Physical Activity Prevents Augmented Body Fat Accretion in Moderately Iron-Deficient Rats¹,²

James P. McClung,³* Nancy E. Andersen,³ Tyson N. Tarr,³ Chad H. Stahl,⁴ and Andrew J. Young³

¹Military Nutrition Division, U.S. Army Research Institute of Environmental Medicine, Natick, MA 01760 and ²Department of Animal Science, North Carolina State University, Raleigh, NC 27695

Abstract

Recent studies describe an association between poor iron status and obesity in humans, although the mechanism explaining this relationship is unclear. The present study aimed to determine the effect of moderate iron deficiency and physical activity (PA) on body composition in an animal model. Male Sprague-Dawley rats consumed iron-adequate (IA; 40 mg/kg) or moderately iron-deficient (ID; 9 mg/kg) diets ad libitum for 12 wk. Rats were assigned to 4 treatment groups (n = 10 per group): IA, sedentary (IAS); IA, PA (IAPA); ID, sedentary (IDS); or ID, PA (IDPA). Activity involved running on motorized running wheels at 4 m/min for 1 h/d for 5 d/wk. After 12 wk, ID rats were not anemic, but body iron stores were reduced as indicated by diminished (P < 0.05) femur iron compared with IA rats. Treatment group did not affect body weight or feed consumption. However, fat mass was greater (P < 0.05) in IDS rats (38.6 ± 6.7%) than IAS (31.8 ± 2.9%), IAPA (31.8 ± 2.0%), and IDPA (32.8 ± 4.5%) rats. Furthermore, lean body mass was diminished in IDS rats (58.7 ± 6.8%) compared with IAS (65.6 ± 3.0%), IAPA (65.6 ± 2.1%), and IDPA (64.7 ± 4.5%) rats. Thus, moderate iron deficiency may cause increased body fat accretion in rats and PA attenuates that effect.  J. Nutr. 138: 1293–1297, 2008.

Introduction

Iron deficiency is the most prevalent micronutrient deficiency in the world (1). The prevalence of iron deficiency and iron deficiency anemia is highest in the developing world; however, suboptimal iron status continues to exist in developed nations (2,3). Iron deficiency and iron deficiency anemia have important public health implications, because diminished iron status affects cognitive development and behavior, energy metabolism, immune function, bone health, and work capacity in humans and animals (4–7).

The best described functions of dietary iron occur through its incorporation into proteins and enzymes necessary for optimal work performance. However, iron may also function in the maintenance of body weight and composition, as a number of studies have suggested an association between iron status and obesity. The first, published in 1962, demonstrated significantly lower serum iron concentrations in obese adolescents compared with controls (8). More recently, in a cross-sectional study, overweight Israeli children and adolescents had lower iron status compared with normal-weight individuals (9). Data from NHANES III supports these findings, as multivariate regression analysis determined that overweight American children were twice as likely to be iron deficient than normal-weight children (10). Similar associations have also been reported in adults (11,12).

The potential role of dietary iron in the maintenance of body weight and composition may not be limited to body fat, as iron is critical for the optimization of bone health in humans and in animals (7,13–16). For example, 1 recent study described a positive association between dietary iron and measures of bone mineral density (BMD) in healthy postmenopausal women (7).

The relationship between iron status, obesity, and bone health may have important public health implications as the prevalence of obesity and obesity-related morbidity continues to climb (17). However, the mechanism explaining reduced iron status in obese individuals remains undefined and the effects of moderate iron deficiency on bone health are poorly understood. In this study we test the hypothesis that moderate iron deficiency may cause changes in body composition in a growing rat model. We studied moderately iron-deficient growing rats, as 1 of our objectives was to develop a model reflective of a human demographic known to demonstrate a relationship between iron status and obesity (8,9). Furthermore, a secondary objective was to determine whether physical activity (PA) could modulate...

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³ To whom correspondence should be addressed. E-mail: james.mcclung@na.amedd.army.mil.

4 Abbreviations used: BMD, bone mineral density; DEXA, dual energy X-ray absorptiometry; FM, fat mass; Hgb, hemoglobin; IA, iron adequate; IAPA, iron adequate, physical activity; IAS, iron adequate, sedentary; ID, iron deficient; IDPA, iron deficient, physical activity; IDS, iron deficient, sedentary; LBMC, lean body mass; PA, physical activity; RDW, red cell distribution width; TIBC, total iron-binding capacity; TS, transferrin saturation.
changes in body composition observed in moderate iron deficiency.

