Exercise reduces oxidative stress but does not alleviate hyperinsulinemia or renal dopamine D1 receptor dysfunction in obese rats

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Muhammad AB, Lokhandwala MF, Banday AA. Exercise reduces oxidative stress but does not alleviate hyperinsulinemia or renal dopamine D1 receptor dysfunction in obese rats. Am J Physiol Renal Physiol 300: F98–F104, 2011. First published October 6, 2010; doi:10.1152/ajprenal.00386.2010.—Impairment of renal dopamine D1 receptor (D1R)-mediated natriuresis is associated with hypertension in humans and animal models, including obese Zucker rats. We have previously reported that treatment of these rats with antioxidants or insulin sensitizers reduced insulin levels and oxidative stress, restored D1R-mediated natriuresis, and reduced blood pressure. Furthermore, the redox-sensitive transcription factor, nuclear factor-κB (NF-κB), has been implicated in impairment of D1R-mediated natriuresis during oxidative stress. In this study, we investigated the effect of exercise on insulin levels, oxidative stress, nuclear translocation of NF-κB, blood pressure, albuminuria, and D1R-mediated natriuresis. The exercise protocol involved treadmill exercise from 3 wk of age for 8 wk. Exercise reduced oxidative stress, nuclear translocation of NF-κB, and albuminuria. However, exercise did not reduce plasma insulin levels or blood pressure. Also, selective D1R agonist (SKF-38393)-mediated increases in sodium excretion and guanosine 5’-O-(3-thiotriphosphate) binding were impaired in obese rats compared with lean rats, and exercise did not restore this defect. We conclude that, while exercise is beneficial in reducing oxidative stress and renal injury, reducing insulin levels may be required to restore D1R-mediated natriuresis in this model of obesity and metabolic syndrome. Furthermore, this study supports previous observations that restoring D1R function contributes to blood pressure reduction in this model.

An increasing number of studies, in several models including old Fischer344 rats (22) and Sprague Dawley (SD) rats treated with prooxidants (8), indicate a critical role of oxidative stress in the impairment of dopamine D1 receptor function. In these models, natriuresis and inhibition of Na⁺-K⁺-ATPase in response to dopamine D1 receptor agonists is reduced (8, 22). This has been attributed to D1 receptor uncoupling from G proteins and receptor downregulation (8, 22). Studies in both animal models and cell cultures have shown the involvement of the redox-sensitive transcription factor nuclear factor-κB (NF-κB) in D1 receptor-G protein uncoupling (7, 21). Also, in these studies, antioxidant treatment prevented the nuclear translocation of NF-κB and restored D1 receptor-G protein coupling, leading to restoration of D1 receptor-mediated inhibition of Na⁺-K⁺-ATPase and natriuresis (7, 21, 22).

Adult obese Zucker rats that are used as a genetic model to study obesity-associated hypertension exhibit hyperglycemia, hyperinsulinemia, and oxidative stress (1). Treatment of adult obese Zucker rats with both insulin sensitizers (rosiglitazone) and the antioxidant 4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl (tempol) decreased oxidative stress, improved insulin sensitivity, restored dopamine D1-receptor mediated natriuresis, and significantly reduced blood pressure (6, 9, 65–67).

Like antioxidants, exercise has been reported to reduce oxidative stress in part by augmenting physiological antioxidant mechanisms (14, 35). Asghar et al. (5) have shown that exercise augments antioxidant defenses, reduces oxidative stress, and restores renal dopamine D1 receptor function in old Fischer344 rats. However, it is not known if exercise can reduce oxidative stress, prevent impairment of dopamine D1 receptor function, and development of hypertension in obese Zucker rats. Therefore, the aim of this study was to investigate the influence of exercise on oxidative stress, insulin sensitivity, D1 receptor function, and development of hypertension in obese Zucker rats. Because obese Zucker rats express the obese phenotype only by 6–7 wk of age, we hypothesized that exercising obese Zucker rats from 3 to 4 wk of age will improve insulin sensitivity and abolish oxidative stress. These changes will prevent nuclear translocation of NF-κB, impairment of D1 receptor-mediated natriuresis, and increase in blood pressure in adult obese Zucker rats.

