

# Aerobic interval training vs. continuous moderate exercise in the metabolic syndrome of rats artificially selected for low aerobic capacity

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**Aims** The recent development of a rat model that closely resembles the metabolic syndrome allows to study the mechanisms of amelioration of the syndrome by exercise training. Here, we compared the effectiveness for reducing cardiovascular risk factors by exercise training programmes of different exercise intensities.

**Methods and results** Metabolic syndrome rats were subjected to either continuous moderate-intensity exercise (CME) or high-intensity aerobic interval training (AIT). AIT was more effective than CME at reducing cardiovascular disease risk factors linked to the metabolic syndrome. Thus, AIT produced a larger stimulus than CME for increasing maximal oxygen uptake ( $VO_{2max}$ ; 45 vs. 10%,  $P < 0.01$ ), reducing hypertension (20 vs. 6 mmHg,  $P < 0.01$ ), HDL cholesterol (25 vs. 0%,  $P < 0.05$ ), and beneficially altering metabolism in fat, liver, and skeletal muscle tissues. Moreover, AIT had a greater beneficial effect than CME on sensitivity of aorta ring segments to acetylcholine (2.7- vs. 2.0-fold,  $P < 0.01$ ), partly because of intensity-dependent effects on expression levels of nitric oxide synthase and the density of caveolae, and a greater effect than CME on the skeletal muscle  $Ca^{2+}$  handling (50 vs. 0%,  $P < 0.05$ ). The two exercise training programmes, however, were equally effective at reducing body weight and fat content.

**Conclusion** High-intensity exercise training was more beneficial than moderate-intensity exercise training for reducing cardiovascular risk in rats with the metabolic syndrome. This was linked to more superior effects on  $VO_{2max}$ , endothelial function, blood pressure, and metabolic parameters in several tissues. These results demonstrate that exercise training reduces the impact of the metabolic syndrome and that the magnitude of the effect depends on exercise intensity.

## 1. Introduction

The metabolic syndrome is a multigenic disorder that encompasses abnormalities such as visceral obesity, insulin resistance, hypertension, dyslipidaemia, and impaired glycaemic control. It has been linked to premature deaths in

cardiovascular disease patients, after other cardiovascular risk factors have been adjusted for.<sup>1</sup> With at least 1.1 billion people or close to 20% of the world's population being overweight, the incidence of the metabolic syndrome is expected to continue to rise.<sup>2</sup> This warrants a thorough mechanistic understanding of the phenomenon to enable optimal treatment at a socioeconomically affordable scale.

Recent data suggest that the presence of cardiovascular risk factors that constitute the metabolic syndrome is linked to the levels of aerobic fitness and endothelial function.<sup>3</sup>

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This notion is further accentuated by the reports, showing that both aerobic fitness and endothelial function are strong and independent predictors of mortality, more so than other established risk factors.<sup>4,5</sup> Although a direct cause-effect relationship has not yet been proven, these observations suggest that impaired aerobic capacity may be a common factor leading to cardiovascular and metabolic disease. It has been established that exercise training partly reverses the metabolic syndrome,<sup>6</sup> but controversy reigns as to the level and format of exercise that would yield optimal beneficial effects. The lack of a mechanistic understanding of the benefit of exercise in individuals with the metabolic syndrome contributes to this controversy. This also halts the development of new and improved options to prevent and treat these conditions. At least partly, the reason for the incomplete understanding is that tissue samples from important organs such as the liver, fat, and aorta are not readily available from humans and that appropriate animal models for studying the metabolic syndrome have been missing.

To address the cardiovascular, cellular, and molecular effects of exercise training with an intensity corresponding to the upper range of current guidelines in humans,<sup>7</sup> we designed a study that aimed to compare the adaptations of two closely supervised, but distinct training programmes: aerobic interval training (AIT) at a high aerobic intensity and continuous moderate exercise (CME) in a unique model of rats with the metabolic syndrome produced after artificial selection for low aerobic capacity over 15 generations.<sup>8</sup> The rats, low capacity runners (LCRs), score high on cardiovascular risk factors that constitute the metabolic syndrome.<sup>3</sup> This model allowed the metabolic syndrome to evolve in a similar fashion as the development of metabolic syndrome in humans, permitting for a complex, multigenic nature of the syndrome. We show that AIT at a high intensity, 85–90% of maximal oxygen uptake ( $VO_{2max}$ ), was more efficient at ameliorating the metabolic syndrome, increasing aerobic fitness, reducing hypertension, improving artery endothelial function, and improving skeletal muscle function, compared with CME at 70% of  $VO_{2max}$ . Thus, we propose a mechanistic link between exercise training and ameliorated metabolic syndrome that is susceptible to exercise intensity.

## 2. Methods

### 2.1 Animal model

In 1996, Koch and Britton<sup>8</sup> started large-scale artificial selective breeding to develop rats that have low intrinsic aerobic treadmill running capacity (LCR). This model has over the subsequent generations developed a phenotype that closely resembles the metabolic syndrome, as these rats score high on cardiovascular risk factors such as obesity, insulin resistance, hypertension, dyslipidaemia, impaired glycaemic control, and reduced cardiac and vascular function.<sup>3</sup> A total of 24 male LCR rats from generation 15 were studied, all 3 months of age at the start of the study. The rats were randomized to either AIT ( $n = 8$ ), CME ( $n = 8$ ), or a sedentary control group (SED,  $n = 8$ ). Animals were maintained as described previously.<sup>3</sup> The investigation conforms to 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 was approved by the Norwegian Council for Animal Research.

### 2.2 Testing of maximal oxygen uptake ( $VO_{2max}$ )

$VO_{2max}$  was measured with rats running uphill (25° inclination) by a treadmill ramp test protocol as previously described in detail.<sup>9</sup>

### 2.3 Training protocols

AIT was performed for 1 h/day, 5 days/week until  $VO_{2max}$  did not increase anymore for 3 consecutive weeks; 8 weeks in total. Uphill (25° inclination) treadmill training was performed, as described previously.<sup>9</sup> Both groups performed a 10 min warm-up at 50–60% of  $VO_{2max}$ , where after AIT ran interval running sessions for 1 h, consisting of successive 4 min periods at 85–90% of  $VO_{2max}$ , interspersed by 3 min recovery periods at 70% of  $VO_{2max}$ , whereas the CME group ran continuously at 70% of  $VO_{2max}$  for the remainder of the session. In order to equalize training volume (i.e. running the same distance at each training session) between the programmes, CME was performed for 1.5 h per session (after the warm-up) at the start of the exercise training period, which gradually extended to 2 h running at the end of the experimental period.  $VO_{2max}$  was measured at the start of every training week, to allow adjustment of the running speed to maintain the relative exercise intensities.

