Long-term voluntary running improves diet-induced adiposity in young adult mice☆

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ABSTRACT

The hypothesis of the present study was that physical activity improves diet-induced obesity in young adult mice. Four-week-old male C57BL/6 mice (n=15/group) were fed the AIN93G diet or a 45% high-fat diet (%kJ) with or without access to in-cage activity wheels for 14 weeks. The high-fat diet increased percentage fat body mass compared to the AIN93G diet ($P=.042$); running reduced percentage fat body mass ($P<.0001$) and increased percentage lean body mass ($P<.0001$) in mice fed either diet. Compared with the AIN93G diet, the high-fat diet increased plasma concentrations of insulin ($P<.05$) and leptin ($P<.05$) in sedentary mice and inflammatory cytokines monocyte chemotactic protein–1 (MCP-1) ($P<.05$) and plasminogen activator inhibitor–1 (PAI-1) ($P<.05$) in both sedentary and running mice. The high-fat diet did not affect angiogenic factors vascular endothelial growth factor and platelet-derived growth factor–BB. Running reduced plasma insulin ($P<.05$) and MCP-1 ($P<.05$) and increased platelet-derived growth factor–BB ($P<.05$) in mice fed the high-fat diet. Running reduced leptin ($P<.05$) and increased plasma vascular endothelial growth factor ($P<.0001$) regardless of diet fed. In summary, consumption of the high-fat diet increased adiposity in young adult mice; running reduced adiposity, normalized plasma insulin and leptin, and reduced MCP-1 despite continued consumption of the high-fat diet. These results suggest that voluntary running may reduce diet-induced obesity and proinflammation and that young mice may be a useful model of their human age equivalents in studying moderate physical exercise and obesity and obesity-related diseases.

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1. Introduction

Overweight and obesity among children and adolescents continue to be a public health concern in the United States. Overweight or obese children often become obese adults [1] who are at risk of chronic diseases such as diabetes, cardiovascular diseases, and certain cancers [2]. The increasing number of obese children and teenagers is of particular concern. In 2007-2008, an estimated 18.1% of adolescents ages 12 to 19 years were obese [3], which was a 13% increase from 1976-1980 [4]. It has been well documented that decreased physical activity and consumption of hypercaloric diets contribute to the prevalence of overweight and obesity [5].

Physical activity is defined as bodily movement due to skeletal muscle contraction that results in energy expenditure [6]. Physical activity exerts anti-inflammatory activities [7] and induces capillary growth in heart [8] and skeletal muscle [9,10]. Laboratory rodents are useful in studies that involve physical exercise because they can be easily trained or managed for various types of activities to meet the study needs. Forced
running on a treadmill and voluntary running on an activity wheel are 2 commonly used models in laboratory animals [11]. The former permits investigators to define exercise duration, frequency, and intensity; and the latter allows animals to determine the duration, frequency, and intensity of the activity. Available studies show that forced physical activity reduces obesity in laboratory rodents [12,13] but that the stress related to its forced nature is a potential study confounder. Voluntary running reduces diet-induced obesity in mice; however, the ages at which the exercises were initiated and the durations of the exercise vary greatly between studies [13–15]. There have not been any studies, to our knowledge, that have assessed the effects of long-term voluntary running on obesity in young mice that mimic their human age equivalents from adolescents to young adults.

Mice sexually mature by 35 days after birth, and maturational growth continues for most biological processes and structures until they are young adults at about 3 months of age [16]. This life phase is comparable to human beings as they transition from teenagers to young adults. Mice in this age group are not affected by senescence, are at their highest survival rate [16], and are used commonly in studies of human diseases. This age group may be useful to model diet-induced body composition changes in its human age equivalents and to study the effects of moderate physical activity on these changes.

The hypothesis of the present study was that physical activity improves diet-induced obesity in young adult mice. The objectives were to investigate the effects of long-term voluntary running on high-fat diet-induced adiposity and body composition changes and to examine changes in related biomarkers including adipokines, inflammatory cytokines, and angiogenic factors in C57BL/6 mice at the life phase from sexual maturation to young adulthood.