EXPERIMENTAL DESIGN AND METHODS

Animals and exercise protocol. The institutional animal care committee approved all protocols and procedures. Three-week-old homozygous Zucker Leprdb/db (obese Zucker) and Zucker Lepr+/+ (lean Zucker) rats were identified by genotyping performed by the vendor (Harlan Sprague Dawley, Indianapolis, IN) and were maintained with a 12:12-h light-dark cycle and provided free access to tap water and standard rat chow containing 0.4% sodium (Labdiet 5001; Purina Mills, St. Louis, MO). The rats were randomly divided into two groups each, an exercise group and a sedentary group. The lean

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exercised group was excluded from some studies specified below. The exercise group was subjected to treadmill exercise for 8 wk according to a previously reported protocol with some modifications (25). Briefly, they were exercised at 0° incline and a speed of 10 m/min for 1 h daily (5-min breaks every 15 min), 5 days/ wk. Body weights were recorded at the beginning of the study and weekly thereafter until the end of the study. The rats were placed in metabolic cages to measure food and water intake from the 5th to 8th wk of age. After 8 wk of exercise, the rats were rested for 48 h, before any further procedures were performed, to eliminate interference from acute effects of exercise.

Surgery and tissue collection. Surgical procedures and tissue collection were performed as described earlier (15). Briefly, rats were fasted overnight and anesthetized with Inactin (100–150 mg/kg ip). Tracheotomy was performed to facilitate breathing. The right carotid artery was catheterized, and blood pressure was recorded using a Statham P23AC pressure transducer connected to Grass Polyview (Astro-Med) following 30 min of stabilization after surgery. After measurement of blood pressure, bladder urine and blood samples were collected. Plasma was separated by centrifuging the blood at 1,500 g for 15 min. A midline abdominal incision was made, and the kidneys were perfused with Krebs buffer and enzyme solution and excised. Proximal tubules were prepared and tested for viability as described earlier (15). Other organs were cleared of fat and connective tissue and weighed after blotting out excess moisture. Tissue samples were stored at −80°C until analysis. Tissue samples analyzed using commercially available kits were treated before storage as directed by the kit manufacturers.

Blood glucose, lipid profile, and insulin sensitivity. Blood glucose and lipid profile were measured using a glucose analyzer (Roche Diagnostics) and Cardiocheck-PA (Polymer Technology Systems, Indianapolis, IN), respectively. Insulin was measured by a radioimmunoassay (RIA) using a sensitive rat insulin RIA kit (catalog no. SRI-13K; Linco Research, St. Charles, MO). Insulin sensitivity was assessed using the quantitative insulin sensitivity check index (QUICKI) (43).

QUICKI = 1/[\log (fasting insulin, μU/ml)]
× [log (fasting glucose, mg/dl)]

Indexes of oxidative stress and urinary albumin. 8-Isoprostanen was measured using an enzyme immunoassay (EIA) kit (catalog no. 516351; Cayman Chemical, Ann Arbor, MI). Malondialdehyde (MDA) was measured using the method of Mihara and Uchiyama (42). Glutathione was measured using a Bioxytech GSH/GSSG-412 kit (catalog no. 21040; Oxis international, Foster City, CA). Superoxide dismutase (SOD) activity was measured using a SOD colorimetric assay kit (catalog no. 706002; Cayman Chemical). Urinary albumin was measured by an EIA using a rat albumin EIA kit (catalog no. A05102; Cayman Chemical).

Since there were no differences in measures of oxidative stress between the lean sedentary and lean exercised groups, the lean exercised group was eliminated from the following studies.