**Materials and Methods**

**Animals and diets.** Male Sprague-Dawley rats (10 wk old) were housed individually in a climate-controlled animal room with a 12-h-light/dark cycle and given free access to feed and distilled water for the 12-wk study period. Rats were assigned to 1 of 4 treatment groups (n = 10): iron adequate (IA); sedentary (IAS); IA, PA (IAPA); iron deficient (ID), sedentary (IDS); and ID, PA (IDPA). Groups were matched for body weight at the beginning of the study. Purified diets were prepared by Research Diets and were based on the AIN-93G laboratory rodent formulation (18) with less ferric citrate added to the mineral mix of the ID diets (Table 1). The final measured iron concentration of the IA diet was 40.1 mg/kg, and 8.9 mg/kg for the ID diet.

Rats assigned to the IAPA and IDPA groups performed regular sessions of PA. Rats were removed from home cages and transferred to cages containing motorized running wheels (Lafayette Instrument) for 60 min/d, 5 d/wk at a speed of 4 m/min, 7 d/wk on sleep in older (22 mo) rats with no reported negative health consequences. In our study, rats were monitored for discomfort while in the running wheels and no obvious negative health consequences of PA were observed. To control for the potential stress associated with movement, rats assigned to the sedentary groups were transferred from home cages to the cages containing running wheels kept in the locked position for 60 min/d, 5 d/wk.

Body weight, feed, and water consumption were measured at regular intervals. Following the 12-wk study period, food-deprived rats were killed by carbon dioxide exposure. Plasma and serum were isolated and stored at −20°C for biochemical assays. Femurs were dissected and wrapped with gauze soaked in saline prior to biomechanical testing and mineral analysis.

All experiments were approved by the Institutional Animal Care and Use Committee at the Army Research Institute of Environmental Medicine. In conducting the research described in this report, the investigators adhered to the Guide for Animal Care and Use of Laboratory Animals as prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources (NRC).

**Iron status and metabolic markers.** We determined iron status using a series of indicators in whole blood and serum. In whole blood, hemoglobin (Hgb) and red cell distribution width (RDW) were measured using a commercial hematology analyzer (Ac T; Beckman Coulter). In serum, iron and total iron-binding capacity (TIBC) were measured using a Beckman Coulter DXC600 pro. Transferrin saturation (TS) was calculated by dividing serum iron by TIBC.

### TABLE 1 Components of rat diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn starch</td>
<td>397.5</td>
</tr>
<tr>
<td>Casein</td>
<td>200.0</td>
</tr>
<tr>
<td>Maltodextrin 10</td>
<td>132.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100.0</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>70.0</td>
</tr>
<tr>
<td>Avicol</td>
<td>50.0</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>3.0</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2.5</td>
</tr>
<tr>
<td>Vitamin mix1</td>
<td>10.0</td>
</tr>
<tr>
<td>Mineral mix2,3</td>
<td>35.0</td>
</tr>
</tbody>
</table>

1 Prepared according to the AIN-93G formulation (18).
2 Prepared according to the AIN-93G formulation (18) with reduced ferric citrate in ID diets.
3 IA, Iron citrate (21.2% Fe), 6.06 g; ID, ferric citrate (21.2% Fe), 0.97 g.

We measured plasma glucose using a commercial colorimetric assay (Quantichrom; BioAssay Systems). Plasma insulin (Crystal Chem) and plasma leptin (Linco Research) were determined using ELISA.

**Body composition.** Body composition was assessed using dual energy X-ray absorptiometry (DEXA) immediately prior to and following the 12-wk treatment period. DEXA has been utilized extensively for the study of mineral nutrition and body composition in small animals by our group and others (16,20,21) and the accuracy and precision of this technique has been assessed (22). Rats were sedated by intraperitoneal injection with a mixture of ketamine (Ketaset; Fort Dodge Animal Health), xylazine (Xyla-Ject; Phoenix Scientific), and acepromazine (Boehringer Ingelheim). Following sedation, rats were transferred to a Prodigy fan beam densitometer (GE Lunar) with a small animal, high resolution scan module. Small animal software (enCore Version 7.53; 2003; GE Lunar) was used for the determination of lean body mass (LBM), fat mass (FM), and BMD.

