

Exercise improves high fat diet-impaired vascular function

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Abstract. The prevalence of metabolic syndrome and cardiovascular disease is increasing due to increases in the consumption of high fat diets (HFDs) and the epidemic of obesity. In the present study, it was hypothesized that swimming exercise may prevent HFD-induced impairment of aortic function and that these changes are associated with reduction of oxidative stress, proinflammatory adipokines/cytokines. Male, 6-week-old C57BL/6J mice were fed a 60% lipid composition HFD with or without swimming exercise (90 min/swim and 2 swims/day) for 16 weeks. Exercise training prevented HFD-induced increases in visceral fat weight, total cholesterol and triglycerides. Furthermore, exercise training improved HFD-impaired aortic endothelium-dependent dilation that was associated with reduction of oxidative stress, leptin, resistin, monocyte chemoattractant protein 1, interleukin (IL)6 and IL8. In addition, exercise inhibited HFD-induced vascular endothelial growth factor expression in gastrocnemius skeletal muscle. These data demonstrate that swimming exercise prevents aortic tissue oxidative stress, inflammation and vascular dysfunction in HFD-induced obesity.

Introduction

The rising prevalence of obesity is associated with an increase in the consumption of high fat diets (HFDs), which contributes to early structural alterations and increases the risk for cardiovascular disease (CVD)-associated morbidity and mortality (1,2). Thus, obesity-induced vascular impairment is markedly associated with damage to target organs, including the heart, kidney, liver and brain (3). For example, aortic relaxation dysfunction, a type of vascular dysfunction,

decreases diastolic pressure and increases systolic pressure, which increases cardiac afterload, left ventricular mass and cardiac oxygen demand (4). In addition, the decrease in diastolic pressure contributes to reduction of coronary blood flow in the period of cardiac diastole (4). Factors, such as increased reactive oxygen species (ROS), release of proinflammatory adipokines/cytokines, and tissue insulin resistance are involved in the abnormal vascular pathophysiological changes and associated CVD (5). The combination of abnormal eating habits and a sedentary lifestyle increase the risk factors of obesity and CVD (6). A previous study has demonstrated that exercise improves vascular function by increasing the vascular mitochondrial respiratory capacity, bioavailable nitric oxide (NO), and redox balance in a sedentary rodent model (7). While prolonged sitting in humans prompts leg endothelial dysfunction, fidgeting improves leg endothelial and vascular function through the intermittent increases in vascular shear stress (8). Thus, the underlying mechanisms and interactions between obesity and exercise in the regulation of vascular function involve a complicated network of interacting factors that require investigation. Therefore, the present study hypothesized that HFD-induced vascular dysfunction is mediated by dyslipidemia, excessive ROS, increased expression levels of proinflammatory adipokines and maladaptive immune responses, and these abnormalities are prevented by swimming exercise. An improved understanding of the underlying mechanisms of vascular function obesity and exercise will be of great clinical significance.

Materials and methods

Animals. A total of 21 male, 6-week-old C57BL/6J mice were purchased from the Beijing HFK Bioscience Co., Ltd. (Beijing, China). For the obese and exercise studies, C57BL/6J male mice were fed a 60% lipid composition HFD with or without swimming exercise (90 min/swim and 2 swims/day) for 16 weeks. The control group mice were age-matched, male mice and were fed regular mouse chow during the same period of time. The mice were ~18 g and individually housed on a 12-h light/dark cycle (6 a.m., lights on and 6 p.m., lights off). The laboratory temperature was 24°C and the humidity was 20.5±3.0%. All of the animal studies and procedures were performed in accordance with the Animal Use and Care Committee at the Xianning Central Hospital and The

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First Clinical Hospital of Hubei University of Science and Technology (Xianning, China). Following completion of the experiments, the mouse aortae were isolated to investigate the vascular activity and the left aortae were maintained in a freezer at -80°C .

Plasma lipid profile assays. During blood sample collection, mice underwent terminal anesthesia, 0.5 ml was collected through cardiac puncture. After the blood was prepared in the heparin-coated tubes, plasma was collected by centrifugation at $604 \times g$ for 15 min. The plasma levels of triglycerides (TG), total cholesterol (TC) and high-density lipoprotein (HDL) were examined using a plasma lipid profile kit (cat. no. MAK043; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) according to manufacturer's instructions. Serum leptin, resistin and adiponectin levels were measured using a mouse serum adipokine milliplex kit (cat. no. MADKMAG-71K; Shanghai Biotechnology Co., Ltd., Shanghai, China) according to manufacturer's instructions.

