an eNOS cofactor, 10 μM) and Cu,Zn SOD (120 U/ml). Incubation of db/db coronary septal arteries with l-Arg + BH4 or SOD significantly improved endothelium-dependent relaxation (Fig. 6A) without affecting ACh-induced vasodilation in arteries from exercised
db/db mice (Fig. 6B) or sedentary and exercised WT mice (data not shown). Incubation with L-Arg + BH4 or SOD did not change EC50 of ACh in db/db arteries (log EC50 = 7.12 ± 0.08, 6.93 ± 0.08, and 7.09 ± 0.11 for untreated db/db arteries, db/db arteries treated with L-Arg + BH4, and SOD-treated db/db arteries, respectively; Fig. 6C) but significantly improved Emax of the ACh response (51.3 ± 1.7, 96.1 ± 3.2, and 91.3 ± 3.9% for untreated db/db arteries, db/db arteries treated with L-Arg + BH4, and SOD-treated db/db arteries, respectively; Fig. 6D).

DISCUSSION

Although the cardiac benefits of lifestyle improvements such as exercise are well known in the management of diabetes, the majority of studies focused on cardiac muscle alterations via improvements in metabolic and mitochondrial activity or upregulation of genes and proteins that lead to cardioprotection (2, 9, 24, 56). The role of improved coronary artery microcirculatory function in exercise-induced cardiac health benefits in mouse models of diabetes is relatively unexplored, largely because of the technical difficulties in studying the coronary resistance arteries from animals such as the db/db mice. Our results indicate a marked endothelial dysfunction in coronary septal arteries of db/db mice, a model of type 2 DM, that can be reversed by exercise. At the time of death (13 wk of age), plasma glucose and insulin were significantly higher in db/db than in WT mice, probably as a result of increased insulin resistance. Endothelial dysfunction is a hallmark of diabetes (10, 21, 46). Reductions in hyperglycemia and insulin resistance improve endothelial function and vasodilation (37). In our study, despite a significant decrease in body weight, 8 wk of chronic moderate-intensity exercise did not decrease plasma insulin or glucose levels in db/db mice, demonstrating that moderate levels of exercise did not change hyperglycemic status. We assessed the effect of exercise on blood sugar by two methods at two different time points, OGTT at 10 wk of age and glucose levels at the time of death; these methods showed that exercise did not have a significant effect on hyperglycemia in db/db mice. The lack of change in OGTT could be related to the exercise protocol, since repeated short periods of daily exercise are more effective in reducing OGTT than a longer period of exercise of the same duration everyday (15). Regarding other metabolic effects of exercise in the mice, we reported previously that the exercise intensity used in the present study lowers triglycerides and LDL cholesterol without changing HDL (34). This effect may be related to improved vascular endothelial function in exercised mice.

Hyperglycemia in diabetes results in increased production of reactive oxygen and nitrogen species in the cell, leading to oxidative stress (38, 41). Free radicals play a major role in endothelial dysfunction during hyperglycemia (10, 21, 47). Oxidative stress arises from an imbalance between the produc-
tion of free radicals and their neutralization by endogenous antioxidants. Because our exercise regimen did not lead to a reduction in high plasma glucose levels in \textit{db/db} mice, we reasoned that the stimulus for production of free oxygen radicals was not likely to have been diminished by exercise. Therefore, we anticipated an increase in antioxidant defenses with exercise. Free radicals such as superoxide quench NO and decrease the bioavailability of this endothelial vasodilator (28).

![Fig. 3. Myogenic tone and passive distensibility in coronary septal arteries. A: myogenic tone was not significantly different in coronary arteries of \textit{db/db} and WT mice. B: passive distensibility of coronary arteries was not statistically different in sedentary and exercised \textit{db/db} and WT mice. C and E: myogenic tone with or without bosentan in coronary arteries of sedentary and exercised \textit{db/db} and WT mice. D and F: myogenic tone with or without bosentan in coronary arteries of sedentary and exercised \textit{db/db} mice. Incubation with bosentan indicated that endogenous endothelin-1 does not have a significant effect on vascular myogenic tone. Values are means ± SE (n = 8–10 in each group).]

