

Poor Wheel-Running Exercise Can Decrease Blood Pressure through Hormonal Control and Increase Endurance Exercise Capacity in Middle-Aged Normal Rats

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Received 14 July 2015; accepted 20 July 2015; published 10 August 2015

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Abstract

The aim of this study was to examine the effects of voluntary wheel-running (WR) on body weight (BW), waist circumference, mesenteric fat mass (MFM), adipocyte size, circulating cytokines/hormones, blood pressure (BP) and exercise endurance capacity in 11-month-old normal rats. Three-week WR with about 0.2 km of daily running distance caused a gradual loss in BW despite an increased intake of food/water. MFM decreased as daily running distance increased. Moreover, there was a positive correlation between MFM and BW, waist circumference or adipocyte size. On the other hand, WR significantly decreased systolic/diastolic BPs, and increased endurance exercise capacity. WR rat sera contained lower concentrations of angiotensin II, aldosterone, vasopressin and endothelin-1 and higher concentration of brain natriuretic peptide compared with sedentary rat sera. Thus, WR-induced reduction in resting BPs may be accomplished by attenuated vasoconstriction, enhanced vasodilatation and reduction in blood volume. In addition, circulating vascular endothelial growth factor and interleukin-6 were higher in WR rats, suggesting angiogenesis, anti-inflammation and insulin-sensitization. These results support a prevalent idea that daily light-exercise is a potential strategy for preventing metabolic syndrome.

Keywords

Adipokine, Blood Pressure, Endurance Exercise Capacity, Hormone, Metabolic Syndrome,

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How to cite this paper: Sakata, S., Hanaoka, T., Ishizawa, R., Iwami, K., Takada, Y., Imagita, H., Minematsu, A., Waki, H. and Nakatani, A. (2015) Poor Wheel-Running Exercise Can Decrease Blood Pressure through Hormonal Control and Increase Endurance Exercise Capacity in Middle-Aged Normal Rats. *Journal of Biosciences and Medicines*, 3, 10-24.

<http://dx.doi.org/10.4236/jbm.2015.38002>

Voluntary Wheel-Running

1. Introduction

The incidence of obesity has increased markedly worldwide during recent decades. At present, consequently, obesity and its associated disorders are serious worldwide problems to threaten the health [1]. Obesity is considered as a major and prevalent risk factor for metabolic syndrome, which is associated with an increased risk of developing atherosclerotic cardiovascular disease, insulin resistance and type 2 diabetes mellitus [2]. In addition to abdominal obesity represented as waist circumference, other risk factors for metabolic syndrome are recognized widely to be atherogenic dyslipidemia, elevated blood pressure and elevated fasting blood glucose [3] [4]. Furthermore, obesity is associated with a chronic inflammatory response, which is characterized by crosstalk of abnormal cytokines produced by both adipocytes and adipocyte tissue macrophages [5] [6]. Therefore, macrophage-related inflammation contributes to the pathogenesis of obesity-induced insulin resistance [7]-[9].

Middle-aged obese people are well known to have a high risk of developing metabolic syndrome. Epidemiological studies showed that physical activity of moderate intensity, like walking and jogging, could reduce the risk of developing type 2 diabetes and coronary heart disease [10] [11]. Every possible mechanism for this risk reduction has been explored, using cages equipped with a running wheel, in several rodent studies, where various genetic models including gene-modified animals [12]-[19] and high-fat diet-sensitive obesity models [20]-[23] start on voluntary wheel running (WR) during juvenile or young period, but not during adult period. Thus, the preventive effects of WR exercise on development of metabolic syndrome have not been fully studied in adult rats as yet. In the present study, therefore, we used 11-month-old genetically normal rats, which would be considered as an experimental animal model of middle-aged obese people, to examine the effects of voluntary WR exercise on body weight (BW), waist circumference, mesenteric fat mass (MFM), adipocyte size, circulating cytokines/hormones, blood pressure (BP) and endurance exercise capacity. We report herein that 3-week WR exercise, in which daily running distance is very short (about 0.2 km), decreases BW, waist circumference, MFM, adipocyte size and circulating adipocyte-secreted cytokines, leptin and resistin, and increases circulating C-reactive protein (CRP), vascular endothelial growth factor (VEGF), interleukine-6 (IL-6) and endurance exercise capacity. In addition, this is the first to report that voluntary WR exercise induces a reduction in resting systolic/diastolic BPs by altering serum concentrations of several hormones that play key roles in regulating BPs.

2. Materials and Methods

2.1. Protocol Approval

All animal experiments in this study were performed with the approval of the Animal Care and Use Committee of Kio University and in accordance with the principles of the National Institute of Health's Guide for the Care and Use of Laboratory Animals.

2.2. Animals

Eleven-month-old male Wistar rats weighing 456 ± 62 g of the normal BW range, purchased from Japan SLC (Hamamatsu, Japan), were housed in cages set in temperature-controlled ($22^{\circ}\text{C} \pm 2^{\circ}\text{C}$) facility with a 12:12-h light-dark cycle (0600-1800 light, 1800-0600 dark). During the experimental period, rats were fed a standard rodent chow (CE-2; Clea Japan, Tokyo, Japan) and water ad libitum. All rats were weighed each week at the same time of day. Rats were randomly divided into the following two groups: 1) control sedentary group with movement confined to the cage space and 2) voluntary wheel-running group.

2.3. Voluntary Wheel Running

Rats in the voluntary wheel-running group were housed in cages equipped with wheels (116 cm circumference, 10 cm inside width; Shinano, Tokyo, Japan), that allowed free access to the wheel, for 3 weeks. The number of revolutions was recorded daily.

2.4. Blood Pressures and Heart Rate

In the conscious and quiet rats, systolic/diastolic blood pressures and heart rate were measured with a tail cuff and a pneumatic pulse transducer (BP-98A, Softron, Tokyo, Japan) [24]. Before the measurements, the animals were restrained for more than 10 min using a temperature-controlled (37°C) warming holder designed for rats. In the quiet state of rats, the sampling of data was usually repeated three times at intervals of about 1 min, and the average of three recordings was used as the data of individual rats.

2.5. Endurance Exercise Capacity

The animals run at a speed of 18 m/min for 5 min on a rodent motor-driven treadmill with a slope of 0°. About ten µl of blood was obtained by sticking a 26-gauge needle in the tip of tail before and just after treadmill exercise. Blood lactate concentrations were measured with a simplified blood lactate test meter (Lactate Pro, Arkray, Kyoto, Japan). Endurance exercise capacity of rats was evaluated by increment in blood lactate concentration at the end of the treadmill exercise.

2.6. Blood Collection and ELISAs

After the rats were anesthetized with intraperitoneal pentobarbital sodium (50 mg/kg; Somnopentyl, Kyoritsu-Seiyaku Corp., Tokyo, Japan) and a thoracotomy was carried out, more than 10 ml of blood was obtained from the left ventricle using a 20-gauge needle. Thirty minutes later, blood in the tubes were centrifuged at 3500 rpm for 10 min to separate serum, which was then stored at -80°C until analyzed with ELISA kits. Serum samples, thus obtained, were analyzed with commercially available ELISA kits for leptin (R & D Systems, Minneapolis, MN), resistin (B-Bridge International, Cupertino, CA), CRP (Life Diagnostics, West Chester, PA), angiotensin II (Assay Designs, Ann Arbor, MI), aldosterone (Enzo life Sciences, Plymouth Meeting, PA), vasopressin (Peninsula Laboratories, San Carlos, CA), endothelin-1 (R & D Systems, Minneapolis, MN), brain natriuretic peptide (BNP-32; Assaypro, St. Charles, MO) and 8 angiogenesis-related cytokines, *i.e.*, VEGF, IL-6, monocyte chemoattractant protein-1 (MCP-1), RANTES, tumor necrosis factor- α (TNF- α), fibroblast growth factor- β (FGF- β), leptin and interferon- γ (IFN- γ) (Rat Angiogenesis ELISA Strip Kit, Signosis, Sunnyvale, CA), using a microplate photometer (Multiskan FC; Thermo Fisher Scientific, Waltham, MA).