2. Methods and materials

2.1. Animals and diets

Three-week-old male C57BL/6 mice (Harlan, Madison, Wisconsin) were housed in a pathogen-free room on a 12:12-hour light-dark cycle at 22°C ± 1°C. Two diets were compared in this study: the AIN93G diet [17] and a modified AIN93G diet with 45% energy from corn oil (% kJ; Table). Both diets were pelleted using a laboratory pellet mill (Model CI, CA Pellet Mill, San Francisco, California) and were stored at −20°C before being provided to mice.

2.2. Experimental design

This study was approved by the Animal Care and Use Committee of the Department of Agriculture, Agricultural Research Service (USDA, ARS), Grand Forks Human Nutrition Research Center. The procedures followed the National Institutes of Health guidelines for the care and use of laboratory animals [18].

Sixty mice were acclimated for 1 week before they were randomly assigned into 4 groups of 15 each. They were fed the AIN93G diet or the 45% fat diet with or without access to in-cage activity wheels (Model 80820; Lafayette Instrument, Lafayette, Indiana). Sedentary mice were individually housed in wire-topped plastic boxes. Running mice were individually housed in cages with freely accessible in-cage activity wheels, and their running distances (kilometers) were recorded daily by a computerized animal wheel monitor system (AWM software version 11.0; Lafayette Instrument). Mice had free access to their diets and deionized water, and they were weighed weekly. Food intake was recorded daily for 10 consecutive days beginning in the ninth week of the experimental feeding. One week before the termination of the experiment, body composition analysis of fat and lean body mass of conscious, immobilized mice was performed using quantitative magnetic resonance (Echo whole-body composition analyzer, Model 100; Echo Medical System, Houston, Texas). At the termination of the experiment (week 14), mice were fasted overnight and then anesthetized with a mixture of ketamine and xylazine. Abdominal adipose tissues (gonadal and perirenal) were collected and weighed. Soleus muscles from both legs and plasma were collected and stored at −80°C for analyses of citrate synthase activity and for plasma concentrations of insulin, triglycerides, cytokines, and angiogenic factors, respectively.

2.3. Citrate synthase activity

Citrate synthase activity, which mediates skeletal muscle oxidative capacity, was used as an index of the exercise status of the mice. Maximal citrate synthase activity was determined spectrophotometrically on soleus muscle homogenates using the method of Kennedy et al [19]. Citrate synthase activity was expressed as micromoles per minute per milligram protein at 25°C.

2.4. Plasma concentrations of insulin, triglycerides, cytokines, and angiogenic factors

Sandwich enzyme-linked immunosorbent assay kits were used to quantify plasma concentrations of insulin, leptin, adiponectin, vascular endothelial growth factor (VEGF), platelet-derived growth factor-BB (PDGF-BB), monocyte chemotactic protein-1 (MCP-1) (all aforementioned were from R&D System, Minneapolis, Minnesota), plasminogen activator

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**Table – Composition of experimental diets**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>AIN93G</th>
<th>High fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>g/kg</td>
<td>kJ</td>
<td>g/kg</td>
</tr>
<tr>
<td>Corn starch</td>
<td>389.6</td>
<td>6519</td>
</tr>
<tr>
<td>Casein</td>
<td>200</td>
<td>3347</td>
</tr>
<tr>
<td>Dextrose</td>
<td>132</td>
<td>2209</td>
</tr>
<tr>
<td>Sucrose</td>
<td>106.2</td>
<td>1778</td>
</tr>
<tr>
<td>Corn oil</td>
<td>70</td>
<td>2636</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Mineral mix</td>
<td>35</td>
<td>96</td>
</tr>
<tr>
<td>Vitamin mix</td>
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<td>163</td>
</tr>
<tr>
<td>L- Cystine</td>
<td>4.4</td>
<td>75</td>
</tr>
<tr>
<td>L- Methionine</td>
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<td>4</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>TBHQ</td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1000</td>
<td>16828</td>
</tr>
</tbody>
</table>

TBHQ: Tert-butyldihydroquinone.
inhibitor–1 (PAI-1) (Molecular Innovations, Southfield, Michigan), and triglycerides (Cayman Chemical, Ann Arbor, Michigan) following the manufacturers’ protocols. Samples were read within the linear range of the assay, and the accuracy of the analysis was confirmed by the controls provided in each enzyme-linked immunosorbent assay kit.