Renal function studies. Renal function studies were performed as described in earlier reports from our laboratory (39). Briefly, after rats were prepared for measurement of blood pressure as described above, the left jugular vein and ureter were catheterized. Subsequently, saline (1% of body wt/h) was infused during a stabilization period (45 min) and two basal periods (30 min each) before the drug period (30 min) during which SKF-38393 (3 mg·kg⁻¹·min⁻¹) in 1% saline was infused. Following the drug period, saline (1% of body wt/h) was infused again during two recovery periods (30 min each). Urine and blood were collected during the basal, drug, and recovery periods and used for measurement of sodium and creatinine. Sodium was measured using a flame photometer (model 2655-10; Cole Parmer Instrument) and creatinine by a colorimetric assay using a creatinine assay kit (K625-100; BioVision).

Effect of exercise on general physiological parameters. Obese rats consumed significantly higher amounts of food and water when compared with lean rats (Fig. 1, A and B, respectively). Exercise did not alter the intake of either food or water in either lean or obese rats (Fig. 1, A and B, respectively). Obese rats displayed significantly higher body weights compared with lean rats from 6 wk of age until the end of the study (Fig. 1C). Exercise did not reduce body weights in either obese or lean rats (Fig. 1C). Obese rats displayed significantly higher blood pressure, fasting blood glucose levels, profound hyperinsulinemia (~9-fold), and decreased insulin sensitivity (QUICKI) when compared with lean rats (Table 1). Exercise had no effect on blood pressure, fasting blood glucose, insulin levels, or insulin sensitivity (QUICKI) in either lean or obese rats (Table 1).

Triglyceride levels were significantly higher (~6-fold) in obese rats compared with lean rats (Table 1). Exercise significantly reduced triglyceride levels in obese rats but not in lean rats (Table 1). Total cholesterol levels were similar in both obese sedentary and exercised groups (Table 1). Total cholesterol levels were undetectable, using the method employed, in lean rats. High-density lipoprotein (HDL) cholesterol was significantly higher in obese rats compared with lean rats (Table 1). Although exercise did not affect HDL cholesterol levels in lean rats, it paradoxically reduced HDL cholesterol in obese rats (Table 1). However, the levels still remained significantly higher compared with lean rats (Table 1). Obese rats compared with lean rats display a significantly higher fat-to-body weight ratio (Table 1). Exercise did not alter the ratio in lean rats but paradoxically increased the ratio in obese rats by ~15% (Table 1).

Effect of exercise on oxidative stress and urinary albumin excretion. Obese rats displayed increased levels of oxidative stress markers like urinary 8-isoprostanen and renal MDA (Table 2). Furthermore, the obese rats also showed decreased blood glutathione levels and decreased SOD activity in plasma and proximal tubular homogenates (Table 2). Exercise decreased levels of urinary 8-isoprostanen and renal MDA in...
that exercise improved several measures of oxidative stress and decreased urinary albumin excretion in obese Zucker rats.

Because exercise did not change any of the above parameters tested in lean rats, the lean exercised group was excluded from the following studies.

Effect of exercise on natriuretic response to selective dopamine D1 receptor agonist. Intravenous infusion of SKF-38393 (3 μg·kg body wt⁻¹·min⁻¹) increased urine flow, urinary sodium excretion (UNaV), and fractional excretion of sodium (FENa) in lean rats but not in obese rats (Fig. 2, A, B, and C, respectively). Exercise failed to restore the response in obese rats, since both UNaV and FENa did not increase in response to SKF-38393 infusion (Fig. 2, B and C, respectively). Glomerular filtration rate (GFR) was similar in all the groups, and SKF-38393 infusion did not alter GFR, blood pressure, or heart rate (data not shown).

Effect of exercise on SKF-38393-induced dopamine D1 receptor-G protein coupling. SKF-38393 (1-100 nM) induced a concentration-dependent increase in [³⁵S]GTPγS binding in proximal tubular membranes from lean rats (Fig. 3). However, it failed to elicit any significant [³⁵S]GTPγS binding in either the sedentary or exercised obese rats (Fig. 3). Thus, obese rats displayed defective dopamine D1 receptor-G protein coupling, and exercise failed to correct this defect in coupling. Basal [³⁵S]GTPγS binding was similar in all groups (lean sedentary, 229.0 ± 9.32 fmol/mg; obese sedentary, 259.1 ± 16.92 fmol/mg; obese exercised, 264.5 ± 10.48 fmol/mg).