2.7. Mesenteric Fat Collection and Adipocyte Size

Mesenteric white adipose tissues were obtained from the carcasses after blood collection and were weighed using a digital scale. Digital photographs were taken at several sites of each mesenteric adipose tissue on tissue glass slide. Each adipocyte area was determined by counting the computerized pixels with the use of software Image J. In each rat, two hundreds of adipocytes were analyzed for adipocyte size.

2.8. Statistical Analysis

All data are presented as mean \pm SD. Means were compared using a Student's t-test or ANOVA. Statistical significance was accepted at the level of $p < 0.05$.

3. Results

3.1. Effects of WR on Food/Water Intake and BW

Figure 1 shows time-courses of total WR distance, BW and food/water intake during the WR exercise period for 3 weeks. Total WR distance was linearly increased during this exercise period. Average daily WR distance of 6 rats was about 0.2 km. This voluntary exercise caused a gradual loss in body weight, despite an increase in food/water intake. The obvious decrease in BW was observed in 5 of 6 WR rats (**Figure 2**). In addition, there was a negative correlation between daily running distance and % body weight change, that was defined by an equation of (post-WR BW/pre-WR BW) X 100 ($r = -0.66$, data not shown).

3.2. Effects of WR on Waist Circumference, MFM, Adipocyte Size, Circulating Adipokines, CRP and Serum Biochemical Parameters

MFM was significantly smaller in the rats subjected to WR exercise for 3 weeks than in the control sedentary

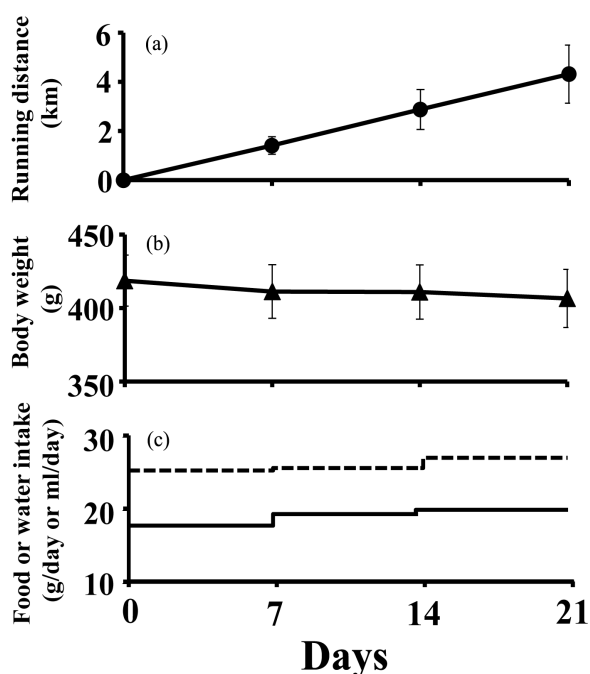


Figure 1. Changes in total voluntary wheel-running distance (a), body weight (b); daily food intake (solid line) and daily water intake (broken line) (c); during 3-week access to voluntary running wheels. Data in A and B are expressed as mean \pm SD. N = 6.

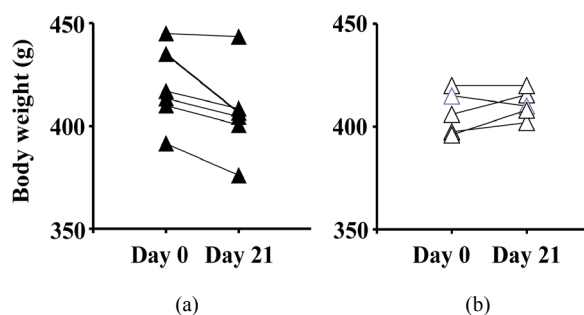


Figure 2. Individual changes in body weight after 3-week voluntary wheel running (a) and 3-week keeping in standard rodent cages (b).

rats (**Figure 3(a)**). In the WR group, MFM decreased as daily running distance increased (**Figure 3(b)**) and correlated positively with BW (**Figure 3(c)**) and waist size (**Figure 3(d)**), which was an abdominal circumference at a distance of 3 cm away from *Xiphisternum*. Mean mesenteric adipocyte size was smaller, although not significantly, in the WR rats than in the control rats (**Figure 4(a)**). In the WR rats, there was a positive correlation between mesenteric adipocyte size and MFM (**Figure 4(b)**). Moreover, histogram of mesenteric adipocyte size in the WR group shifted leftward compared with that of the control group (**Figure 4(c)**). The WR rats showed a significant decrease in serum leptin levels and a marginal decrease in serum resistin levels, which were determined by a specific ELISA, as compared with the control sedentary rats (**Figure 5**). On the other hand, serum CRP levels were slightly higher in the WR rats than in the control rats (**Figure 5**). There was no difference in hematologic and serum biochemical parameters between the WR and control rats: WR vs. control; blood hemoglobin, 14.7 ± 0.4 vs. 14.6 ± 1.1 g/dl; hematocrit, 44.4 ± 0.9 vs. $43.8\% \pm 3.6\%$; total protein, 6.3 ± 0.2 vs. 6.4 ± 0.1 g/dl; albumin, 4.0 ± 0.1 vs. 4.0 ± 0.1 g/dl; creatinine, 0.32 ± 0.02 vs. 0.33 ± 0.02 mg/dl; triglyceride, 141 ± 50

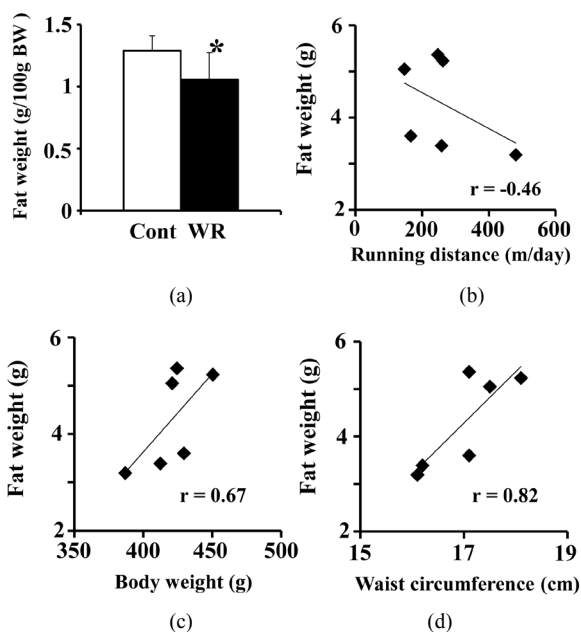


Figure 3. Mesenteric fat weight after 3-week voluntary wheel running (WR) and 3-week keeping in standard rodent cages (Cont) (a), and relationship between mesenteric fat weight and daily running distance (b) or body weight (c) or waist circumference (d) in “WR” rats. * $p < 0.05$ compared to “Cont” group. $N = 5$ in “Cont” and $n = 6$ in “WR”.

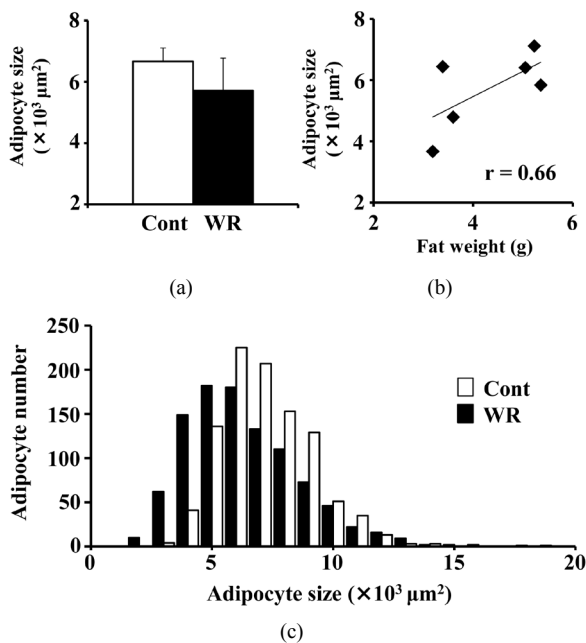


Figure 4. Mean adipocyte size after 3-week voluntary wheel running (WR) and 3-week keeping in standard rodent cages (Cont) (a), relationship between mean adipocyte size and mesenteric fat weight in “WR” rats (b), and histogram of adipocyte size in “Cont” and “WR” rats (c). Histogram is shown as net data of 4 rats for “Cont” and 5 rats for “WR”. In (a) $n = 5$ in “Cont” and $n = 6$ in “WR”.