### 2.4 Endothelial function

Animals were anaesthetized, and the abdominal aorta between the kidney and iliac arteries was carefully dissected by a 'non-touch' technique to leave the endothelium intact. Two to four millimetre long segments were connected to a force transducer and immersed in a 10 mL organ bath containing Krebs buffer and indomethacin. Segments were subjected to a gradual increase in tension to 1.0 g over 75 min, exposed to 60 mM  $K^+$ ,  $3 \cdot 10^{-7}$  M phenylephrine, and  $10^{-4}$  M acetylcholine and equilibrated for 30 min before the start of the experiments. Four segments from each animal were pre-contracted with  $3 \cdot 10^{-7}$  M phenylephrine. Two of these segments were relaxed with cumulative doses ( $10^{-9}$ – $3 \cdot 10^{-5}$  M) of acetylcholine: one with  $Na^+$  nitroprusside (SNP) ( $10^{-9}$ – $3 \cdot 10^{-5}$  M) and the other was treated with  $10^{-4}$  M  $N_{\omega}$ -nitro-L-arginine methyl ester (L-NAME) before exposure to acetylcholine.

### 2.5 Caveolae density

Ring segments from the common carotid artery were immersed in a mixture of glutaraldehyde (2%) for 24 h in room temperature, then post-fixed for 1 h in 2% osmium tetroxide, dehydrated in 50, 70, 90, and 100% ethanol solutions and propyleneoxyd, and embedded in Epoxy Resin LX 112 (Ladd, Ladd Research Industries, Williston, VT, USA). Ultra-thin sections (100 nm) were contrasted with uranyl acetate and lead citrate and examined in a Jeol Jem-100 X electron microscope. Endothelial cells were measured at 27 300 $\times$  and photographed; distinct flask-shaped caveolae (50–100 nm in diameter) were counted.

### 2.6 Tissue samples

Retroperitoneal fat, liver, and abdominal aortic samples were collected, frozen in liquid nitrogen, and stored at  $-80^{\circ}C$ . Pre-freezing, the retroperitoneal fat mass was weighed. The *m. soleus* was carefully free-dissected, and a small sample was used immediately for Serca-1 and 2  $Ca^{2+}$  transport measurements, whereas the rest was frozen in liquid nitrogen and stored at  $-80^{\circ}C$ .

### 2.7 Skeletal muscle Serca-1 and 2 $Ca^{2+}$ transport

Because the mostly oxidative *m. soleus* is an important contributor to uphill treadmill running, we permeabilized tissue samples by saponin (50  $\mu$ g/mL, 30 min,  $4^{\circ}C$ ), before transferring to a solution containing (millimolar) 0.05 ethylene glycol tetraacetic acid, 5 adenosine triphosphate (ATP), 10 creatine phosphate, 25 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid, 100 KCl, and 5.5 MgCl. Fura-2 (10  $\mu$ M; Molecular Probes) was used to measure  $Ca^{2+}$ , oxalate (10  $\mu$ M) to stabilize intra-sarcoplasmic reticulum (SR)  $[Ca^{2+}]$ , and ruthenium red (3  $\mu$ M) to inhibit SR  $Ca^{2+}$  efflux and mitochondrial  $Ca^{2+}$  buffering. The experiment was initiated by stirring tissue samples in a 150  $\mu$ L cuvette while monitoring

extra-SR  $\text{Ca}^{2+}$  using an inverted epi-fluorescence microscope (Diaphot-TMD, Nikon, Tokyo, Japan), to monitor  $[\text{Ca}^{2+}]$  by counting 510 nm emission after alternating excitation at 340 and 380 nm wavelengths.  $\text{Ca}^{2+}$  (50  $\mu\text{M}$ ) was added to induce a rapid increase in  $[\text{Ca}^{2+}]$ , and kinetics of the subsequent decline in  $\text{Ca}^{2+}$  were analysed to assess SR  $\text{Ca}^{2+}$  ATPase (Serca)-1 and -2  $\text{Ca}^{2+}$  transport capacity. About 10  $\mu\text{M}$  thapsigargin was added at the end of the protocol to assay any SR  $\text{Ca}^{2+}$  leakage.<sup>10</sup>

## 2.8 Insulin receptor phosphorylation

Frozen tissue samples were homogenized in ice-cold lysis buffer (1% Triton X-100 in the presence of phosphatase and protease inhibitors), and the lysates were treated with 100 nM insulin (Sigma, St Louis, MO, USA) or buffer alone for 10 min prior to phosphorylation in the presence of cold ATP. Proteins were immunoprecipitated with an anti-insulin receptor (IR) antibody (Santa Cruz Biotechnology Inc.), analysed on 7%  $\text{Na}^+$  dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), and transferred to nitrocellulose membranes prior to detecting phosphorylated residues with a monoclonal anti-phosphotyrosine antibody (Cell Signalling Technology Inc.). The blots were reprobbed with anti-IR  $\beta$ -subunit ( $\text{IR}_\beta$ ) antibodies (Santa Cruz Biotechnology Inc.) to account for the amount of IRs in the immunopellets. Enhanced chemiluminescence (ECL; Amersham Pharmacia Biotech) and densitometry were used for detection and quantification.

## 2.9 Protein expression

For other westerns, 75  $\mu\text{g}$  of total lysates was analysed by 4–12% SDS-PAGE prior to transferring proteins on nitrocellular membranes and probing with specific antibodies. These include antibodies against endothelial nitric oxide (NO) synthase (eNOS) (Cell Signalling Technology Inc.), caveolin-1, fatty acid transport protein-1 (FATP-1), peroxisome proliferator-activated receptor- $\gamma$ -coactivator-1 $\alpha$  (PGC-1 $\alpha$ ), heat-shock protein 90 (HSP90) (Santa Cruz Biotechnology Inc.), and fatty acid synthase (FAS).<sup>11</sup> Blots were then incubated with horseradish peroxidase-conjugated anti-IgG antibody, and proteins were detected by ECL and quantified by densitometry.