**Bone strength and mineral analysis.** Bone strength was determined in femur bones using a 5-kN Flexure Fixture configured for 3-point bend tests and attached to an Instron Universal Testing Machine (Model 4502). Maximum tolerated force and bone stiffness were determined as described previously (20) using commercially available software (Series IX, v 8.08.00; Instron).

Mineral concentrations were measured in bone using inductively coupled plasma-optical emission spectrometry (Advanced Laboratories). Bones were digested in concentrated trace metal grade nitric acid (Fisher Scientific) prior to mineral analysis.

**Statistical analysis.** Data are presented as means ± SD. For body weight data, changes were assessed using 2-factor ANOVA with repeated measures. All other data were analyzed using 2-factor ANOVA. Post hoc testing was performed using Tukey’s test with commercially available statistical software (SPSS 13.0).

**Results**

**Body weight and feed intake.** Body weight did not differ among the treatment groups at the end of the study (IAS, 485 ± 44 g; IDS, 490 ± 41 g; IAPA, 479 ± 38 g; and IDPA, 484 ± 30 g). There were no consistent differences in weekly feed intakes among the treatment groups (data not shown). Furthermore, total measured feed intake did not differ over the course of the experiment (IAS, 798 ± 70 g/12 wk; IDS, 799 ± 82 g/12 wk; IAPA, 760 ± 49 g/12 wk; and IDPA, 797 ± 35 g/12 wk).

**Body composition.** At the start of the study, DEXA analysis indicated no differences in FM, LBM, or BMD (data not shown). At the end of the study, there was a positive interaction (P < 0.05) between diet and PA on FM (Fig. 1A). The IDS rats had greater (P < 0.05) FM than did rats from all other groups. Similar to FM, there was an interaction (P < 0.05) between diet and PA on LBM (Fig. 1B). LBM was lower (P < 0.05) in the IDS rats than in all other groups. There was an effect (P < 0.05) of PA on BMD, although there was no interaction between diet and PA (Fig. 1C).

**Iron status.** There was an effect (P < 0.05) of diet on early indicators of iron depletion, including serum iron, RDW, and TS (Table 2). There was an effect of PA (P < 0.05) on Hgb but not on any other iron status indicators.

**Metabolic markers.** There were no significant treatment-associated differences in plasma glucose following the 12-wk study period, although levels tended to be lower (P < 0.1) in PA rats (Table 3). There was an effect of PA (P < 0.05) on plasma insulin. Although there was no main effect of treatment group
on plasma leptin, IDS rats tended (P < 0.1) to have elevated levels compared with all other treatment groups.

**Bone strength and mineral analysis.** Neither diet nor PA affected maximum tolerated force or bone stiffness in excised femurs (Table 4). However, diet did affect bone mineral concentrations. There was an effect (P < 0.05) of diet on femur levels of iron, calcium, phosphorous, and zinc. Furthermore, there was an interaction of diet and PA (P < 0.05) on femur iron levels.

**Discussion**

Recent reports have described a relationship between iron status and obesity in human populations (8–12), although definitive mechanisms have not been elucidated. The potential role of diminished iron status as a causative factor in the development of obesity and the accretion of body fat has not been studied in an animal model. As such, the objective of the present study was to examine the effect of moderate iron deficiency on body composition in growing rats. The major findings indicate that moderate iron deficiency, without anemia, causes increased fat accretion and reduced LBM. Furthermore, moderate PA prevented the accretion of FM in moderately ID rats, suggesting an interaction between iron status and PA in the biochemical or metabolic regulation of the systems responsible for the maintenance of body composition.

Accretion of FM, as opposed to LBM, in moderately ID rats is a phenomenon that, to our knowledge, has not been previously described. Although the ID rats in this study did not become heavier than their counterparts, it would be interesting to follow them beyond the growth phase to determine whether the observed changes in body composition persisted and whether moderately ID rats would become obese. Obesity has not been described in ID rats in the past, although few studies have investigated the effects of moderate iron deficiency beyond the growth phase. Altered feed efficiency and diminished growth rates have been observed in anemic Sprague-Dawley rats, although moderate iron deficiency is different from anemia in animal models, as anemia is known to cause reduced body weight and feed intake (23,24).