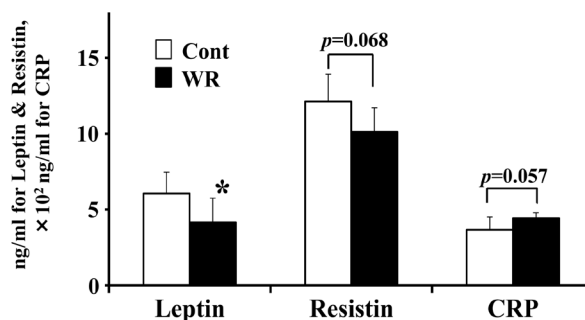


Figure 5. Serum concentrations of leptin, resistin and C-reactive protein after 3-week voluntary wheel running (WR) and 3-week keeping in standard rodent cages (Cont). * p < 0.05 compared to “Cont” group. N = 5 in “Cont” and n = 6 in “WR”. CRP: C-reactive protein.

vs. 147 ± 46 mg/dl; nonesterified fatty acid, 298 ± 101 vs. 407 ± 94 μ Eq/l; total cholesterol, 66 ± 8 vs. 77 ± 8 mg/dl.

3.3. Effects of WR on HR, BPs and BP-Related Hormones

The WR for 3 weeks caused little or no change in HR but a significant decrease in systolic, diastolic and mean BPs at rest, while the sedentary condition for 3 weeks did not cause any changes in HR and BPs (**Figures 6(a)-(d)**). In the WR group, the decrement in resting systolic BP was augmented as daily running distance increased (data not shown). On the other hand, the WR rat serum showed the significantly lower levels of angiotensin II, vasopressin and endothelin-1 and the detectable levels of BNP as compared with the undetectable levels of the sedentary rat serum (**Figure 7(a)** and **Figure 7(b)**). In addition, the WR rat serum contained a lower, but insignificant, mean value of aldosterone concentration (**Figure 7(a)**).

3.4. Effects of WR on Endurance Exercise Capacity

The treadmill exercise for 5 min, performed before 3-week WR or sedentary condition, induced about threefold increase in blood lactate concentrations (**Figure 8**). At the end of 3-week WR period, however, this treadmill exercise induced only about twofold increase in blood lactate concentrations, which were about half the post-treadmill exercise levels before WR (**Figure 8**). Furthermore, about twofold increase in blood lactate by the treadmill exercise was observed even at the end of 1 week-sedentary condition after 3-week WR (data not shown).

3.5. Effects of WR on Angiogenesis-Related Cytokines

In the WR and control groups, angiogenesis-related cytokines in the serum were tested with an ELISA kit for simultaneously profiling 8 cytokines; VEGF, IL-6, MCP-1, RANTES, TNF- α , FGF- β , leptin and IFN- γ . The circulating levels of VEGF and IL-6 were found to increase about twofold in the WR group compared with the control group (**Figure 9**). In the WR group, however, the increased levels of serum MCP-1 and RANTES were slight and insignificant (**Figure 9**). On the other hand, there was no difference in optical density at 450nm (around 0.1) of TNF- α , FGF- β and IFN- γ between the WR and control groups (data not shown).

4. Discussion

This study was designed to investigate the effects of voluntary WR exercise on the adipose tissue, resting BPs and endurance exercise capacity in the middle-aged normal rats, considered as an experimental animal model of middle-aged obese people. We, for the first time, provide direct evidence that the 3-week WR exercise can induce the reduction in resting systolic/diastolic BPs by altering the circulating levels of several hormones which play pivotal roles in regulating BPs.

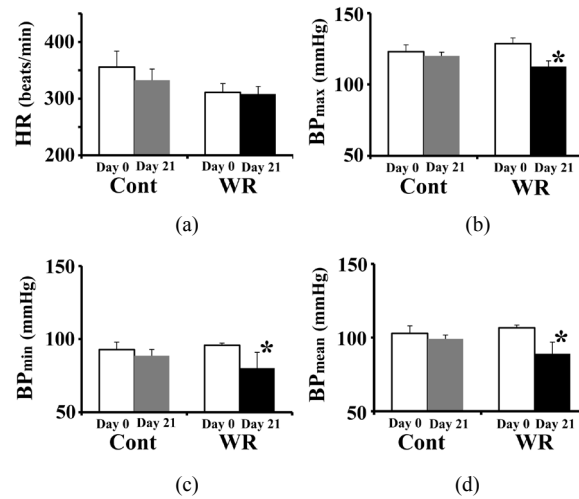


Figure 6. Heart rate (a); maximum blood pressure (b); minimum blood pressure (c) and mean blood pressure (d) before/after 3-week voluntary wheel running (WR) and 3-week keeping in standard rodent cages (Cont). * $p < 0.05$ compared to “Day 0” data. $N = 4$ in “Cont” and $n = 6$ in “WR”. HR: heart rate; BP_{max}: maximum blood pressure; BP_{min}: minimum blood pressure; BP_{mean}: mean blood pressure.

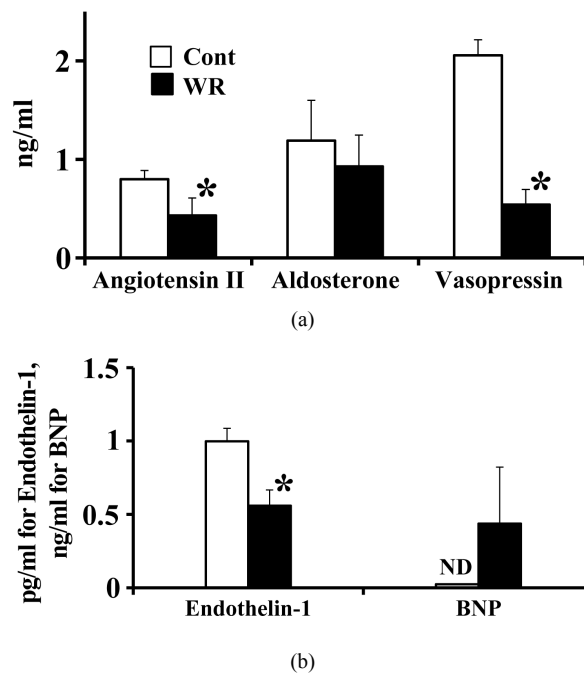


Figure 7. Serum concentrations of angiotensin II, aldosterone and vasopressin (a) and of endothelin-1 and BNP (b) after 3-week voluntary wheel running (WR) and 3-week keeping in standard rodent cages (Cont). * $p < 0.05$ compared to “Cont” group. $N = 5$ in “Cont” and $n = 6$ in “WR”. BNP: brain natriuretic peptide; ND: not detected.

4.1. Effects of WR on Food/Water Intake and BW

The middle-aged normal rats used herein showed a very poor WR performance (about 0.2 km of daily running

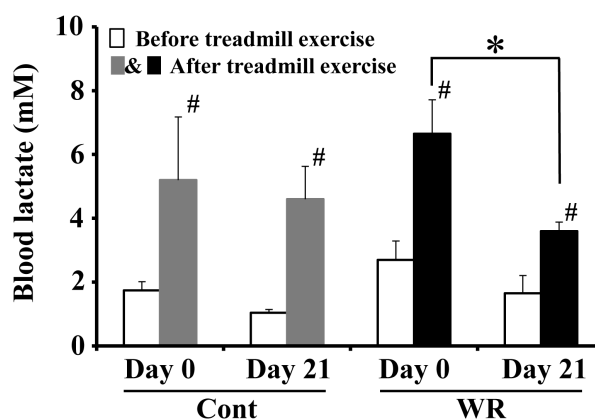


Figure 8. Blood lactate concentrations increased by treadmill exercise before/after 3-week voluntary wheel running (WR) and 3-week keeping in standard rodent cages (Cont). * $p < 0.05$ compared to “Day 0” data. # $p < 0.05$ compared to pre-treadmill exercise data. $N = 5$ in “Cont” and “WR”.