## 2.10 Blood pressure

Blood pressure was measured 48 h after the last exercise session using a non-invasive tail-cuff monitor (NIBP-8, Columbus Instruments, Columbus, OH, USA).

## 2.11 Blood analyses

Following an overnight fast, blood glucose was measured between 0900 and 1000 h in blood from the tail vein (Accu-Check Sensor, Roche Diagnostics, Indianapolis, IN, USA). For measuring glucose tolerance, animals were given 0.8 g glucose in a 1 mL aqueous solution through a gastric tube 2 h prior to blood sampling. After an overnight fast, rats were anaesthetized with diethyl ether between 0600 and 0800 h, and venous blood was drawn from the inferior vena cava to measure plasma free fatty acids (FFAs) by the NEFA C Kit (Wako Diagnostics, Richmond, VA, USA) and triglycerides by the Infinity Triglycerides Reagent Kit (Sigma).

## 2.12 Statistical analyses

All values are expressed as mean  $\pm$  standard error of the mean (SEM). For endothelial function, dose-response curves were drawn and  $\text{EC}_{50}$  values were obtained, as described by Ariens *et al.*<sup>11</sup> A univariate repeated-measures general linear model analysis of variance (ANOVA) including Scheffe *post hoc* test verified the differences in arterial relaxation, whereas the Kruskal-Wallis with Scheffe *post hoc* test determined differences between groups for other variables.

One-way ANOVA confirmed these results. *P*-value less than 0.05 was considered statistically significant.

## 3. Results

### 3.1 Baseline characteristics

Rats were matched for age, body weight, and metabolic parameters prior to different exercise programmes (Table 1).

### 3.2 Aerobic capacity

$\text{VO}_{2\text{max}}$  increased by 45 and 10% after AIT and CME, respectively (Figure 1A).

### 3.3 Body and fat weight

AIT and CME caused a 5 and 7% reduction in body weight and a 54 and 55% reduction in retroperitoneal fat weight, respectively (Table 1).

### 3.4 Blood pressure

AIT reduced systolic blood pressure more than CME (20 vs. 6 mmHg,  $P < 0.01$ , Table 1), whereas both training regimens decreased diastolic blood pressure to a similar extent (7–9 mmHg).

### 3.5 PGC-1 $\alpha$ and Serca: skeletal muscle molecular mechanisms

Decreased exercise capacity in individuals with the metabolic syndrome may be linked to abnormal mitochondrial biogenesis and function and to excitation-contraction coupling in the skeletal muscle. We therefore determined the levels of PGC-1 $\alpha$ , a critical factor coordinating the activation of metabolic genes required for substrate utilization and mitochondrial biogenesis and the re-uptake of  $\text{Ca}^{2+}$  into the SR, in order to assess and mechanistically understand skeletal muscle function and fatigue.<sup>3,12</sup> AIT increased the PGC-1 $\alpha$  content in *m. soleus* to a higher extent than CME (12- vs. 6-fold increase compared with SED; Figure 1B). Additionally, AIT, but not CME, increased the maximal rates of  $\text{Ca}^{2+}$  removal via Serca-1 and 2 in the skeletal muscle fibres by  $\sim 50\%$  (Figure 1C;  $P < 0.01$ ).

### 3.6 Endothelial function

AIT and CME significantly increased the endothelial function 2.7- and 2.0-fold, respectively ( $P < 0.01$ , group differences  $P < 0.05$ , Figure 2A and B), as indicated by improved sensitivity of aorta ring segments to acetylcholine; measured as  $\text{EC}_{50}$  (the concentration of acetylcholine that provoked a half-maximal relaxation response). However, the maximal absolute relaxation was comparable in AIT, CME, and SED. The inhibition of NO production by L-NAME in combination with indomethacin abolished the relaxation in all three groups (Figure 2C), suggesting that NO mediated the differential aortic relaxation after exercise training. This occurs in the absence of altered intrinsic sensitivity of vascular smooth muscle to NO, as demonstrated by comparable responses between the different groups to stimulation with the exogenous NO-donor SNP (Figure 2D).

Table 1. Physiological characteristics of the rats with metabolic syndrome

	SED		CME		AIT	
	Pre	Post	Pre	Post	Pre	Post
Weight (g)	430 ± 17	478 ± 21**	393 ± 12	377 ± 14 <sup>§</sup>	417 ± 16	391 ± 14 <sup>§</sup>
Retropitoneal fat (g)	—	12.5 ± 3.5	—	5.8 ± 1.8 <sup>§§</sup>	—	5.6 ± 2.0 <sup>§§</sup>
Systolic blood pressure (mmHg)	151.8 ± 3.2	148.1 ± 4.0	148.7 ± 6.2	142.2 ± 4.8 <sup>§§</sup>	150.9 ± 5.1	131.1 ± 4.8 <sup>§§</sup>
Diastolic blood pressure (mmHg)	88.9 ± 4.1	90.1 ± 3.4	91.1 ± 4.2	84.1 ± 2.9 <sup>§§</sup>	90.0 ± 3.2	81.1 ± 2.9 <sup>§§</sup>
MAP (mmHg)	109.9 ± 3.6	109.3 ± 4.1	110.4 ± 4.2	103.4 ± 2.9 <sup>§§</sup>	110.2 ± 4.4	97.7 ± 3.1 <sup>§§</sup>
HDL (mmol/L)	1.51 ± 0.2	1.50 ± 0.3	1.52 ± 0.3	1.56 ± 0.2	1.52 ± 0.2	2.0 ± 0.2 <sup>§§</sup>
Triglycerides (mEq/mL)	40.3 ± 3.1	41.2 ± 3.0	41.6 ± 2.7	34.2 ± 4.1 <sup>§§</sup>	40.4 ± 2.9	35.3 ± 2.8 <sup>§§</sup>
Free fatty acids (mEq/mL)	1.44 ± 0.17	1.52 ± 0.12	1.38 ± 0.15	0.52 ± 0.08 <sup>§§</sup>	1.42 ± 0.10	0.56 ± 0.09 <sup>§§</sup>
Fasting glucose (mmol/L)	5.5 ± 0.2	5.8 ± 0.2	5.5 ± 0.2	4.9 ± 0.1*	5.5 ± 0.2	4.7 ± 0.2*
2 h post-glucose load (mmol/L)	6.1 ± 0.1	6.3 ± 0.2	6.5 ± 0.2	5.3 ± 0.2 <sup>§§</sup>	6.1 ± 0.2	4.9 ± 0.1 <sup>§§</sup>

Data are presented as mean ± SEM. SED, sedentary; CME, continuous moderate-intensity exercise; AIT, aerobic interval training; MAP, mean arterial blood pressure; HDL, high density lipoprotein.