Table 3. \textit{Vasomotor responses of isolated coronary arteries}

<table>
<thead>
<tr>
<th>Group</th>
<th>Passive diameter at 80 mmHg, %constriction</th>
<th>Myogenic tone at 80 mmHg, %constriction</th>
<th>U-46619 (-\log EC_{50}, M)</th>
<th>E\textsubscript{max}, %constriction</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>168.4±2.7</td>
<td>34.4±4.1</td>
<td>6.56±0.15</td>
<td>75.91±6.18</td>
</tr>
<tr>
<td>WT exe</td>
<td>174.3±4.9</td>
<td>34.0±3.9</td>
<td>6.80±0.14</td>
<td>72.60±4.78</td>
</tr>
<tr>
<td>\textit{db/db}</td>
<td>167.0±4.8</td>
<td>41.1±4.5</td>
<td>6.52±0.18</td>
<td>68.20±3.34</td>
</tr>
<tr>
<td>\textit{db/db} exe</td>
<td>168.2±4.9</td>
<td>37.5±5.2</td>
<td>6.76±0.13</td>
<td>75.38±4.65</td>
</tr>
</tbody>
</table>

Values are means ± SE for 8–10 mice in each group. Data are from 13-wk-old animals.
Additionally, superoxides also lead to the formation of peroxynitrite, a potent inducer of irreversible oxidative damage (41). SODs are endogenous antioxidants: they compete with NO for reaction with superoxides and, thus, are able to neutralize them. Exercise decreases whole body oxidative stress, as indicated by lowered plasma 8-isoprostane levels in exercised db/db mice. Inasmuch as the total protein levels in coronary arteries were too low for direct immunoblotting experiments, we used whole hearts from db/db mice to demonstrate that exercise increased Mn SOD expression and decreased nitrotyrosine levels (used as a biomarker for increased peroxynitrite activity) in the heart (41). Using immunofluorescence techniques, we further confirmed our finding of increased Mn SOD in coronary arteries after exercise in WT and db/db hearts. Our finding of potentiation of Mn SOD expression by exercise in diabetes is important, since increased mitochondrial SOD eliminates oxygen free radicals generated by mitochondria, which is the primary source of these compounds in diabetes (5). This will reduce the extent of free radical-induced cellular damage by mitigating the four major sources of vascular/endothelial dysfunction in diabetes, including the polyol pathway, advanced glycation end products, protein kinase C, and activated hexosamine pathway (5). The mechanisms whereby exercise induces Mn SOD expression are unknown; however, there are some probable explanations. For example, bouts of exercise-induced oxidative stress increase Mn SOD (57). Mn SOD can also be induced by cytokines such as TNFα and IL-1β (57), which are increased secondary to elevation of NF-κB (54) in altered redox states (22), such as exercise. It appears that only moderate-intensity (and not low-intensity) exercise increases Mn SOD (34). This could be related to
the higher oxidative stress threshold required for induction of Mn SOD (57). Other than the heart muscle, it is likely that oxidative stress in coronary arteries from \textit{db/db} mice is also related to reduced SOD expression/activity, since addition of exogenous SOD completely restored ACh-induced vasodilation in arteries from sedentary \textit{db/db} mice; this finding is consistent with previous reports of reversal of endothelial dysfunction in diabetic vessels by exogenous SOD (4, 43, 47). Exercise probably increases SOD expression/activity in diabetic coronary arterioles (49), which is evident from reduced effectiveness of exogenous SOD on endothelium-dependent vasodilation in coronary arteries from exercised \textit{db/db} mice.

Although unlikely in the light of unchanged catalase levels, it is also possible that accumulation of H$_2$O$_2$, formed from the increased dismutation of superoxides by various SODs after exercise, may also be partly responsible for vasorelaxation in the study groups. H$_2$O$_2$, a product of O$_2^-$ dismutation generated from uncoupled eNOS, plays an important role in endothelium-dependent relaxation under conditions of BH$_4$ deficiency (11). Therefore, conversion of H$_2$O$_2$ to H$_2$O and O$_2$ by catalase significantly decreases endothelium-dependent relaxation in BH$_4$-deficient states (11). Under such conditions, SOD can improve endothelium-dependent vasodilation in coronary arteries from exercised \textit{db/db} mice.

Fig. 5. Concentration-response curves for endothelium-dependent (ACh-mediated, A) and endothelium-independent (SNP-mediated, B) vasorelaxation in coronary arteries. ACh-induced vasodilation declined markedly in \textit{db/db} mice compared with WT mice. Values are means ± SE (n = 8–10 in each group). *P < 0.05 (repeated-measures ANOVA). Exercise preserved endothelium-dependent ACh-mediated vasodilation in \textit{db/db} mice. C and D: EC$_{50}$ and E$_{\text{max}}$ of ACh and SNP response in coronary arteries. EC$_{50}$ was not statistically different among groups for ACh and SNP response. E$_{\text{max}}$ was significantly decreased in \textit{db/db} compared with WT mice. Values are means ± SE (n = 8–10 in each group). *P < 0.05 (repeated-measures ANOVA). Exercise significantly increased E$_{\text{max}}$ in \textit{db/db} mice. For SNP response there was no significant difference in E$_{\text{max}}$ among groups.