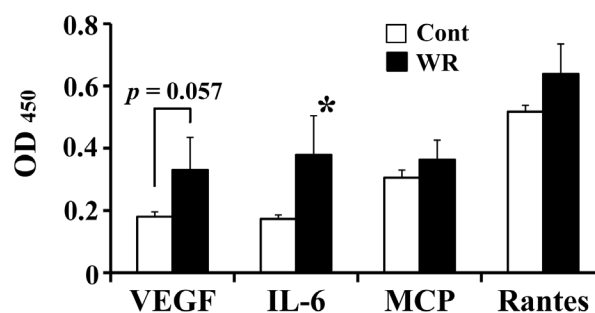


Figure 9. OD₄₅₀ of serum VEGF, IL-6, MCP-1 and RANTES in an ELISA after 3-week voluntary wheel running (WR) and 3-week keeping in standard rodent cages (Cont). Serum concentrations of these cytokines are directly proportional to OD₄₅₀. * $p < 0.05$ compared to “Cont” group. $N = 5$ in “Cont” and $n = 6$ in “WR”. OD₄₅₀: optical density at 450 nm; VEGF: vascular endothelial growth factor; IL-6: interleukine-6; MCP-1: monocyte chemoattractant protein-1.

distance) and an increased weekly food intake during the period of 3-week WR exercise, but nevertheless five of six rats showed a substantial reduction in BW after 3-week WR. In the previous studies using 4- - 6-week-old genetic model rats [12] [14] [15] [17] or high-fat diet-induced obesity rats [20] [23], on the other hand, these juvenile rats showed an extremely high WR activity (3-12 km of daily running distance). To compensate the increased energy expenditure by the WR exercise, they also showed a significantly higher caloric intake compared with the control sedentary rats [17] [23]. However, the WR rats gained less weight and consequently their growth curve was shifted downward compared with that of the sedentary rats [12] [17] [23]. In high-fat diet-induced obesity model rats, moreover, 3-week WR that started at 4 weeks of age was found to sustain reduction both in BW gain and in total fat pad mass during an additional 10 weeks of being sedentary, suggesting that only 3 weeks of WR is an exercise period sufficient to prevent obesity [22]. Therefore, we applied 3-week WR to our middle-aged fat rats.

4.2. Effects of WR on Waist Circumference, MFM, Adipocyte Size, Circulating Adipokines and CRP

In the previous studies, 4- or 7-week WR exercise that started at 3 - 5 weeks of age caused a decrease in various

fat pad mass [20] [23]. In this study, likewise, MFM weighed less in the WR exercised than in the sedentary animals. Moreover, MFM of our WR rats, which is correlated positively with BW or waist circumference regarded as one of risk factors for metabolic syndrome, was found to decrease as daily running distance increased, suggesting that voluntary WR exercise mainly utilized energy released from the augmented lipid metabolism. In addition, adipocyte size distribution in the present WR rats shifted leftward compared with that of the sedentary rats, as seen in WR exercised OLETF [17] and Osborne-Mendel rats [20]. In agreement with several previous studies of voluntary WR exercise [16] [17] [20]-[23], circulating levels of adipocyte-secreted leptin were significantly lower in our WR than in the sedentary rats, resulting from the decreased MFM of the WR rats. In fact, the reduction in leptin secretion and its mRNA expression was reported to be closely associated with smaller adipocyte size [25] [26].

Another adipokineresistin was reported to be a hormone that potentially links obesity to type 2 diabetes and contributes to insulin resistance *in vivo* [27]. In humans, however, there is considerable controversy as to the pathophysiological role of resistin [28]. In rodent, on the other hand, one of us previously reported that resistin, highly expressed in type 2 diabetic rat hearts, may cause cardiac hypertrophy and mechanical dysfunction [29]. Thus, the lower levels of serum resistin, which were found for the first time in our WR exercised rats, may contribute toward reducing the risk of developing insulin resistance and cardiac hypertrophy in obese rats.

Since CRP was reported to be a powerful inflammatory biomarker to predict the development of type 2 diabetes mellitus [30], peripheral arterial disease [31] and cardiovascular disease events [32], serum CRP concentration was measured in both WR exercised and sedentary animals in this study. Contrary to our expectations, however, the slightly higher levels of CRP were found in our WR rats. This higher CRP levels may be ascribable to the hepatic CRP production stimulated by IL-6 [33] [34], of which the higher serum levels were also found in our WR rats.

4.3. Effects of WR on BPs and BP-Related Hormones

In the present study, the 3-week WR caused a significant reduction in resting systolic/diastolic BPs of middle-aged normal rats in agreement with the previous reports in which resting systolic BP was lowered by the 4 - 10 week-WR in spontaneously hypertensive rats (SHRs) [12] [13] [15] and TG(mREN2)27 rats [14]. In both normotensive and hypertensive individuals, likewise, moderate-intensity exercise equivalent to half maximum oxygen consumption was reported to lower resting BPs [11] [35]-[37]. The most important finding of this study is that the voluntary WR for 3 weeks altered the circulating levels of five BP-related hormones (decrease in Angiotensin II, aldosterone, vasopressin and endothelin-1, and increase in BNP), which play pivotal roles in controlling BPs, and, consequently, induced a reduction in systolic/diastolic BPs.

The renin-angiotensin system is well-known to play a major role in the control of resting BPs. Angiotensin II increases BP both by constricting peripheral vessels and by directly stimulating adrenal production of aldosterone. We found that 3-week WR rats show a significantly lower level of serum angiotensin II compared to sedentary rats, and have a significantly decreased systolic/diastolic BP at rest. Similarly, 10 week-WR could prevent an increase in plasma angiotensin II and attenuate the elevation of systolic BP in young SHRs [15]. Our WR rats showed a decrease in mesenteric fat mass and adipocyte size, as compared with the control sedentary rats. In adipose tissue of rats, angiotensinogen messenger RNA was identified and angiotensinogen was produced [38] [39]. Further, systolic BP was modulated by fasting and refeeding parallel to adipocyte angiotensinogen expression [39]. Moreover, in mice with targeted overexpression of angiotensinogen in adipose tissue [40] and with a deficiency of endogenous angiotensinogen in adipocytes [41], adipocyte-derived angiotensinogen was demonstrated to contribute to systemic angiotensinogen concentrations and BP control. Based on these results, it is conceivable that adipocyte-derived angiotensinogen plays an important role in controlling resting BPs not only in normal, but also in exercise physiology. Endothelin-1 and vasopressin are also well-known as potent vasoconstrictors that contribute to hypertension. In addition to angiotensin II, our WR rat sera showed lower concentrations of endothelin-1 and vasopressin, as compared with the sedentary rat sera. Thus, WR exercise-induced reduction in resting BPs appears to be ascribable in part to attenuated vasoconstriction by these hormones.

On the other hand, aldosterone and vasopressin promote the body's retention of water and increase the blood volume, resulting in hypertension. Moreover, BNP with natriuretic effect is well-known to induce not only a reduction in blood volume, but also vasodilatation, and, consequently, to lower systemic BPs. Our WR rats

showed a decrease in circulating aldosterone and vasopressin levels and an increase in circulating BNP levels. Thus, it appears obvious that resting BPs lowered by the WR exercise are attributable in part to both vasodilatation and reduction in blood volume, which would be induced by the changed concentrations of these circulating hormones. In fact, the reduction in blood volume was reported to be closely linked to the antihypertensive effect of moderate-intensity exercise training using a bicycle ergometer in essential hypertensive subjects [37]. In juvenile SHR, furthermore, moderate-intensity exercise training on a motor-driven treadmill decreased cardiac output and, consequently, attenuated development of hypertension [42] [43].