\*Significantly different within each group from pre to post ( $P < 0.05$ ).

\*\*Significantly different within each group from pre to post ( $P < 0.01$ ).

§Significantly different from SED ( $P < 0.01$ ).

§§Significantly different from SED and CME ( $P < 0.01$ ).

†Significantly different from CME ( $P < 0.05$ ). Each group consisted of eight rats.

### 3.7 Endothelial molecular mechanisms

Consistent with a larger improvement in endothelial function after AIT, this training programme also increased eNOS protein levels in aorta when compared with CME (Figure 3A). However, CME increased the HSP90 protein content similar to AIT (Figure 3B), which suggests that eNOS activity may have also increased after CME. In accordance with increased shear stress during exercise,<sup>13</sup> AIT and CME increased the endothelial luminal caveolae density by 65 and 27%, respectively (Figure 3C), whereas the levels of caveolin-1 increased by approximately two-fold in both training groups (Figure 3D).

### 3.8 Insulin action in skeletal muscle, fat, and liver tissue

IR phosphorylation in *m. soleus* was not different between the groups (Figure 4A). In fat tissue, AIT was associated with a greater IR phosphorylation in response to insulin than CME and SED groups, although CME also showed a greater phosphorylation of the IR than the SED group (Figure 4B). Both endurance training programmes increased IR phosphorylation in the liver to a similar extent (Figure 4C,  $P < 0.01$ ).

### 3.9 Fatty acid transporter-1 and fatty acid synthase

Neither AIT or CME affected the contents of FATP-1 in the *m. soleus* (data not shown). In contrast, fat tissue FATP-1 levels decreased markedly (by approximately three to four-fold) after AIT, but not CME (Figure 5A). In accordance with this, AIT caused a more marked decrease in the protein content of the lipogenic enzyme FAS in fat tissue than CME, although CME also reduced the protein levels of FAS (Figure 5B). Endurance training did not affect FAS or FATP-1 protein contents in the liver (data not shown).

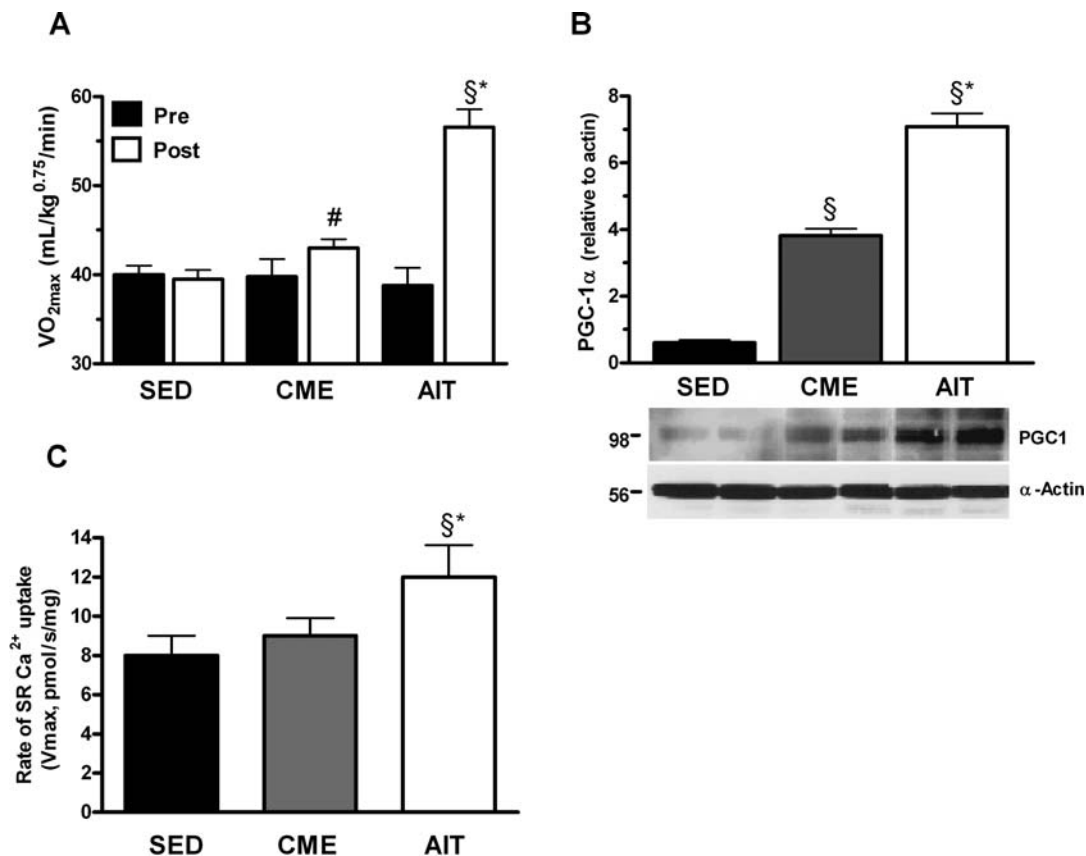
### 3.10 Blood analysis

Both AIT and CME reduced overnight-fasting blood glucose levels by 10–15% ( $P < 0.05$ ) (Table 1). Oral glucose ingestion increased blood glucose levels, suggesting glucose intolerance before the training period (Table 1). Both AIT and CME removed the increase in blood glucose after the oral glucose ingestion to a similar extent, indicating improved glucose tolerance.

HDL cholesterol increased by ~25% after AIT ( $P < 0.05$ ), but remained unaltered in the two other groups (Table 1). Both endurance training programmes reduced plasma triglyceride and FFA levels to an equal extent (Table 1).

## 4. Discussion

The current study demonstrates the superior effect of high-intensity aerobic exercise on aerobic capacity, endothelial function, insulin action, lipogenesis, and improvement of the cardiovascular risk profile in rats with the metabolic syndrome, when compared with a moderate-intensity exercise programme. The study presents novel mechanistic insights into the causes of the differential effects of high- and moderate-intensity exercise training programmes and how they may correct metabolic and vascular abnormalities associated with the metabolic syndrome.