Aortic oxidative stress. The formation of ROS in the aortae was evaluated by chemiluminescence. Aortae were homogenized in a glass homogenizer with sucrose buffer (pH 7.5 protease inhibitor tablet, 0.5 mM EDTA, 50 mM HEPES and 250 mM sucrose) (9). The homogenates were centrifuged at $1,500 \times g$ for 10 min at 4°C . Supernatants in the whole homogenate were removed and added to 1.4 ml 50-mM phosphate (KH_2PO_4) buffer (100 μM NADPH, 1 mM EGTA, 5 μM lucigenin and 150 mM sucrose) for incubation at room temperature for 1 h in dark-adapt counting vials (9). Following dark adaptation, samples were placed in a scintillation counter to count every 30 sec for 10 min using a spectrophotometer (wavelength, 450 nm) and normalized to total protein in the whole homogenate. The ROS values were taken as counts per min per milligram (cpm/mg) of protein (9).

Analysis of vascular function. The thoracic aorta was dissected and sliced in 2 mm long aortic rings that were suspended in 95% O_2 /5% CO_2 aerated organ chambers filled with modified Krebs-Ringer bicarbonate solution (118 mM NaCl, 1.2 mM MgCl_2 , 4.7 mM KCl, 11.2 mM NaH_2PO_4 , 2.5 mM CaCl_2 , 1.2 mM Na_2SO_4 , 25 mM NaHCO_3 , 10 mM glucose; pH 7.4; Sigma-Aldrich; Merck KGaA) (10). Aortic contractile state was ascertained by KCl (80 mM). Rings were pre-constricted with norepinephrine and relaxations to acetylcholine (ACh, 10^{-9} - 10^{-4} M) or sodium nitroprusside (SNP; 10^{-9} - 10^{-4} M; Sigma-Aldrich; Merck KGaA) were obtained in a cumulative fashion.

Western blot analysis. After the mice were under deep anesthesia with sodium pentobarbital (50 mg/kg) intraperitoneal injection, a scalpel cut through the skin all around and just above the ankle, the gastrocnemius muscles were removed from mouse hind limbs and lysed with protein lysis buffer (Sigma-Aldrich; Merck KGaA). After the protein concentration of the lysate was determined by Bio-Rad protein assay (Bio-Rad Laboratories Ltd., Shanghai, China). The proteins (20 μg) were separated on 10% SDS-PAGE gels and transferred onto nitrocellulose membranes at 90 V for 90 min (Sigma-Aldrich; Merck KGaA). Membranes were incubated overnight at 4°C

Table I. Effects of exercise on characteristics of mice fed with a HFD.

Parameter	Control	HFD	HFD + exercise
Body weight (g)	4.12 \pm 0.28	11.34 \pm 1.08 ^a	9.58 \pm 1.66 ^a
Visceral fat weight (g)	0.49 \pm 0.08	1.71 \pm 0.31 ^a	1.08 \pm 0.11 ^{a,b}
Total cholesterol (mg/dl)	99.34 \pm 8.32	148.23 \pm 6.77 ^a	120.08 \pm 7.11 ^{a,b}
Triglycerides (mg/dl)	70.11 \pm 5.28	97.13 \pm 6.25 ^a	80.71 \pm 4.01 ^{a,b}
High-density lipoprotein (mg/dl)	63.65 \pm 8.63 (4)	62.81 \pm 7.56	64.22 \pm 7.93

Values are presented as means \pm standard error of the mean (n=7).

^aP<0.05 vs. control; ^bP<0.05 vs. HFD group. HFD, high fat diet.