Moreover, vasodilation is induced not only by hormone, but also by endothelial nitric oxide (NO) formation. Short-term running on a treadmill was found to stimulate the formation of endothelial NO, resulting in reduced BP [44] [45]. In addition, endothelial NO synthase (eNOS) mRNA and protein expression were elevated in rats with exercise training on the treadmill [46]. Likewise, voluntary WR was reported to increase NO formation and to decrease plasma angiotensin II concentration, and consequently, attenuated the elevation of systolic BP in SHR [15]. In addition, positive immunostaining for phospho-specific eNOS in cross sections of white gastrocnemius muscle was increased in OLETF rats with voluntary WR [18]. Thus, a WR-induced reduction in resting BP appears to be ascribable in part to peripheral vascular resistance decreased by enhanced endothelium-dependent vasodilation, which is mediated via the NO signaling pathway.

In addition, there seem three other possible mechanisms for the WR-induced reduction in resting BPs. First, BW loss caused by the present WR might have lowered resting BPs through diminished sympathetic nervous activity (SNA), because BW gain-induced sympathetic nervous activation was found to be tightly linked to BW gain-induced BP elevation [47]. In fact, the reduction both in resting BPs and in plasma norepinephrine concentrations that reflect SNA were observed in patients with essential hypertension, who were subjected to moderate-intensity aerobic exercise therapy for 10 or 20 weeks [35] [37]. Second, lowered serum leptin levels in our WR rats might have caused the fall in BPs at rest by decreased SNA. In Sprague-Dawley rats, acute intravenous infusion of leptin increased SNA to adrenal gland [48], and, furthermore, chronic leptin infusion caused a sustained increase in mean arterial pressure [49]. Therefore, leptin may play a crucial role not only in energy expenditure but also in cardiovascular regulation via sympathetic nervous system. Finally, the WR exercise may improve the abnormal expression of hypertension-related genes in the nucleus tractus solitarius (NTS), which is considered a central site that integrates the descending and ascending inputs in regulating baroreceptor function during exercise [50] [51]. Recently, one of us has reported that altered gene expression of the inflammatory molecules, but not of histamine receptor, may be related to the antihypertensive effects in exercise-trained SHR [52]. Thus, WR exercise-induced reduction in resting BPs may be accomplished via multiple mechanisms, *i.e.*, a combination of 1) hormone- and/or NO-induced vasodilatation, 2) attenuated vasoconstriction by hormones, 3) hormone-induced reduction in blood volume, and 4) diminished SNA by BW loss, lower leptin levels and/or improvement on abnormal gene expression of hypertension-related molecules in the NTS.

4.4. Effects of WR on Endurance Exercise Capacity

In the present study, we found that endurance exercise capacity of the middle-aged rats, assessed by the increase in blood lactate concentration, can be substantially augmented even by the poor WR exercise for 3 weeks, and the endurance exercise capacity, thus augmented, can be preserved in sedentary condition at least for a week after 3-week WR. It is well known that exercise-training can induce lots of adaptations in skeletal muscle. These include not only increases in the mitochondrial content, mitochondrial enzyme activity and the GLUT-4 isoform of the glucose transporter [53] [54] but also a fast-to-slower fiber type transformation of myosin heavy chain isoforms [55] [56]. In addition to such histochemical and metabolic adaptations, adaptive increase in skeletal muscle vascular transport capacity [57] appears to contribute to the increased maximal oxygen consumption, resulting in the enhanced endurance performance. In fact, voluntary WR exercise elicits increases in both citrate synthase activity and GLUT-4 protein expression in rat skeletal muscles [58]-[60], and consequently increases the maximal oxygen consumption [12] [14] [42] [43].

4.5. Effects of WR on Angiogenesis-Related Cytokines

The present WR exercise caused no change in the circulating levels of two inflammatory chemokines, *i.e.*, MCP-1 that contributes to macrophage infiltration into adipose tissue and insulin resistance [61] [62], and RANTES that plays an active role in accumulation of T cells in adipose tissue [63] [64]. In contrast to little or

no response of these chemokines, the WR exercise produced about twofold elevation in the circulating levels of VEGF and IL-6. The angiogenic growth factor VEGF, whose mRNA expression is up-regulated in the active muscles, is considered central in the angiogenic process in response to exercise [65] [66]. In fact, voluntary WR for 4 weeks [67] and treadmill exercise for 24 days [68] increased the mRNA and protein expression of VEGF, resulting in the development and formation of new capillaries in skeletal muscle. On the other hand, IL-6 is known to be expressed in both adipocytes and macrophages in adipose tissue [8], and is recently regarded, like VEGF, as one of myokines that is produced in and released from contracting skeletal muscles [34] [69]. The increased circulating IL-6, found in our WR rats, seems to be due mainly to the release of IL-6 from working skeletal muscles into the circulation and in part due to the IL-6 release from adipocyte tissue, because the adipocyte tissue mass reduced by the WR exercise is likely to produce a lesser amount of IL-6. Exercise-induced IL-6, which has both anti-inflammatory and insulin-sensitizing effects, may be involved in mediating the health beneficial effects of exercise and play key roles in protecting against diseases associated with low-level inflammation, insulin resistance and hyperlipidemia [34] [69].

5. Conclusion

In the middle-aged normal rats, the 3-week WR exercise with about 0.2 km of daily running distance decreased BW, the waist circumference, MFM, adipocyte size and circulating adipokines, *i.e.*, leptin and resistin, and induced the reduction in resting systolic/diastolic BPs by altering circulating BP-related hormones. Such a reduction in resting BPs may be accomplished via multiple mechanisms, *i.e.*, a combination of 1) hormone- and/or NO-induced vasodilatation, 2) attenuated vasoconstriction by hormones, 3) hormone-induced reduction in blood volume, and 4) diminished SNA by BW loss, lower leptin levels and/or improvement on abnormal gene expression of hypertension-related molecules in the NTS. Moreover, the endurance exercise capacity was enhanced even by this poor WR exercise. In addition, circulating VEGF and IL-6 were higher in the WR rats, suggesting angiogenesis, anti-inflammation and insulin-sensitization. These results support a prevalent idea that daily light-exercise, like walking and jogging, is a potential strategy for preventing metabolic syndrome.

Acknowledgements

We thank Ms. Y. Nishii for technical assistance and Prof. T. Yamamoto for critical reading of the manuscript. This study was supported in part by Grant-in-Aid for Scientific Research (C) from Japan Society for the Promotion of Science; 20590215 and 24590290 (S.S).

References

- [1] Despres, J.-P. and Lemieux, I. (2006) Abdominal Obesity and Metabolic Syndrome. *Nature*, **444**, 881-887. <http://dx.doi.org/10.1038/nature05488>
- [2] Kahn, S.E., Hull, R.L. and Utzschneider, K.M. (2006) Mechanisms Linking Obesity to Insulin Resistance and Type 2 Diabetes. *Nature*, **444**, 840-846. <http://dx.doi.org/10.1038/nature05482>
- [3] National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) (2001) Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA*, **285**, 2486-2497.
- [4] Grundy, S.M., Cleeman, J.I., Daniels, S.R., Donato, K.A., Eckel, R.H., Franklin, B.A., Gordon, D.J., Krauss, R.M., Savage, P.J., Smith Jr., S.C., Spertus, J.A. and Costa, F. (2005) Diagnosis and Management of the Metabolic Syndrome. An American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation*, **112**, 2735-2752. <http://dx.doi.org/10.1161/CIRCULATIONAHA.105.169404>
- [5] Wellen, K.E. and Hotamisligil, G.S. (2005) Inflammation, Stress, and Diabetes. *Journal of Clinical Investigation*, **115**, 1111-1119. <http://dx.doi.org/10.1172/JCI200525102>
- [6] Tilg, H. and Moschen, A.R. (2006) Adipocytokines: Mediators Linking Adipose Tissue, Inflammation and Immunity. *Nature Reviews Immunology*, **6**, 772-783. <http://dx.doi.org/10.1038/nri1937>
- [7] Wellen, K.E. and Hotamisligil, G.S. (2003) Obesity-Induced Inflammatory Changes in Adipose Tissue. *Journal of Clinical Investigation*, **112**, 1785-1788. <http://dx.doi.org/10.1172/jci20514>
- [8] Weisberg, S.P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R.L. and Ferrante Jr., A.W. (2003) Obesity Is Associated with Macrophage Accumulation in Adipose Tissue. *Journal of Clinical Investigation*, **112**, 1796-1808.