**Figure 1** Maximal oxygen uptake (VO<sub>2max</sub>) (A). Expression of peroxisome proliferator-activated receptor- $\gamma$  co-activator-1 $\alpha$  (PGC-1 $\alpha$ ) in samples from *m. soleus* (B). Maximal rate of re-uptake of calcium into sarcoplasmic reticulum in *m. soleus* (C). \*Significantly different between high-intensity aerobic interval training (AIT) and continuous moderate-intensity exercise (CME) programmes ( $P < 0.05$ ). <sup>§</sup>Significantly different from sedentary (SED) ( $P < 0.01$ ). <sup>#</sup>Significantly different from SED ( $P < 0.05$ ). Each group consisted of eight rats.

#### 4.1 Aerobic capacity

Of all the established risk factors for cardiovascular disease, low aerobic exercise capacity appears to be the strongest predictor of mortality.<sup>4,5</sup> Hence, improving aerobic capacity seems to be an important clinical target, particularly in a population with a higher than normal risk of developing cardiovascular disease, such as patients with metabolic syndrome. We herein demonstrated that high-intensity exercise increased VO<sub>2max</sub> in rats with the metabolic syndrome more than continuous moderate intensity.

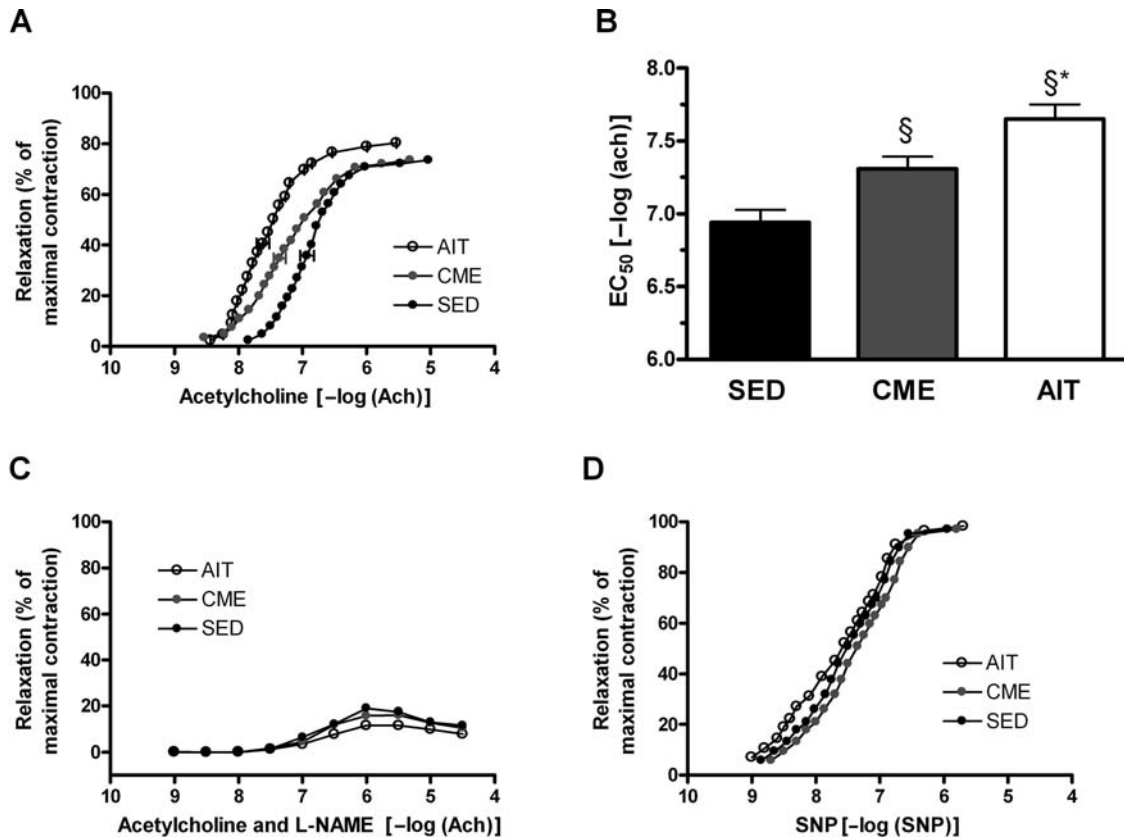
Larger improvement in VO<sub>2max</sub> with high-intensity training has normally been attributed to a higher increase in stroke volume.<sup>14</sup> This study suggests that also vascular and peripheral changes may contribute to the intensity-dependent effects, as arterial endothelial function and its regulatory mechanisms, and mitochondrial biogenesis, as evaluated by PGC-1 $\alpha$  levels in skeletal muscle, increased more after AIT than CME. Furthermore, AIT, but not CME, increased Serca capacity, suggesting that Ca<sup>2+</sup> cycling in skeletal muscle contributed to the increased exercise tolerance after AIT, in line with other recent publications.<sup>15,16</sup>

#### 4.2 Endothelial function

High-intensity exercise training improved endothelial function more often than moderate-intensity exercise, perhaps because of differential effects on NO bioavailability. In line with the hypothesis that the exercise-induced improvement in vessel relaxation is mainly mediated by NO,<sup>17,18</sup>

experiments using NO inhibitors (L-NAME) or NO donors (SNP) abolished the differences in vasorelaxation between groups. Additionally, exercise-induced improvements in endothelial function and NO bioavailability were associated with increased expression of aortic eNOS levels and eNOS activity, as indicated by the increased expression of HSP90.

The low- and high-intensity exercise training programmes produce differences in shear stress exerted on the walls of blood vessels, and it is thought that this mechanical stress induces the increase in the gene expression of eNOS. However, the molecular identity of the link between sensing the shear stress and nuclear gene transcription is unknown.<sup>19</sup> A central hypothesis emphasizes the role of the vesicular transporters, caveolae, in the shear stress-mediated regulation of NO production.<sup>20,21</sup> Shear stress stimulates caveolae formation by translocating caveolin-1 from the Golgi apparatus to the luminal plasma membrane,<sup>22</sup> leading to enhanced sensitivity to shear stress with increased phosphorylation of eNOS.<sup>23</sup> Because disruption of the caveolar system due to metabolic and cardiovascular alterations<sup>24,25</sup> impairs acetylcholine-mediated vasorelaxation,<sup>24,26</sup> structural enhancement of the caveolae system of LCR rats by exercise suggests that this mechanism may contribute to improve endothelial function. However, as increased levels of caveolin-1 may also sequester and inhibit eNOS,<sup>24,27</sup> it is possible that increased HSP90 with exercise training counteracts this seemingly detrimental side effect.<sup>28</sup> To the best of our knowledge, this is the first study to report a dose-response relationship between caveolae density and endurance training.