2.5. Statistical analyses

Differences in running distances and citrate synthase activity were tested using Student t test. The effects of diet (AIN93G or high fat) and running status (sedentary or running) and their interaction were tested using two-way analysis of variance (ANOVA). When the interaction between diet and running status was statistically significant, Tukey contrasts were performed to compare the 4 groups. All data are presented as means±SEM. Differences with a P value of .05 or less were considered statistically significant. All statistical analyses were performed using SAS software (version 9.2; SAS Institute, Inc, Cary, North Carolina).

3. Results

3.1. Daily running distance, citrate synthase activity, body weight, and food intake

Mice assigned to the 2 running groups ran 4 to 7 km/d voluntarily for the duration of the experiment, which is similar to the reported daily running distance for the C57BL/6 strain [20]. There was no significant difference in daily running distance between the 2 groups (Fig. 1A). Citrate synthase activity was 1.24±0.06 and 1.82±0.05 μmol/(min mg) protein for sedentary and running mice (P <.0001; n=20, 10 from each of the sedentary groups and 10 from each of the running groups), respectively. Consumption of the high-fat diet increased body weight in sedentary mice compared with their AIN93G-fed counterparts at week 14 of the experiment feeding (P =.048; Fig. 1B). After 10 weeks, voluntary running reduced body weight in mice fed either diet compared with their sedentary counterparts (P <.0001; Fig. 1B); and the significant reduction continued throughout the experiment. There were no differences in food intake among the groups. The overall mean food intake was 3.44±0.04 g/d.

3.2. Body weight gain and body composition

Consumption of the high-fat diet and voluntary running significantly affected body composition. The high-fat diet increased (P =.035) and running reduced (P <.0001) weight gain compared with their respective controls (Fig. 2A). Similarly, the high-fat diet increased (P =.042) and running reduced (P <.0001) percentage fat body mass compared with their respective controls (Fig. 2B). Similar results were observed with fat mass and lean mass weights (data not shown) and with abdominal adipose tissue weights (gonadal and perirenal; data not shown). The high-fat diet did not affect percentage lean body mass compared with the AIN93G diet (P =.306), but running mice had greater percentage lean body mass compared to sedentary mice (P <.0001; Fig. 2C).

3.3. Plasma insulin, adipokines, and triglycerides

Dietary fat supplementation and voluntary running affected plasma concentrations of insulin and adipokines. In sedentary mice, consumption of the high-fat diet resulted in a 2.8-fold increase in plasma insulin concentrations compared with the AIN93G diet (P <.05). Running did not affect plasma insulin in the AIN93G-fed mice, but significantly reduced plasma insulin in the high-fat diet-fed mice (P <.05) compared with their respective sedentary controls (Fig. 3A). Feeding sedentary mice the high-fat diet resulted in a 2-fold increase in plasma leptin concentrations compared with the AIN93G diet (P <.05). Running resulted in a 65% reduction in leptin in the AIN93G-fed mice (P <.05) and an 80% reduction in the high-fat diet-fed mice (P <.05) compared with their respective sedentary controls (Fig. 3B). The high-fat diet did not affect plasma adiponectin

Fig. 1 – Average daily running distance (A) and body weight changes in mice (B) during the experiment. There was no significant difference in daily running distance between the groups (A). Consumption of the high-fat diet increased body weight in sedentary mice compared with their AIN93G-fed counterparts at week 14 of the experiment feeding (P =.048); running reduced body weight in mice fed both diets compared with their sedentary counterparts after 10 weeks of running (P <.0001), and the significant reduction continued throughout the experiment (B). Two-way ANOVA and Tukey contrasts were performed for statistical analysis. AIN: AIN93G diet; AIN+Run: AIN93G diet with running; HFD: high-fat diet; HFD+Run: high-fat diet with running. Values are means±SEM (n=15/group).
concentrations compared with the AIN93G diet; running resulted in a 42% increase in adiponectin in the AIN93G-fed mice ($P<.05$) but not in mice fed the high-fat diet (Fig. 3C). Furthermore, the high-fat diet increased ($P<.0001$) and running reduced ($P=.004$) plasma levels of triglycerides compared with their respective controls (Fig. 3D).