with blocking solution containing antibodies targeting vascular endothelial growth factor (VEGF; cat. no. 07-1420, 1:1,000 dilution; Sigma-Aldrich; Merck KGaA). The membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (cat. no. A9542, 1:5,000 dilution; Sigma-Aldrich; Merck KGaA) at room temperature for 1 h. The HRP activity was detected using an enhanced chemiluminescence kit (cat. no. 1705060; Bio-Rad Laboratories Ltd.). GAPDH was run in parallel and served as a loading control.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The genes associated with expression of pro-inflammatory cytokines were examined by (RT-qPCR) (11). Total RNA was isolated from the aortae using RNAzol (Sigma-Aldrich; Merck KGaA) and cDNA synthesis was completed by using 1 μg total RNA with 5X reaction buffer, oligo(dT) (1 μg), RNase inhibitor, MgCl_2 , dNTP mix, and ImProm II reverse transcriptase as per the ImProm II reverse transcription kit (cat. no. 11939823001; Sigma-Aldrich; Merck KGaA). Following first strand cDNA synthesis, qPCR was performed using 8 μl cDNA, 10 μl SYBR-Green PCR master mix (Sigma-Aldrich; Merck KGaA) and forward and reverse primers (10 pM/ μl ; Sigma-Aldrich; Merck KGaA) using a real-time PCR system (CFX96; Bio-Rad Laboratories, Inc., Hercules, CA, USA). The following primer sequences were used: Forward, 5'-GCGATAGTTAATGCAACACT-3' and reverse, 5'-TTGGTTACCGGGTGCAGCAC-3' for monocyte chemoattractant protein 1 (MCP1); forward, 5'-ACACAGTGTGCTCACGCATGA-3' and reverse, 5'-GCAAAGTTATATGCCGACAGC-3' for interleukin (IL)6; forward, 5'-CGGAATGGGGTTCATTTGT-3' and reverse, 5'-ACTTGCAGTCCGATGCAAGC-3' for IL8; forward, 5'-GGTAATTAACGCCAAGTCTGA-3' and reverse, 5'-TGGTCAGCCTAGCTGAGGA-3' for 18S. The PCR cycling conditions for the gene expression were as follows: 5 min at 95°C for initial denaturation, 39 cycles of 30 sec at 95°C , 30 sec at 58°C and 30 sec at 72°C . The qPCR data were obtained from five different samples in triplicate. Calculations of relative normalized gene expression were performed according to the ΔCt method as

Table II. Exercise improved aortic relaxation (%) in mice fed with a HFD.

Dose (mol)	Control		HFD		HFD + exercise	
	Ach	SNP	Ach	SNP	Ach	SNP
1x10 ⁻⁹	0.78±0.53	0.45±0.35	5.93±5.16	3.52±1.97	2.49±1.26	0.42±0.21
3x10 ⁻⁹	3.29±1.76		6.72±4.91		7.13±2.18	
1x10 ⁻⁸	9.36±3.33	3.39±1.74	10.73±4.87	9.36±3.68	11.72±2.59	7.48±1.69
3x10 ⁻⁸	20.67±5.03		12.61±4.63		15.99±3.40	
1x10 ⁻⁷	36.51±4.22	46.92±6.91	20.27±7.41	38.39±5.38	34.83±3.95	33.31±5.03
3x10 ⁻⁷	41.16±5.71		32.61±6.46		34.14±4.92	
1x10 ⁻⁶	50.45±5.45	66.06±8.64	44.18±6.30	65.91±3.45	49.84±5.82	62.07±5.14
3x10 ⁻⁶	62.67±6.66		49.59±5.28		53.10±6.83	
1x10 ⁻⁵	67.80±4.90	83.44±8.50	50.70±3.05 ^a	79.67±3.64	60.17±4.06 ^b	78.81±4.23
3x10 ⁻⁵	69.52±4.73		51.55±2.83 ^a		65.84±3.55 ^b	
1x10 ⁻⁴	71.85±4.36	85.18±8.00	58.53±2.20 ^a	83.21±3.72	66.10±4.49	80.28±4.36

Values are presented as means ± standard error (n=7). ^aP<0.05 vs. CD; ^bP<0.05 vs. HFD group. HFD, high fat diet; Ach, acetylcholine; SNP, sodium nitroprusside.