**Figure 2** Endothelial function measured as a relaxation response to accumulating concentrations of acetylcholine (ach) in isolated ring segments of aorta (A) and as the concentration of acetylcholine that provoked a half-maximal response ( $EC_{50}$ ) (B). Endothelial function was also measured after incubation of  $10^{-4}$  M L-NAME (C) and with accumulating concentrations of  $Na^+$  nitroprusside (D). \*Significantly different between high-intensity aerobic interval training (AIT) and continuous moderate-intensity exercise (CME) programmes ( $P < 0.05$ ). <sup>§</sup>Significantly different from sedentary (SED) ( $P < 0.01$ ). Each group consisted of eight rats.

In agreement with a previous study,<sup>29</sup> exercise training reduced blood glucose. Also, high, but not moderate, intensity exercise training increased the levels of plasma HDL cholesterol, which inhibits LDL oxidation and increases eNOS activity.<sup>30</sup> Thus, elevation in the eNOS protein content increased plasma HDL cholesterol, and reduced glucose levels appear to mediate, at least partly, the superior effect of high-intensity training on arterial reactivity, compared with moderate-intensity exercise training.

### 4.3 Skeletal muscle adaptation

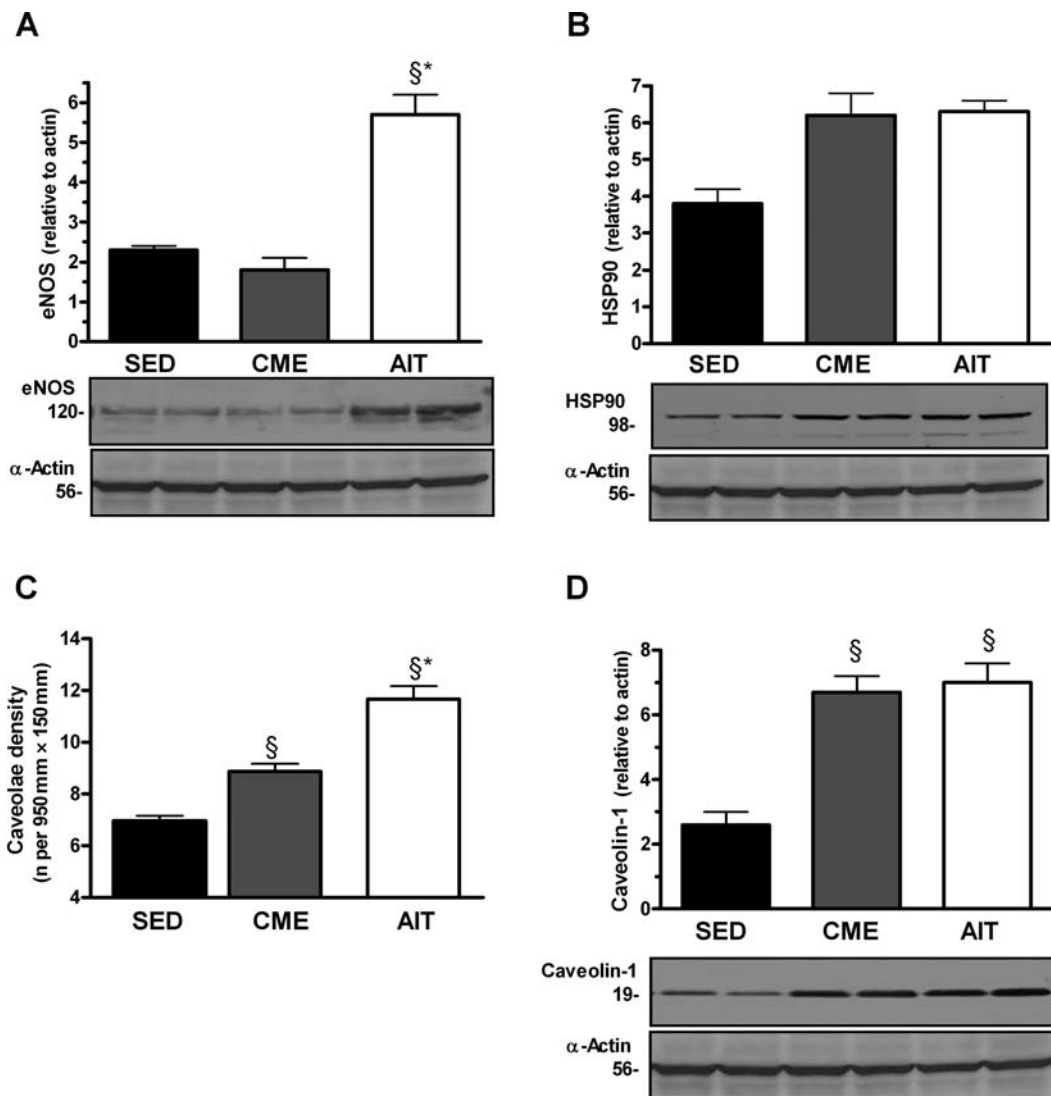
Markers of skeletal muscle metabolic and contractile states showed that adaptations originating here most likely contribute to the overall improvement of aerobic capacity and health profile. First, the increase in PGC-1 $\alpha$  levels suggests that exercise training induces biogenesis and increases mitochondrial function by its selective control over expression of genes that modulate mitochondrial metabolism. In contrast, exercise training did not affect phosphorylation of IR or the expression levels of FATP-1 and FAS in the skeletal muscle. This suggests that the adaptation of the skeletal muscle may be specific to some, but not all metabolic pathways, possibly because not all are rate-limiting for energy production during exercise. Moreover, as metabolic processes also affect the contractile ability of the skeletal muscle, we studied this aspect through the function of the intracellular  $Ca^{2+}$  transporter Serca. Serca removes the bulk of intracellular  $Ca^{2+}$  during relaxation of the muscle and

loads the SR for the subsequent  $Ca^{2+}$  release event.<sup>10</sup> It is also particularly interesting here because it is an ATPase and may thus be directly linked to its metabolic environment. In fact, it has been shown to co-localize with mitochondria.<sup>31</sup> Hence, Serca serves as a marker of contractile status in the muscle, and importantly, the activity of the Serca may be studied in tissue samples, as established previously by our group.<sup>10</sup> In contrast, studying the contractile function and  $Ca^{2+}$  handling of the whole muscle fibre are technically difficult, as this requires intact fibres.