Effect of exercise on nuclear translocation of NF-κB. Significantly higher levels of the redox-sensitive NF-κB (p65) were observed in proximal tubular nuclear extracts of obese rats compared with lean rats (Fig. 4). Exercise significantly decreased the nuclear content of NF-κB in obese rats (Fig. 4). The decrease in nuclear content was accompanied with a corresponding increase in the cytosolic content of NF-κB, indicating that exercise reduced the nuclear translocation of NF-κB in obese rats (Fig. 4).

DISCUSSION

Our results show that, in obese Zucker rats, the exercise protocol employed in this study reduces oxidative stress by augmenting antioxidant defenses and also reduces albuminuria. However, it fails to reduce blood glucose levels or improve insulin sensitivity, does not restore renal dopamine D1 receptor-mediated natriuresis or renal D1 receptor coupling to G proteins, and does not prevent the development of hypertension. This is despite the observation that the reduction in oxidative stress levels was enough to prevent the nuclear translocation of redox-sensitive transcription factor NF-κB that has been implicated in the impairment of the natriuretic response to D1 receptor agonists in several models of hypertension, diabetes, and aging (7, 8, 21, 22). This study provides evidence that reducing oxidative stress per se is not enough to restore renal D1 receptor function in this model. Reducing insulin and/or blood glucose levels may play an important role in restoring renal D1 receptor function. Furthermore, this study provides additional evidence that restoring D1 receptor function contributes to reducing blood pressure in obesity-associated hypertension.

The exercise protocol employed in our study did not reduce food intake or the gain in weight in obese rats. Although some studies have reported moderate reductions in weight gain in

Fig. 1. Effect of exercise on body weight (C), food intake (A), and water intake (B). Data are expressed as means ± SE of n = 8–10. *p < 0.05 vs. lean rats was considered significant using ANOVA followed by the Newman-Keuls multiple test.

obese rats but did not affect levels in lean rats (Table 2). Exercise did not cause any changes in glutathione levels in either lean or obese rats (Table 2). However, exercise augmented SOD activity in both plasma and proximal tubular homogenates of obese rats while the lean exercised group did not show any significant changes (Table 2).

Obese rats displayed ~20-fold higher urinary albumin excretion compared with lean rats (Table 2). Exercise decreased urinary albumin excretion by ~50% in obese rats but did not have any affect on lean rats (Table 2). These results indicate
female obese rats with swim training (70, 71), several other groups have reported results that are similar to our study (36, 41, 61). Nevertheless, even in studies that reported reductions in weight gain, exercise only delayed but did not prevent the development of obesity (70). Inability of exercise to reduce weight gain, in this model, might be related to lack of attenuation of leptin resistance and the associated hyperphagia and impaired thermogenesis. In a clinical trial involving children 8-14 yr of age, Reinehr et al. (56) have reported that plasma leptin concentrations were not affected by exercise (14-22 wk) has been shown to reduce systolic blood pressure in this patient subset (64). A longer duration of exercise (14-22 wk) has been shown to reduce systolic blood pressure in these rats using the tail cuff method (4, 54). It is possible that the shorter duration of exercise (8 wk) in our study precluded us from observing a decrease in blood pressure. However, stress due to the restraining procedure involved in the tail cuff method can limit the interpretation of data by providing false high values of blood pressure (69). Because exercised rats are more accustomed to handling and restraining in a narrow space compared with sedentary rats, it is possible that they displayed lesser stress during measurement of blood pressure. This might have been responsible for the apparent lower blood pressures in both the lean and obese exercised groups. Xiang et al. (74) have reported a decrease in insulin levels with exercise. However, a number of other studies have reported no reductions in plasma insulin levels with exercise in obese rats (11, 14, 19, 33, 35, 63) as well as humans (64). Although some studies have reported that insulin resistance in this model is refractory to exercise (41), others have reported improvements (36, 71). Furthermore, even in studies reporting an improvement in insulin sensitivity, there were no reductions in fasting plasma insulin concentrations (18, 36, 71). While our study involved 8 wk of exercise at 10 m/min, Xiang et al. (74) exercised the rats for 4-5 wk at 24 m/min. However, the difference in intensity of exercise may not be responsible for the difference in the effect on insulin levels. Our results are in agreement with other authors who, using intensities ranging 20-26 m/min for >5 wk, have reported no reductions in insulin levels with exercise (11, 18, 19, 33).