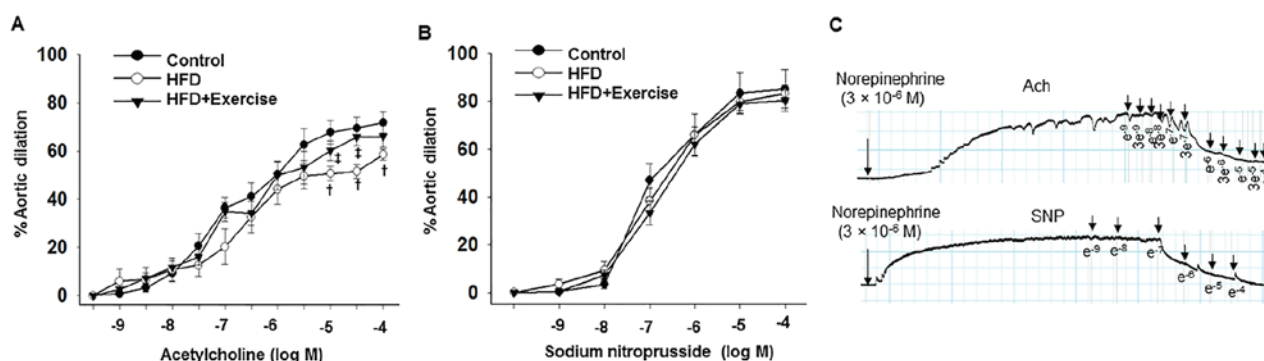


Figure 1. Exercise improves aortic function in mice fed a HFD. Vasodilator responses of isolated aortic rings to (A) Ach in an endothelium-dependent manner and to (B) SNP in an endothelium independent manner. (C) Representative figures of the aortic activity induced by Ach and SNP (n=7) per group. [†]P<0.05 vs. control; [‡]P<0.05 vs. HFD. HFD, high fat diet; Ach, acetylcholine; SNP, sodium nitroprusside.

described before (7). The data were normalized to house-keeping gene, 18S ribosomal RNA.

Statistical analysis. All data are reported as the mean ± standard error. Statistical analysis was performed using one-way analysis of variance (ANOVA) and analyzed variance using the IBM software SPSS v22.0 (IBM Corp., Armonk, NY, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

Effect of HFD and exercise on body weight, visceral fat weight and plasma lipid profiles. As hypothesized, HFD induced a 2.75-fold increase in body weight, an ~3.5-fold increase in visceral fat body weight compared with the control C57BL/6J mice that were fed regular mouse chow (P<0.05; Table I). The levels of plasma TC and TG were higher in the HFD group than those mice fed with regular mouse chow. However, swimming exercise prevented HFD-induced increases in visceral fat weight, plasma TC and TG levels (Table I). Notably, no significant difference in

body weight was noted between HFD alone and HFD with exercise. In addition, no significant difference in HDL level was observed between the three groups of mice.

Exercise prevented HFD-induced impairment of vascular function. Aortic endothelium-dependent dilation responses to acetylcholine were decreased in the HFD mice compared with the control C57BL/6J mice fed with regular mouse chow, and these abnormalities were prevented in the HFD with swimming exercise group (Fig. 1A and Table II). However, no differences in sodium nitroprusside-induced aortic endothelium-independent dilation responses were observed between the three groups (Fig. 1B and Table II), indicating that exercise prevented HFD-induced impairment of the aortic endothelium-dependent relaxation function.

Exercise prevented HFD-induced oxidative stress and proinflammatory adipokine expression. HFD induced increases in oxidant stress, assessed by ROS assay in the aortic tissues compared with the control C57BL/6J mice fed with regular mouse chow (Fig. 2A). In addition, HFD increased the