3.4. Plasma inflammatory cytokines and angiogenic factors

Obesity is accompanied with increased inflammation and angiogenesis. Plasma concentrations of MCP-1 in the high-fat diet–fed mice were 6.6-fold greater than those in the AIN93G-fed mice ($P<.05$; Fig. 4A); running reduced plasma MCP-1 in the high-fat diet–fed mice by 37% ($P<.05$), but did not affect MCP-1 in the AIN93G-fed mice (Fig. 4A). Consumption of the high-fat diet resulted in significantly greater plasma concentrations of PAI-1 compared with the AIN93G-fed mice ($P<.05$; Fig. 4B). Running resulted in a 4-fold increase in PAI-1 in the AIN93G-fed mice ($P<.05$), but did not affect plasma PAI-1 levels in the high-fat diet–fed mice (Fig. 4B). The high-fat diet did not affect plasma concentrations of either VEGF or PDGF-BB compared with the AIN93G diet (Fig. 4C and D). Running resulted in a 40% to 50% increase in VEGF in mice fed either diet compared with their respective sedentary controls ($P<.0001$; Fig. 4C). Furthermore, running resulted in an 83% increase in PDGF-BB in the AIN93G-fed mice ($P>0.05$) and an approximately 3-fold increase in the high-fat diet–fed mice compared with their respective sedentary controls ($P<.05$; Fig. 4D).

4. Discussion

Mice voluntarily ran when activity wheels were made available. Voluntary running at 4 to 7 km/d reduced fat body mass in the high-fat diet–fed mice despite continued consumption of the high-fat diet. This reduction was not due to changes in food consumption, as there were no significant differences in food intake among the groups.

Increases in fat body mass were responsible for the significant weight gain in mice fed the high-fat diet, as lean body mass was similar between the AIN93G and high-fat diet–fed mice. Running reduced fat body mass of the high-fat diet–fed mice to the levels of AIN93G-fed running mice, indicating that long-term voluntary running fully reversed the increased fat body mass by the high-fat diet in these young adult mice.

The reduction in fat body mass in the high-fat diet–fed running mice was associated with a complete reversal of the high plasma insulin concentrations. Fasting plasma insulin levels were significantly greater in the high-fat diet–fed sedentary mice, and running reduced it to the levels of the AIN93G-fed controls. This normalization persisted despite continued intake of the high-fat diet. Moderate physical activity increases insulin utilization and caloric metabolism [14]; and thus, the reduction in fat body mass is secondary to the insulin normalization in the high-fat diet–fed running mice.

Leptin and adiponectin are adipose-produced hormones. Leptin regulates body weight through its effects on food intake and energy expenditure [21], and circulating leptin concentrations are directly proportional to the total amount of visceral adiposity in the body [22]. Consistent with the reduction in fat body mass, voluntary running in the present study significantly reduced plasma leptin concentrations in the high-fat diet–fed mice. Adiponectin modulates glucose regulation and fatty acid catabolism [23]; and in humans, plasma adiponectin levels are reduced in overweight and obese adults and elevated in those with normal body weight [24]. In laboratory animals, increases in blood adiponectin concentrations have been reported in mice fed high-fat diets [25, 26], which may be an initial response to counteract diet-induced obesity and insulin resistance [26]. The observations that neither the high-fat diet reduced nor running increased plasma adiponectin levels in the high-fat diet–fed mice are consistent with findings from a 6-week running study with C57BL/6 mice [14], suggesting that adiponectin may not be involved in running-induced reduction in fat body mass.

Triglycerides are major components of very low density lipoproteins and chylomicrons [27]. High levels of triglycerides in the bloodstream are associated with atherosclerosis, a risk factor of heart disease and stroke [27]. Greater amounts of fat body mass in the high-fat diet–fed mice were accompanied by
greater plasma triglyceride concentrations, and running significantly reduced triglyceride levels compared with the sedentary controls. These results indicate the benefits of moderate physical activity in improving blood lipid profiles in these young adult mice.