Previously, we have reported that anti-oxidant treatment of Fisher344 rats (22), obese rats (9, 40,) and pro-oxidant-treated SD rats (8) reduced oxidative stress and restored dopamine D1 receptor function. Furthermore, exercise has been reported to augment physiological antioxidant mechanisms and improve renal dopamine D1 receptor function in old Fisher344 rats (5, 25). A critical role of NF-κB nuclear translocation in impairment of dopamine D1 receptor function and its restoration following anti-oxidant treatment has been demonstrated in primary cultures (21) and pro-oxidant-treated SD rats (8). In the present study, exercise reduced oxidative stress in obese rats as evidenced by an increase in SOD activity in both plasma and proximal tubular homogenates and reductions in levels of the oxidative stress markers, urinary 8-isoprostane and renal MDA. These reductions in oxidative stress were enough to prevent the nuclear translocation of redox-sensitive transcription factor NF-κB that has been implicated in the development of renal dopamine D1 receptor dysfunction. However, we did not observe an improvement in dopamine D1 receptor-mediated sodium excretion and GTPγS binding or reduction of blood pressure in this study.

Table 2. Effect of exercise on oxidative stress

<table>
<thead>
<tr>
<th></th>
<th>Lean Sedentary</th>
<th>Obese Sedentary</th>
<th>Lean Exercised</th>
<th>Obese Exercised</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary 8-isoprostan</td>
<td>0.84 ± 0.10</td>
<td>1.20 ± 0.12</td>
<td>0.003*</td>
<td>0.002#</td>
</tr>
<tr>
<td>Malondialdehyde, nmol/mg protein</td>
<td>2.41 ± 0.42</td>
<td>3.30 ± 0.17*</td>
<td>2.26 ± 0.37</td>
<td>2.63 ± 0.44#</td>
</tr>
<tr>
<td>Total glutathione, µM</td>
<td>653 ± 24.68</td>
<td>542.2 ± 20.02*</td>
<td>639.5 ± 19.14</td>
<td>550.6 ± 27.13*</td>
</tr>
<tr>
<td>Plasma SOD activity, U/mg</td>
<td>20.84 ± 0.60</td>
<td>7.71 ± 1.13*</td>
<td>18.3 ± 1.04</td>
<td>13.85 ± 1.28#</td>
</tr>
<tr>
<td>Proximal tubular homogenate SOD activity, U/mg protein</td>
<td>55.29 ± 6.20</td>
<td>39.51 ± 2.61*</td>
<td>44.04 ± 4.15</td>
<td>49.53 ± 3.319#</td>
</tr>
<tr>
<td>Urinary albumin, µg/mg creatinine</td>
<td>310.1 ± 202</td>
<td>635 ± 2685*</td>
<td>174.6 ± 48.25</td>
<td>2911 ± 593.4*</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE of n = 8–10. SOD, superoxide dismutase. P < 0.05 vs. lean rats (*) and vs. obese sedentary (#) was considered significant using ANOVA followed by Newman-Keuls multiple test.
Exercise reduced urinary albumin excretion in our study. In contrast, Boor et al. (11) reported an increase in urinary protein levels with exercise. In this particular study, the rats were placed in metabolic cages right after the last exercise session to collect urine used for measuring protein. Boor et al. acknowledge that this is a drawback of their study design, since it does not eliminate the acute effects of exercise. In addition, the authors report a decrease in number of glomeruli exhibiting protein droplets in their histopathological studies, and this might actually indicate an improvement in terms of reducing proteinuria. In our study, we collected urine from the bladder 48 h after the last bout of exercise to eliminate the acute effect of exercise on proteinuria. Furthermore, as pointed out by Boor et al., a number of other studies support our observation that exercise reduces proteinuria (5, 17, 72).