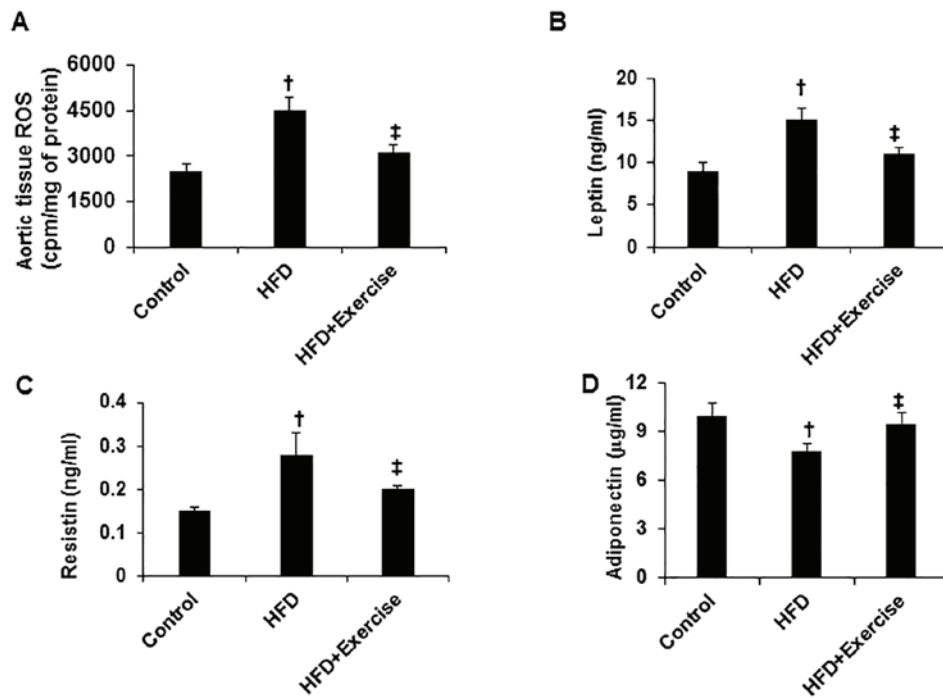


Figure 2. Exercise decreases HFD-induced aortic oxidative stress and proinflammatory adipokines. HFD induced increases in oxidant stress, assessed by (A) ROS assay in the aortic tissue samples compared with the control group. HFD also increased the proinflammatory adipokine expression in (B) leptin and (C) resistin and (D) inhibited the anti-inflammatory adipokine expression in adiponectin. However, swimming exercise significantly prevented the HFD-induced abnormalities (n=7/group). [†]P<0.01 vs. control; [‡]P<0.01 vs. HFD. HFD, high fat diet.

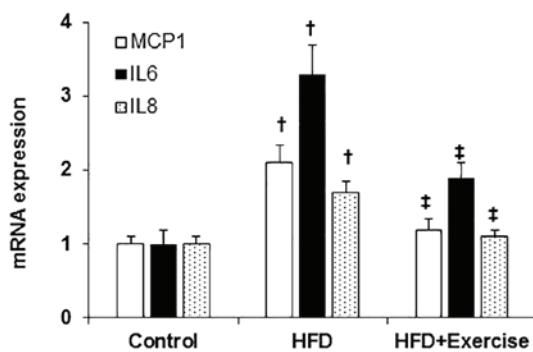


Figure 3. Exercise inhibits HFD-induced expression of MCP1, IL6 and IL8. Expression levels of the pro-inflammatory transcripts in MCP1, IL6 and IL8 were elevated in aortic extracts of HFD when compared with those of the control group. However, swimming exercise significantly decreased HFD-induced expression of MCP1, IL6, and IL8 in aortae (n=7/group). [†]P<0.01 vs. control; [‡]P<0.05 vs. HFD. MCP1, monocyte chemoattractant protein 1; IL, interleukin; HFD, high fat diet.

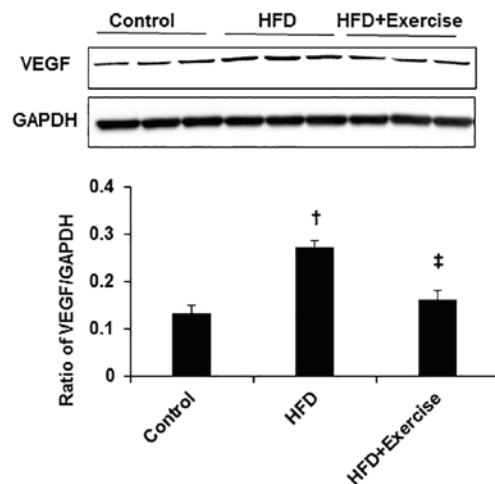


Figure 4. Exercise inhibits HFD-induced VEGF expression in skeletal muscle (n=3/group). [†]P<0.01 vs. control; [‡]P<0.01 vs. HFD. HFD, high fat diet; VEGF, vascular endothelial growth factor.

proinflammatory adipokine expression of leptin and resistin, and inhibited the anti-inflammatory adipokine expression of adiponectin (Fig. 2B-D). However, swimming exercise significantly prevented these HFD-induced abnormalities (Fig. 2).

Exercise prevented HFD-induced proinflammatory response. The levels of the pro-inflammatory transcripts, MCP1, IL6 and IL8 were elevated in the aortic samples from HFD mice compared with those of the control mice fed with regular mouse chow (Fig. 3). However, swimming exercise significantly decreased the HFD-induced aortic proinflammatory

response demonstrated by decreased expression levels of MCP1, IL6 and IL8 (Fig. 3).