<http://dx.doi.org/10.1172/JCI200319246>

- [9] Xu, H., Barnes, G.T., Yang, Q., Tan, G., Yang, D., Chou, C.J., Sole, J., Nichols, A., Ross, J.S., Tartaglia, L.A. and Chen, H. (2003) Chronic Inflammation in Fat Plays a Crucial Role in the Development of Obesity-Related Insulin Resistance. *Journal of Clinical Investigation*, **112**, 1821-1830. <http://dx.doi.org/10.1172/JCI200319451>
- [10] Hu, F.B., Sigal, R.J., Rich-Edwards, J.W., Colditz, G.A., Solomon, C.G., Willett, W.C., Speizer, F.E. and Manson, J.E. (1999) Walking Compared with Vigorous Physical Activity and Risk of Type 2 Diabetes in Women. A Prospective Study. *JAMA*, **282**, 1433-1439. <http://dx.doi.org/10.1001/jama.282.15.1433>
- [11] Bassuk, S.S. and Manson, J.E. (2005) Epidemiological Evidence for the Role of Physical Activity in Reducing Risk of Type 2 Diabetes and Cardiovascular Disease. *Journal of Applied Physiology*, **99**, 1193-1204. <http://dx.doi.org/10.1152/jappphysiol.00160.2005>
- [12] Overton, J.M., Tipton, C.M., Matthes, R.D. and Leininger, J.R. (1986) Voluntary Exercise and Its Effects on Young SHR and Stroke-Prone Hypertensive Rats. *Journal of Applied Physiology*, **61**, 318-324.
- [13] LaPier, T.L.K., Swislocki, A.L.M., Clark, R.J. and Rodnick, K.J. (2001) Voluntary Running Improves Glucose Tolerance and Insulin Resistance in Female Spontaneously Hypertensive rats. *American Journal of Hypertension*, **14**, 708-715. [http://dx.doi.org/10.1016/S0895-7061\(01\)01285-7](http://dx.doi.org/10.1016/S0895-7061(01)01285-7)
- [14] Kinnick, T.R., Youngblood, E.B., O'keefe, M.P., Saengsirisuwan, V., Teachey, M.K. and Henriksen, E.J. (2002) Selected Contribution: Modulation of Insulin Resistance and Hypertension by Voluntary Exercise Training in the TG(mREN2)27 Rat. *Journal of Applied Physiology*, **93**, 805-812. <http://dx.doi.org/10.1152/jappphysiol.00236.2002>
- [15] Kohno, H., Furukawa, S., Naito, H., Minamitani, K., Ohmori, D. and Yamakura, F. (2002) Contribution of Nitric Oxide, Angiotensin II and Superoxide Dismutase to Exercise-Induced Attenuation of Blood Pressure Elevation in Spontaneously Hypertensive Rats. *Japanese Heart Journal*, **43**, 25-34. <http://dx.doi.org/10.1536/jhj.43.25>
- [16] Haskell-Luevano, C., Schaub, J.W., Andreasen, A., Haskell, K.R., Moore, M.C., Koerber, L.M., Rouzaud, F., Baker, H.V., Millard, W.J., Walter, G., Litherland, S.A. and Xiang, Z. (2009) Voluntary Exercise Prevents the Obese and Diabetic Metabolic Syndrome of the Melanocortin-4 Receptor Knockout Mouse. *The FASEB Journal*, **23**, 642-655. <http://dx.doi.org/10.1096/fj.08-109686>
- [17] Laye, M.J., Rector, R.S., Warner, S.O., Naples, S.P., Perretta, A.L., Uptergrove, G.M., Laughlin, M.H., Thyfault, J.P., Booth, F.W. and Ibdah, J.A. (2009) Changes in Visceral Adipose Tissue Mitochondrial Content with Type 2 Diabetes and Daily Voluntary Wheel Running in OLETF Rats. *The Journal of Physiology*, **587**, 3729-3739. <http://dx.doi.org/10.1113/jphysiol.2009.172601>
- [18] Mikus, C.R., Rector, R.S., Arce-Esquivel, A.A., Libla, J.L., Booth, F.W., Ibdah, J.A., Laughlin, M.H. and Thyfault, J.P. (2010) Daily Physical Activity Enhances Reactivity to Insulin in Skeletal Muscle Arterioles of Hyperphagic Otsuka Long-Evans Tokushima Fatty Rats. *Journal of Applied Physiology*, **109**, 1203-1210. <http://dx.doi.org/10.1152/jappphysiol.00064.2010>
- [19] Rector, R.S., Uptergrove, G.M., Borengasser, S.J., Mikus, C.R., Morris, E.M., Naples, S.P., Laye, M.J., Laughlin, M.H., Booth, F.W., Ibdah, J.A. and Thyfault, J.P. (2010) Changes in Skeletal Muscle Mitochondria in Response to the Development of Type 2 Diabetes or Prevention by Daily Wheel Running in Hyperphagic OLETF Rats. *The American Journal of Physiological Society: Endocrinology and Metabolism*, **298**, E1179-E1187. <http://dx.doi.org/10.1152/ajpendo.00703.2009>
- [20] Zachwieja, J.J., Hendry, S.L., Smith, S.R. and Harris, R.B.S. (1997) Voluntary Wheel Running Decreases Adipose Tissue Mass and Expression of Leptin mRNA in Osborne-Mendel Rats. *Diabetes*, **46**, 1159-1166. <http://dx.doi.org/10.2337/diab.46.7.1159>
- [21] Bradley, R.L., Jeon, J.Y., Liu, F.-F. and Maratos-Flier, E. (2008) Voluntary Exercise Improves Insulin Sensitivity and Adipose Tissue Inflammation in Diet-Induced Obese Mice. *The American Journal of Physiological Society: Endocrinology and Metabolism*, **295**, E586-E594. <http://dx.doi.org/10.1152/ajpendo.00309.2007>
- [22] Patterson, C.M., Dunn-Meynell, A.A. and Levin, B.E. (2008) Three Weeks of Early-Onset Exercise Prolongs Obesity Resistance in DIO Rats after Exercise Cessation. *American Journal of Physiology—Regulatory, Integrative and Comparative Physiology*, **294**, R290-R301. <http://dx.doi.org/10.1152/ajpregu.00661.2007>
- [23] Gollisch, K.S.C., Brandauer, J., Jessen, N., Toyoda, T., Nayer, A., Hirshman, M.F. and Goodyear, L.J. (2009) Effects of Exercise Training on Subcutaneous and Visceral Adipose Tissue in Normal- and High-Fat Diet-Fed Rats. *The American Journal of Physiological Society: Endocrinology and Metabolism*, **297**, E495-E504. <http://dx.doi.org/10.1152/ajpendo.90424.2008>
- [24] Matsumura, Y., Kita, S. and Okui, T. (2001) Potentiation by Endothelin-1 of Vasoconstrictor Response in Stroke-Prone Spontaneously Hypertensive Rats. *European Journal of Pharmacology*, **415**, 45-49. [http://dx.doi.org/10.1016/S0014-2999\(01\)00817-2](http://dx.doi.org/10.1016/S0014-2999(01)00817-2)