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Exaggerated coronary myogenic tone or increased arterial stiffness in coronary arteries from diabetic hearts may lead to increased cardiac ischemia. (50). Lagaud et al. (30) and Frisbee et al. (17), in separate studies on db/db mouse and Zucker rat models of type 2 DM, reported that myogenic tone is increased in mesenteric and skeletal muscle arterioles, respectively. Crijns et al. (13) demonstrated increased arterial stiffness in streptozocin-induced type 1 diabetic rats. Our results suggest that, in the coronary vascular bed, the myogenic response and passive distensibility are the same in db/db and WT mice. Exercise did not have a significant effect on myogenic tone or arterial passive distensibility in WT or db/db mice. Moreover, our study shows that the constrictor responses of smooth muscle cells to pressure and a thromboxane agonist were similar in diabetic and WT mice. Therefore, an increased myogenic response or decreased vascular compliance cannot completely account for reduced cardiac function in db/db mice (23).

We observed a marked reduction in ACh-induced, endothelium-dependent NO-mediated vasodilation in coronary septal arteries isolated from db/db mice. Decreased ACh-induced vasodilation was significantly improved after incubation with L-Arg + BH4 or SOD in db/db (A and B), but not exercised db/db, mice. C and D: Emax and EC50 values for ACh concentration-response curves before and after L-Arg + BH4 and SOD incubation. Values are means ± SE (n = 8–10 in each group). *P < 0.01 (repeated-measures ANOVA).

Fig. 6. Effect of L-Arg + tetrahydrobiopterin (BH4) and SOD incubation on ACh-mediated vasorelaxation in coronary septal arteries of sedentary and exercised db/db mice. Decreased ACh-induced vasodilation was significantly improved after incubation with L-Arg + BH4 or SOD in db/db (A and B), but not exercised db/db, mice. C and D: Emax and EC50 values for ACh concentration-response curves before and after L-Arg + BH4 and SOD incubation. Values are means ± SE (n = 8–10 in each group). *P < 0.01 (repeated-measures ANOVA).
However, the concentration-response curves to U-46619 were similar in sedentary and exercised db/db and WT mice in our study. Alterations in ACh receptor activity in DM may also lead to decreases in ACh-mediated relaxation in db/db mice (7, 8). However, our finding of a decline in ACh E_{max} in the absence of a change in EC_{50} indicates that ACh receptor function remained relatively unaltered in diabetic arteries.

The reduced NO-mediated relaxation in arteries of db/db mice was not due to decreased expression of NO-generating enzyme protein (eNOS), in agreement with other studies showing an unaltered or even upregulated eNOS protein expression in DM (12, 40). Although eNOS is present, it is possible that the activity and/or regulation of this enzyme may be negatively altered in DM (16). Moreover, it is unlikely that smooth muscle cell sensitivity to NO or activation of vascular smooth muscle cell guanylate cyclase is altered in diabetic mice, since the SNP-mediated vasodilatation was unaltered by exercise.

Alterations in the cofactor and substrate regulation in eNOS activity have been reported in diabetes (6, 20). Moderate levels of exercise in db/db mice corrected the relative deficiency of L-Arg and BH4 in db/db coronary arteries. In sedentary db/db mice, incubation of coronary arteries with L-Arg reversed the impaired ACh response. Such a deficiency of cofactors (6, 42, 43) and substrates (44, 45) of NOS was reported previously and considered to be due to enhanced consumption of the substrate (20) or direct degradation of BH4 by excessive free radicals such as peroxynitrite (33), a common feature of DM (41). It is likely that exercise ameliorates oxidative stress in the whole body and the heart (e.g., reduction in nitrotyrosine, a biomarker for peroxynitrite activity) and coronary arteries (e.g., endothelium-dependent relaxation was not further improved by incubation with L-Arg + BH4 or SOD) in exercised db/db mice.

The incidence of ischemic heart disease is greater in diabetic patients (50, 52). Alterations in myogenic regulation of arterial diameter are likely to detrimentally affect regional myocardial blood flow. Since the pressure-constriction curves were similar in coronary arteries from control and diabetic mice, we speculate that a greater myogenic tone in coronary arteries of diabetic mice is unlikely to be the primary cause of cardiac ischemia in this model of type 2 DM (1). The markedly reduced ACh-induced, NO-mediated coronary vasodilation in db/db mice was related to a greater oxidative stress and reduced NO bioavailability in diabetes, likely leading to an imbalance between cardiac oxygen supply and demand during activity. Moreover, during ischemia-reperfusion during myocardial infarction in diabetes, poorer outcome is often predicted because of more extensive myocardial damage as a consequence of low antioxidant levels (19, 51). According to our study, a moderate level of exercise not only increases myocardial antioxidant levels but also increases NO bioavailability, thus leading to improved endothelium-dependent vasodilation and better perfusion of the diabetic heart.

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Exercise improves coronary dilation in diabetic mice


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