Obesity dysregulates production of several inflammatory cytokines that contribute to chronic inflammation in obese individuals [28]. We quantified plasma levels of 2 key inflammatory markers: MCP-1 and PAI-1. Monocyte chemoattractant protein–1 is elevated in obesity [29,30], and its elevation may promote systemic proinflammatory cellular events [31]. In the present study, running significantly reduced MCP-1 levels in the high-fat diet–fed mice, indicating an inhibition of obesity-mediated proinflammatory changes.

Plasminogen activator inhibitor–1 is a major component of the plasminogen activator system, and its principle physiological function is to inhibit tissue-type and urokinase-type plasminogen activators [32]. Adipose tissues produce PAI-1 [33,34], and the expression of PAI-1 is positively correlated with body mass index in humans [35] and is increased with diet-induced obesity in mice [36]. Plasminogen activator inhibitor–1 deficiency prevents fat accumulation in mice in both diet-induced [36] and genetic obese murine models [37]. Results from the present study are consistent with the existing knowledge that increases in adiposity elevate plasma PAI-1 levels. That voluntary running increased plasma PAI-1 in AIN93G-fed mice suggests exercise-induced angiogenesis in skeletal muscles. Both vascular endothelial [38] and smooth muscle cells [39] produce PAI-1. In vascular endothelial cells, PAI-1 is induced by hypoxia [40]. That running did not affect plasma PAI-1 levels in the high-fat diet–fed mice suggests the possibility that voluntary running, by reducing fat body mass, may attenuate the adipose-mediated increases in PAI-1, but this portion of reduction may be compensated by the increases due to muscular angiogenesis.

Angiogenesis, a complex process that involves the participation of multiple angiogenic factors, plays an important role in adipogenesis [41]. Vascular endothelial growth factor accounts for most of the angiogenic activity in adipose tissues [42], and PDGF-BB and VEGF have a potent synergistic effect in inducing neovascularization [43]. Interestingly, the high-fat diet did not affect plasma concentrations of either VEGF or PDGF-BB compared with the AIN93G diet; however, it significantly increased plasma levels of leptin in sedentary mice and MCP-1 and PAI-1 in sedentary and running mice. Both leptin [44] and MCP-1 [45,46] have direct proangiogenic activities, and PAI-1 contributes to angiogenesis by regulating plasmin-mediated proteolysis or by modulating cell migration by affecting cell-matrix interactions [47]. Thus, the angiogenic activities during the high-fat diet-induced adipogenesis were likely through...
mechanisms that were not mediated by VEGF and PDGF-BB, but by others including leptin, MCP-1, and PAI-1.

Increases in plasma levels of VEGF and PDGF-BB by voluntary running are an adaptive response to physical activities [48,49]. Capillary growth in skeletal muscles is a direct response to exercise [9], and angiogenesis occurs as an adaptation to long-term exposure to hypoxia [10]. Significant increases in plasma PDGF-BB in the high-fat diet–fed running mice compared with those in the AIN93G-fed counterparts indicate that adipose-derived factors may be involved in or stimulate the production of PDGF-BB.

We chose the voluntary running model because mice run in a self-controlled, physically capable manner, which is an advantage over the treadmill running model due to the stress associated with its forced nature. A potential limitation of the study is that the rodent life span differs from humans and mice mature faster than their human age equivalents [16]. Thus, the results obtained in this study of long-term voluntary running during this rapid maturation phase may not be able to be extrapolated directly to humans.

In summary, feeding mice a 45% high-fat diet from sexual maturation to 18 weeks of age significantly increased fat body mass and plasma concentrations of insulin, leptin, triglycerides, MCP-1 and PAI-1; running significantly reduced the diet-induced increases in fat body mass and related biomarkers. These results support our hypothesis that physical activity improves diet-induced obesity in young adult mice, and indicate that voluntary running benefits young adult mice in reducing diet-induced adiposity and proinflammation changes. Furthermore, our results indicate that young mice respond to both a high-fat diet and nonmotorized voluntary running and suggest that young mice may be useful as a model of their human age equivalents in studying moderate physical activity and obesity and obesity-related diseases.

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