- [25] Skurk, T., Alberti-Huber, C., Herder, C. and Hauner, H. (2007) Relationship between Adipocyte Size and Adipokine Expression and Secretion. *The Journal of Clinical Endocrinology and Metabolism*, **92**, 1023-1033. <http://dx.doi.org/10.1210/jc.2006-1055>
- [26] Miyazaki, S., Izawa, T., Ogasawara, J., Sakurai, T., Nomura, S., Kizaki, T., Ohno, H. and Komabayashi, T. (2010) Effect of Exercise Training on Adipocyte-Size-Dependent Expression of Leptin and Adiponectin. *Life Sciences*, **86**, 691-698. <http://dx.doi.org/10.1016/j.lfs.2010.03.004>
- [27] Steppan, C.M., Balley, S.T., Bhat, S., Brown, E.J., Banerjee, R.R., Wright, C.M., Patel, H.R., Ahima, R.S. and Lazar, M.A. (2001) The Hormone Resistin Links Obesity to Diabetes. *Nature*, **409**, 307-312. <http://dx.doi.org/10.1038/35053000>
- [28] Rosen, E.D. and Spiegelman, B.M. (2006) Adipocytes as Regulators of Energy Balance and Glucose Homeostasis. *Nature*, **444**, 847-853. <http://dx.doi.org/10.1038/nature05483>
- [29] Kim, M., Oh, J.K., Sakata, S., Liang, L., Park, W.J., Hajjar, R.J. and Lebeche, D. (2008) Role of Resistin in Cardiac Contractility and Hypertrophy. *Journal of Molecular and Cellular Cardiology*, **45**, 270-280. <http://dx.doi.org/10.1016/j.yjmcc.2008.05.006>
- [30] Pradhan, A.D., Manson, J.E., Rifai, N., Buring, J.E. and Ridker, P.M. (2001) C-Reactive Protein, Interleukin 6, and Risk of Developing Type 2 Diabetes Mellitus. *The Journal of the American Medical Association*, **286**, 327-334. <http://dx.doi.org/10.1001/jama.286.3.327>
- [31] Ridker, P.M., Stampfer, M.J. and Rifai, N. (2001) Novel Risk Factors for Systemic Atherosclerosis. A Comparison of C-Reactive Protein, Fibrinogen, Homocysteine, Lipoprotein(a), and Standard Cholesterol Screening as Predictors of Peripheral Arterial Disease. *The Journal of the American Medical Association*, **285**, 2481-2485. <http://dx.doi.org/10.1001/jama.285.19.2481>
- [32] Ridker, P.M., Buring, J.E., Cook, N.R. and Rifai, N. (2003) C-Reactive Protein, the Metabolic Syndrome, and Risk of Incident Cardiovascular Events. An 8-Year Follow-Up of 14719 Initially Healthy American Women. *Circulation*, **107**, 391-397. <http://dx.doi.org/10.1161/01.CIR.0000055014.62083.05>
- [33] Van Gaal, L.F., Mertens, I.L. and De Block, C.E. (2006) Mechanisms Linking Obesity with Cardiovascular Disease. *Nature*, **444**, 875-880. <http://dx.doi.org/10.1038/nature05487>
- [34] Pedersen, B.K. and Febbraio, M.A. (2008) Muscle as an Endocrine Organ: Focus on Muscle-Derived Interleukin-6. *Physiological Reviews*, **88**, 1379-1406. <http://dx.doi.org/10.1152/physrev.90100.2007>
- [35] Kiyonaga, A., Arakawa, K., Tanaka, H. and Shindo, M. (1985) Blood Pressure and Hormonal Responses to Aerobic Exercise. *Hypertension*, **7**, 125-131. <http://dx.doi.org/10.1161/01.HYP.7.1.125>
- [36] Jennings, G., Nelson, L., Nestel, P., Esler, M., Korner, P., Burton, D. and Bazelmans, J. (1986) The Effects of Changes in Physical Activity on Major Cardiovascular Risk Factors, Hemodynamics, Sympathetic Function, and Glucose Utilization in Man: A Controlled Study of Four Levels of Activity. *Circulation*, **73**, 30-40. <http://dx.doi.org/10.1161/01.CIR.73.1.30>
- [37] Urata, H., Tanabe, Y., Kiyonaga, A., Ikeda, M., Tanaka, H., Shindo, M. and Arakawa, K. (1987) Antihypertensive and Volume-Depleting Effects of Mild Exercise on Essential Hypertension. *Hypertension*, **9**, 245-252. <http://dx.doi.org/10.1161/01.HYP.9.3.245>
- [38] Campbell, D.J. and Habener, J.F. (1987) Cellular Localization of Angiotensinogen Gene Expression in Brown Adipose Tissue and Mesentery: Quantification of Messenger Ribonucleic acid Abundance Using Hybridization *in Situ*. *Endocrinology*, **121**, 1616-1626. <http://dx.doi.org/10.1210/endo-121-5-1616>
- [39] Frederich, R.C., Kahn, B.B., Peach, M.J. and Flier, J.S. (1992) Tissue-Specific Nutritional Regulation of Angiotensinogen in Adipose Tissue. *Hypertension*, **19**, 33-344. <http://dx.doi.org/10.1161/01.HYP.19.4.339>
- [40] Massiera, F., Bloch-Faure, M., Ceiler, D., Murakami, K., Fukamizu, A., Gasc, J.-M., Quignard-Boulangé, A., Negrel, R., Ailhaud, G., Seydoux, J., Meneton, P. and Teboul, M. (2001) Adipose Angiotensinogen Is Involved in Adipose Tissue Growth and Blood Pressure Regulation. *The FASEB Journal*, **15**, 2727-2729. <http://dx.doi.org/10.1096/fj.01-0457fje>
- [41] Yiannikouris, F., Karounos, M., Charnigo, R., English, V.L., Rateri, D.L., Daugherty, A. and Cassis, L.A. (2012) Adipocyte-Specific Deficiency of Angiotensinogen Decreases Plasma Angiotensinogen Concentration and Systolic Blood Pressure in Mice. *American Journal of Physiology—Regulatory, Integrative and Comparative Physiology*, **302**, R244-R251. <http://dx.doi.org/10.1152/ajpregu.00323.2011>
- [42] Tipton, C.M., Sebastian, L.A., Overton, J.M., Woodman, C.R. and Williams, S.B. (1991) Chronic Exercise and Its Hemodynamic Influences on Resting Blood Pressure of Hypertensive Rats. *Journal of Applied Physiology*, **71**, 2206-2210.
- [43] Veras-silva, A.S., Mattos, K.C., Gava, N.S., Brum, P.C., Negrao, C.E. and Krieger, E.M. (1997) Low-Intensity Exercise Training Decreases Cardiac Output and Hypertension in Spontaneously Hypertensive Rats. *American Journal of*

Physiology, **273**, H2627-H263.