Our results suggest that in order to improve contractile function of the skeletal muscle, high intensity of exercise training is required, as moderate-intensity exercise did not induce any significant effects. However, a small trend towards improvement after CME may suggest that a continuation of this exercise programme may have had the potential to beneficially alter the contractile function, but only over a considerably longer time period.

### 4.4 Insulin action

Antecedent exploration and the current results show that this rat model exhibits compromised insulin action in fat tissue and the liver and an abnormal metabolic state.<sup>5</sup> Regular exercise training was able to improve insulin action, with a stronger effect of high- vs. moderate-intensity exercise training in fat tissue. This was also associated with a stronger lowering effect of high intensity on FFA uptake and lipogenesis in fat tissue, as suggested by the



**Figure 3** Relative expressions of the endothelial nitric oxide synthase (eNOS) (A) and heat shock protein 90 (HSP90) (B) in aorta. Caveolae density measured with an electron microscope in endothelial cells from aorta (C) and the relative expression of caveolin-1 in aorta (D). \*Significantly different between high-intensity aerobic interval training (AIT) and continuous moderate-intensity exercise (CME) programmes ( $P < 0.05$ ). <sup>§</sup>Significantly different from sedentary (SED) ( $P < 0.01$ ). Each group consisted of eight rats.

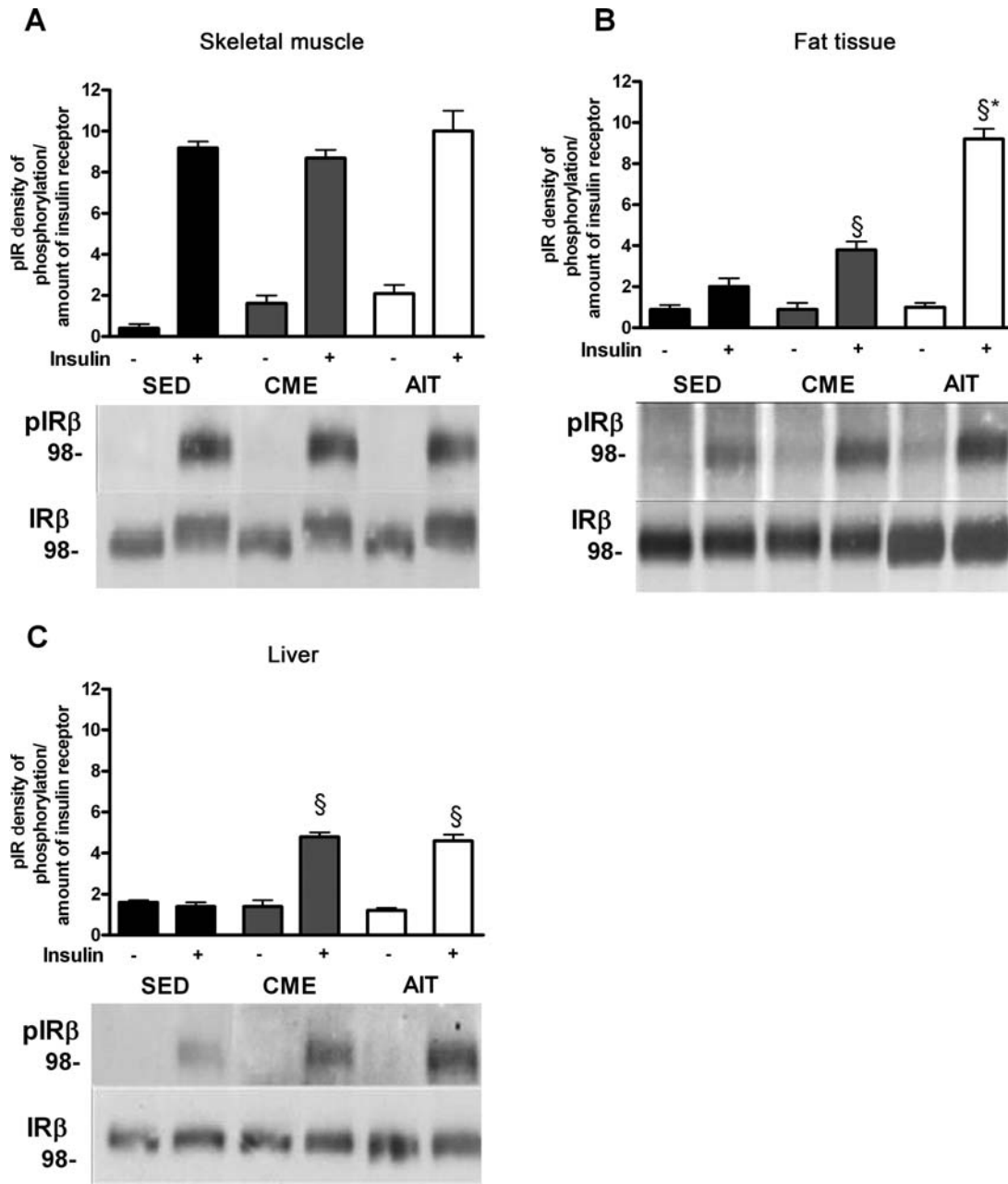
greater reductions of FATP-1 and FAS levels, respectively. However, both exercise training programmes lowered plasma FFA and triglyceride levels to a similar extent. This was in accordance with the similar effects of the exercise training programmes on visceral obesity and insulin action in the liver. Moreover, both AIT and CME reduced fasting glucose levels and increased the glucose tolerance to a similar extent. These results suggest that exercise training, particularly high-intensity exercise training, serves to correct abnormalities in insulin action and metabolic parameters. This should have an impact on the clinical scenario, given the link this phenotype of rats has to the metabolic syndrome and disease,<sup>3</sup> and also contribute to a reduction in the risk of developing cardiovascular disease.