Exercise inhibited HFD-induced angiogenesis in skeletal muscle. To reveal the effect of HFD on angiogenesis, the protein expression level of angiogenic factor, VEGF was measured. The protein expression level of VEGF was greater in the skeletal muscles of HFD mice compared with the control mice (Fig. 4). However, swimming exercise significantly decreased HFD-induced VEGF expression levels in the gastrocnemius skeletal muscle samples (Fig. 4).

Discussion

The primary finding in the current study is that was that consumption of a HFD for 16 weeks induced increases in plasma dyslipidemia, oxidative stress, proinflammatory adipokines/cytokines, as well as skeletal muscle angiogenesis. These pathophysiological changes contributed to impairment of aortic endothelium-dependent dilation. Furthermore, swimming exercise prevented HFD-increased dyslipidemia, ROS, proinflammatory adipokines/cytokines, skeletal muscle VEGF expression and associated aortic dysfunction. The current findings are of considerable translational importance, as obesity and sedentary lifestyles are strongly associated with vascular dysfunction and CVD.

Dyslipidemia, one of the pathophysiological characteristics in obese patients, includes high levels of plasma TC and TG, low levels of HDL, and is key in the development of CVD in obese patients (12). For example, in a large series of 26,000 overweight children, 14.1% of patients had abnormal TC levels, 15.8% of patients had abnormal LDL-C, 11.1% of patients had abnormal HDL-C and 14.3% of patients had abnormal TG levels (13). Consistent with these data, the present study demonstrated that HFD induced increases in body weight and visceral fat body weight using abnormal lipid profile assays. The coexistence of these pathophysiological changes markedly aggravated the lipid accumulation and impaired aortic endothelium-dependent dilation in the present study. Thus, acetylcholine may induce aortic dilation via NO production and bioavailability, and sodium nitroprusside may induce vascular smooth muscle cell relaxation via NO/cyclic guanosine monophosphate/protein kinase G signaling pathways (14,15).

Increased oxidative stress is one of the underlying mechanisms in obesity-induced CVD (16). The Framingham study demonstrated that there was an increase in urinary levels of 8-epi-prostaglandin F_{2α}, which is a systemic oxidative stress marker and was significantly linked to body mass index in 2,828 obese individuals (17). ROS are produced during mitochondrial respiration, as well as from outside the mitochondria, via free fatty acid β -oxidation, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase, lipoxygenase and cyclooxygenases (18,19). As anticipated, HFD increased the level of aortic ROS, serum leptin and resistin, which further prompted aortic tissue maladaptive immune response and release of proinflammatory cytokines, including MCP1, IL6 and IL8. It has been demonstrated that HFD and high-fructose beverages increased body weight, leptin, inflammation and insulin resistance that are associated with steatosis, and oxidative stress in plasma and tissues, indicating that oxidative stress and inflammation are important contributors to the pathogenesis of CVD (20,21).

Recent studies have shown that physical activity decreases the risk of chronic diseases, and improves quality of life, as well as increasing life expectancy (22,23). For example, exercise increases atheroprotection by inhibition of oxidative stress and inflammation through two distinct signaling pathways (24). Exercise increases laminar shear stress activation to decrease ROS activity and to preserve endothelial NO bioavailability (25). Furthermore, exercise increases expression levels of anti-inflammatory cytokines in adipose tissue (25). One study indicated that exercise

improves mitochondrial performance, and thus decreases ROS production and maladaptive immune response (26). Consistent with these studies, the present study further identified that exercise increased anti-inflammatory adipokine adiponectin expression levels and inhibited proinflammatory adipokine expression levels of leptin and resistin. Therefore, exercise or physical activity exerts protective effects in the pathologic conditions of over-nutrition and obesity, as well as other CVDs.

In the present study, HFD increased VEGF expression levels in skeletal muscle, which is an important marker for angiogenesis, indicating that HFD prompts mouse obesity and endothelial function impairment that further induce VEGF expression and angiogenesis with a feed-back mechanism. Other factors, such as VEGFs, PDGFs, angiopoietins and fibroblast growth factors are involved in regulating angiogenesis (27).

In conclusion, these data demonstrate that exercise prevented HFD-induced impairment of aortic dysfunction, and these protective effects were accompanied by a reduction in ROS and expression of inflammatory cytokines. The link between increased ROS, maladaptive immune responses, and aortic dysfunction offers the potential for identifying the origins of the impairment of vascular function and contributes to further development of novel therapeutic strategies.

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