- [44] Sun, M.-W., Qian, F.-L., Wang, J., Tao, T., Guo, J., Wang, L., Lu, A.-Y. and Chen, H. (2008) Low-Intensity Voluntary Running Lowers Blood Pressure with Simultaneous Improvement in Endothelium-Dependent Vasodilatation and Insulin Sensitivity in Aged Spontaneously Hypertensive Rats. *Hypertension Research*, **31**, 543-552. <http://dx.doi.org/10.1291/hypres.31.543>
- [45] Sun, D., Huang, A., Koller, A. and Kaley, G. (1994) Short-Term Daily Exercise Activity Enhances Endothelial NO Synthesis in Skeletal Muscle Arterioles of Rats. *Journal of Applied Physiology*, **76**, 2241-2247.
- [46] Spier, S.A., Delp, M.D., Meininger, C.J., Donato, A.J., Ramsey, M.W. and Muller-Delp, J.M. (2004) Effects of Ageing and Exercise Training on Endothelium-Dependent Vasodilatation and Structure of Rat Skeletal Muscle Arterioles. *The Journal of Physiology*, **556**, 947-958. <http://dx.doi.org/10.1113/jphysiol.2003.060301>
- [47] Masuo, K., Mikami, H., Ogihara, T. and Tuck, M.L. (2000) Weight Gain-Induced Blood Pressure Elevation. *Hypertension*, **35**, 1135-1140. <http://dx.doi.org/10.1161/01.HYP.35.5.1135>
- [48] Haynes, W.G., Sivitz, W.I., Morgan, D.A., Walsh, S.A. and Mark, A.L. (1997) Sympathetic and Cardiorenal Actions of Leptin. *Hypertension*, **30**, 619-623. <http://dx.doi.org/10.1161/01.HYP.30.3.619>
- [49] Shek, E.W., Brands, M.W. and Hall, J.E. (1998) Chronic Leptin Infusion Increases Arterial Pressure. *Hypertension*, **31**, 409-414. <http://dx.doi.org/10.1161/01.HYP.31.1.409>
- [50] Mueller, P.J. and Hasser, E.M. (2006) Putative Role of the NTS in Alterations in Neural Control of the Circulation Following Exercise Training in Rats. *American Journal of Physiology—Regulatory, Integrative and Comparative Physiology*, **290**, R383-R394. <http://dx.doi.org/10.1152/ajpregu.00455.2005>
- [51] Michelini, L.C. and Stern, J.E. (2009) Exercise-Induced Neuronal Plasticity in Central Autonomic Networks: Role in Cardiovascular Control. *Experimental Physiology*, **94**, 947-960. <http://dx.doi.org/10.1113/expphysiol.2009.047449>
- [52] Waki, H., Gouraud, S.S., Bhuiyan, M.E.R., Takagishi, M., Yamazaki, T., Kohsaka, A. and Maeda, M. (2013) Transcriptome of the NTS in Exercise-Trained Spontaneously Hypertensive Rats: Implications for NTS Function and Plasticity in Regulating Blood Pressure. *Physiological Genomics*, **45**, 58-67. <http://dx.doi.org/10.1152/physiolgenomics.00074.2012>
- [53] Holloszy, J.O. and Coyle, E.F. (1984) Adaptations of Skeletal Muscle to Endurance Exercise and Their Metabolic Consequences. *Journal of Applied Physiology*, **56**, 831-838.
- [54] Holloszy, J.O. (2011) Regulation of Mitochondrial Biogenesis and GLUT 4 Expression by Exercise. *Comprehensive Physiology*, **1**, 921-940.
- [55] Fitzsimons, D.P., Diffie, G.M., Herrick, R.E. and Baldwin, K.M. (1990) Effects of Endurance Exercise on Isomyosin Patterns in Fast- and Slow-Twitch Skeletal Muscles. *Journal of Applied Physiology*, **68**, 1950-1955.
- [56] Sullivan, V.K., Powers, S.K., Criswell, D.S., Tumer, N., Larochelle, J.S. and Lowenthal, D. (1995) Myosin Heavy Chain Composition in Young and Old Rat Skeletal Muscle: Effects of Endurance Exercise. *Journal of Applied Physiology*, **78**, 2115-2120.
- [57] Sexton, W.L. (1995) Vascular Adaptations in Rat Hindlimb Skeletal Muscle after Voluntary Running-Wheel Exercise. *Journal of Applied Physiology*, **79**, 287-296.
- [58] Rodnick, K.J., Henriksen, E.J., James, D.E. and Holloszy, J.O. (1992) Exercise Training, Glucose Transporters, and Glucose Transport in Rat Skeletal Muscles. *American Journal of Physiology*, **262**, C9-C14.
- [59] Henriksen, E.J. and Halseth, A.E. (1995) Adaptive Responses of GLUT-4 and Citrate Synthase in Fast-Twitch Muscle of Voluntary Running Rats. *American Journal of Physiology*, **268**, R130-R134.
- [60] Hokama, J.Y., Streeper, R.S. and Henriksen, E.J. (1997) Voluntary Exercise Training Enhances Glucose Transport in Muscle Stimulated by Insulin-Like Growth Factor I. *Journal of Applied Physiology*, **82**, 508-512.
- [61] Kamei, N., Tobe, K., Suzuki, R., Ohsugi, M., Watanabe, T., Kubota, N., Ohtsuka-Kowatari, N., Kumagai, K., Sakamoto, K., Kobayashi, M., Yamauchi, T., Ueki, K., Oishi, Y., Nishimura, S., Manabe, I., Hashimoto, H., Ohnishi, Y., Ogata, H., Tokuyama, K., Tsunoda, M., Ide, T., Murakami, K., Nagai, R. and Kadowaki, T. (2006) Overexpression of Monocyte Chemoattractant Protein-1 in Adipose Tissue Causes Macrophage Recruitment and Insulin Resistance. *The Journal of Biological Chemistry*, **281**, 26602-26614. <http://dx.doi.org/10.1074/jbc.m601284200>
- [62] Kanda, H., Tateya, S., Tamori, Y., Kotani, K., Hiasa, K., Kitazawa, R., Kitazawa, S., Miyachi, H., Maeda, S., Egashira, K. and Kasuga, M. (2006) MCP-1 Contributes to Macrophage Infiltration into Adipose Tissue, Insulin Resistance, and Hepatic Steatosis in Obesity. *Journal of Clinical Investigation*, **116**, 1494-1505. <http://dx.doi.org/10.1172/JCI26498>
- [63] Matter, C.M. and Handschin, C. (2007) RANTES (Regulated on Activation, Normal T Cell Expressed and Secreted), Inflammation, Obesity, and the Metabolic Syndrome. *Circulation*, **115**, 946-948. <http://dx.doi.org/10.1161/CIRCULATIONAHA.106.685230>
- [64] Wu, H., Ghosh, S., Perrard, X.D., Feng, L., Garcia, G.E., Perrard, J.L., Sweeney, J.F., Peterson, L.E., Chan, L., Smith,

- C.W. and Ballantyne, C.M. (2007) T-Cell Accumulation and Regulated on Activation, Normal T Cell Expressed and Secreted Upregulation in Adipose Tissue in Obesity. *Circulation*, **115**, 1029-1038. <http://dx.doi.org/10.1161/CIRCULATIONAHA.106.638379>
- [65] Prior, B.M., Yang, H.T. and Terjung, R.L. (2004) What Makes Vessels Grow with Exercise Training? *Journal of Applied Physiology*, **97**, 1119-1128. <http://dx.doi.org/10.1152/jappphysiol.00035.2004>
- [66] Bloor, C.M. (2005) Angiogenesis during Exercise and Training. *Angiogenesis*, **8**, 263-271. <http://dx.doi.org/10.1007/s10456-005-9013-x>
- [67] Waters, R.E., Rotevatn, S., Li, P., Annex, B.H. and Yan, Z. (2004) Voluntary Running Induces Fiber Type-Specific Angiogenesis in Mouse Skeletal Muscle. *American Journal of Physiology-Cell Physiology*, **287**, C1342-C1348. <http://dx.doi.org/10.1152/ajpcell.00247.2004>
- [68] Lloyd, P.G., Prior, B.M., Yang, H.T. and Terjung, R.L. (2003) Angiogenic Growth Factor Expression in Rat Skeletal Muscle in Response to Exercise Training. *The American Journal of Physiology: Heart and Circulatory Physiology*, **284**, H1668-H1678. <http://dx.doi.org/10.1152/ajpheart.00743.2002>
- [69] Pedersen, B.K. (2009) Muscle as an Endocrine Organ: IL-6 and Other Myokines. *Journal of Applied Physiology*, **107**, 1006-1014. <http://dx.doi.org/10.1152/jappphysiol.00734.2009>

Abbreviation

BNP, brain natriuretic peptide; BP, blood pressure; BP_{max}, maximum blood pressure; BP_{mean}, mean blood pressure; BP_{min}, minimum blood pressure; BW, body weight; CRP, C-reactive protein; eNOS, endothelial NO synthase; FGF- β , fibroblast growth factor- β ; IFN- γ , interferon- γ ; IL-6, interleukine-6; MCP-1, monocyte chemoattractant protein-1; MFM, mesenteric fat mass; NO, nitric oxide; NTS, nucleus tractussolitariae; SHR, spontaneously hypertensive rat; SNA, sympathetic nervous activity; TNF- α , tumor necrosis factor- α ; VEGF, vascular endothelial growth factor; WR, wheel running.