Also, obese rats display higher HDL cholesterol levels, and exercise paradoxically decreased HDL cholesterol levels in these rats. Higher HDL levels have normally been associated with lower low-density lipoprotein (LDL) oxidation and better anti-inflammatory and anti-atherogenic profile (26). However, recent research suggests that HDL levels per se might not correlate with these beneficial effects (2, 12). Conditions like hyperglycemia, oxidative stress, and chronic acute phase response seen in obesity and diabetes (both type I and II) can alter HDL composition and structure and actually promote LDL oxidation and inflammation (29, 45–48, 51–53, 68). In addition, our results are similar to those of Roberts et al. (57), who reported that aerobic exercise in humans exhibiting metabolic syndrome actually decreases HDL levels while improving its anti-inflammatory properties. Although exercise signif-

![Fig. 2. Effect of exercise on urine flow (A), urinary sodium excretion ($U_{\text{Na}}V$), (B), and fractional excretion of sodium ($\text{FENa}$) (C). Data are expressed as means ± SE of $n = 5–6$. $P < 0.05$ vs. basal (*) and vs. SKF-38393 ($) was considered significant using ANOVA followed by the Newman-Keuls multiple test.](http://ajprenal.physiology.org/)

![Fig. 3. Effect of exercise on membrane $[^{35}S] \text{Guanosine 5\prime-O-(3-thiotriphosphate)} (\text{GTP}\gamma\text{S})$ binding in response to SKF-38393. Data are expressed as means ± SE of $n = 5$. *$P < 0.05$ vs. obese rats was considered significant using ANOVA followed by the Newman-Keuls multiple test.](http://ajprenal.physiology.org/)

![Fig. 4. Effect of exercise on nuclear translocation of nuclear factor-κB (NF-κB) (p65). Bars represent cytosolic and nuclear NF-κB levels as a percentage of cytosolic and nuclear NF-κB levels, respectively, in lean rats. Data are expressed as means ± SE of $n = 4$. *$P < 0.05$ vs. lean (*) and vs. obese sedentary (#) was considered significant using ANOVA followed by the Newman-Keuls multiple test.](http://ajprenal.physiology.org/)
icantly reduced triglyceride levels in obese rats, it failed to decrease blood cholesterol levels in our study. A similar effect of exercise on blood cholesterol levels has been reported in type II diabetic patients (64). Boor et al. (11) did not observe any effect on triglyceride levels with 10 wk of exercise in obese Zucker rats. The reason for differences between our study and the above study is not clear. However, we suspect that this might be due to the overnight fasting protocol employed in our study. Boor et al. do not mention if the rats were fasted overnight, and the triglyceride levels in their study might reflect postprandial/-random levels. In addition, our results are similar to those of Frisbee et al. (24) who, using 10 wk of exercise and an overnight fasting period, observed a similar decrease in triglyceride levels.

In conclusion, this study offers evidence that exercise is beneficial in reducing oxidative stress, nuclear translocation of NF-κB, and renal injury in obese Zucker rats. However, the reductions in oxidative stress are not enough to prevent renal dopamine D1 receptor dysfunction and development of hypertension. In addition, considering previous studies, we predict that reducing insulin levels may be an important prerequisite to prevent renal dopamine D1 receptor dysfunction that contributes to development of hypertension in this model.

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DISCLOSURES

The authors do not have any financial interests to disclose.

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