#### 4.5 Blood pressure and body weight

AIT and CME reduced systolic blood pressure by 20 and 6 mmHg and diastolic blood pressure by 9 and 7 mmHg, respectively. Reduced hypertension would alone reduce the risk of developing cardiovascular disease substantially.

Exercise remains a primary treatment option for hypertension, but the published guidelines for exercise intensity have so far not recommended high exercise intensity; rather, moderate intensity has been promoted.<sup>30</sup> Our results suggest that this aspect of blood pressure control should be studied more rigorously. The causes of exercise-induced lowering of blood pressure are not entirely clear because of its complexity,<sup>30</sup> but the improved endothelial function, insulin action, and metabolic control are likely contributors.

Both exercise training programmes slightly reduced body weight, but substantially reduced fat weights. Even though obesity is linked to cardiovascular disease and predicts cardiovascular mortality, the link between aerobic capacity and mortality seems to be stronger.<sup>4</sup> This suggests that treatment should target aerobic capacity rather than loss of body weight *per se*.<sup>32</sup> Furthermore, evolution has preserved or evolved complex biological mechanisms to avoid weight loss. Thus, the likelihood of a pharmacological solution is small.<sup>33</sup> In fact, there is no ideal drug at the present time



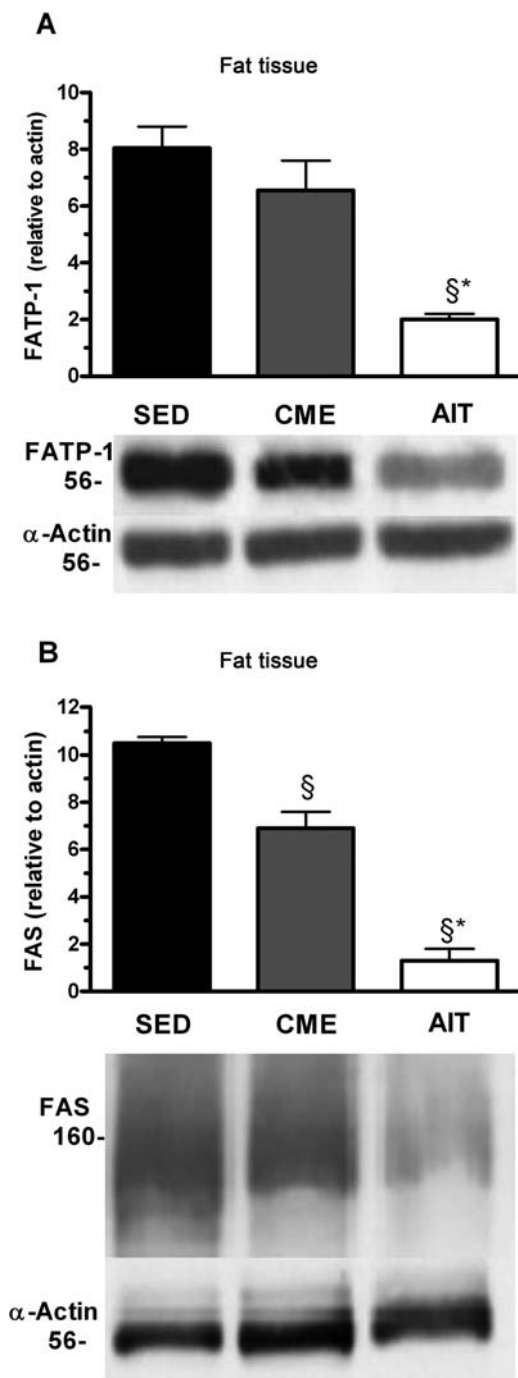
**Figure 4** Insulin action in *m. soleus* (A), fat (B), and liver tissue (C). pIR, phosphorylated insulin receptor. \*Significantly different between high-intensity aerobic interval training (AIT) and continuous moderate-intensity exercise (CME) programmes ( $P < 0.05$ ). <sup>§</sup>Significantly different from sedentary (SED) ( $P < 0.01$ ). Each group consisted of eight rats.

for the majority of patients with the metabolic syndrome.<sup>34</sup> The current study suggests that high-intensity training should be considered as a part of the treatment plan in the metabolic syndrome.

#### 4.6 Clinical translation

Taken together, these results suggest that the importance of the exercise intensity should be investigated in clinical trials of the metabolic syndrome. We have recently studied the effects of high vs. moderate-intensity exercise, both in patients diagnosed with the metabolic syndrome<sup>35</sup> and in obese subjects not fulfilling the criteria of the metabolic syndrome,<sup>36</sup> albeit at a small scale. Both studies confirmed

the intensity dependence of effects after aerobic exercise training. The current study provides molecular insights into the aetiology of the metabolic syndrome, mechanisms of which may contribute to the exercise-induced reduction of the syndrome and improvement in function, and to what degree changes in these underlying mechanisms depend on the intensity of the exercise. However, the clinical role of exercise intensity remains unresolved. Large clinical trials have shown that the more intense exercise is more beneficial in reducing cardiovascular risk,<sup>37</sup> but other trials have reported that moderate levels of exercise may confer a greater reduction in cardiovascular risk.<sup>38</sup> Different study populations and clinical scenarios may have contributed to the differences.



**Figure 5** Relative expression of fatty acid transporter protein 1 (FATP-1) (A) and fatty acid synthase (FAS) (B) in fat tissue. \*Significantly different between high-intensity aerobic interval training (AIT) and continuous moderate-intensity exercise (CME) programmes ( $P < 0.05$ ). <sup>§</sup>Significantly different from sedentary (SED) ( $P < 0.01$ ). Each group consisted of eight rats.

#### 4.7 Conclusions

This study demonstrates that high-intensity exercise training was better than moderate-intensity training in reversing risk factors related to the metabolic syndrome. This is in line with previous results from our laboratory, showing that similar exercise protocols also confer intensity-dependent effects to normal rats.<sup>9</sup> The closely supervised training regimens and the comparable training volumes between the two exercise groups demonstrate the dependence of exercise

intensity for ameliorating the cardiovascular risk factors. Important mechanisms that appear to contribute to the improved aerobic capacity and vascular function, reduced blood pressure and obesity, and improved metabolic control seem to be related to regulatory systems in the artery endothelium, the skeletal muscle, the liver, and fat tissue. This study also confirms that the ability to respond to exercise in a dose-response dependent manner is intact, even with the phenotype of the metabolic syndrome.

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