The mechanism by which reduced iron status results in increased body fat in rats remains unclear. In our study, moderate PA attenuated the effects of dietary iron on the accretion of FM. This finding suggests that moderate iron deficiency affects a metabolic or biochemical signal that may also be affected by PA. A number of iron-dependent enzymes function in energy metabolism, including aconitase, NADH dehydrogenase, and succinate dehydrogenase (4). For example, aconitase, an enzyme that converts citrate to isocitrate in the tricarboxylic acid cycle, is affected by iron regulatory proteins (25) and may also be modulated by PA (26). It is also possible that reduced volitional activity in the IDS rats could have contributed to increased FM, especially as PA attenuated the affect of moderate iron deficiency.

Other potential mechanisms to explain our findings may include the impact of PA on plasma volume, which would affect the interpretation of DEXA data. In fact, increased plasma volume could confound our findings, because DEXA does not distinguish fluid from LBM. Other biochemical changes to explain our findings could include modulation of growth hormones, including insulin-like growth factor-1 (27), or iron and PA responsive changes in thyroid hormone metabolism (24).

The novel interaction of PA and iron status on body composition could have important public health implications, because both iron deficiency (1) and obesity (17) affect a large portion of the population. Although the health benefits of regular PA are well known (28), few have demonstrated the ability of PA to compensate for functional decrements caused by single nutrient deficiencies. Others have reported decrements in iron status in response to significant sessions of exercise in humans (29–31).

In our study, moderate PA did not have a significant negative effect on most iron status indicators, although Hgb levels were

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**TABLE 2** Iron status indicators in sedentary or physically active rats fed ID or IA diets for 12 wk1

<table>
<thead>
<tr>
<th></th>
<th>IAS</th>
<th>IAPA</th>
<th>IDS</th>
<th>IDPA</th>
<th>Effect2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hgb, g/L</td>
<td>180 ± 8.3</td>
<td>156 ± 8.2</td>
<td>159 ± 3.9</td>
<td>154 ± 5.0</td>
<td>PA</td>
</tr>
<tr>
<td>RDW, %</td>
<td>13.8 ± 0.93</td>
<td>13.8 ± 1.1</td>
<td>15.1 ± 0.53</td>
<td>14.1 ± 0.76</td>
<td>D</td>
</tr>
<tr>
<td>Serum iron, μmol/L</td>
<td>30.8 ± 3.9</td>
<td>35.1 ± 7.5</td>
<td>28.5 ± 3.6</td>
<td>29.5 ± 3.4</td>
<td>D</td>
</tr>
<tr>
<td>TIBC</td>
<td>488 ± 63</td>
<td>508 ± 50</td>
<td>500 ± 59</td>
<td>514 ± 56</td>
<td></td>
</tr>
<tr>
<td>TS, %</td>
<td>35.5 ± 5.0</td>
<td>39.2 ± 11</td>
<td>32.1 ± 5.1</td>
<td>32.2 ± 3.7</td>
<td>D</td>
</tr>
</tbody>
</table>

1 Values are means ± SD, n = 9–10.
2 Significant effects from 2-way ANOVA. D, Diet.

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**TABLE 3** Metabolic markers in plasma from sedentary or physically active rats fed ID or IA diets for 12 wk1

<table>
<thead>
<tr>
<th></th>
<th>IAS</th>
<th>IAPA</th>
<th>IDS</th>
<th>IDPA</th>
<th>Effect2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mmol/L</td>
<td>8.4 ± 3.2</td>
<td>6.9 ± 2.6</td>
<td>8.2 ± 3.9</td>
<td>5.8 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>Insulin, pmol/L</td>
<td>454 ± 290</td>
<td>325 ± 86</td>
<td>368 ± 140</td>
<td>243 ± 86</td>
<td>PA</td>
</tr>
<tr>
<td>Leptin, pmol/L</td>
<td>321 ± 81</td>
<td>319 ± 110</td>
<td>501 ± 260</td>
<td>334 ± 110</td>
<td></td>
</tr>
</tbody>
</table>

1 Values are means ± SD, n = 8–10.
2 Significant effects from 2-way ANOVA.
diminished in PA groups. Future research should strive to determine the optimal diet and exercise prescription for the maintenance of body composition in moderately ID individuals.

Dietary iron is critical for the maintenance of optimal bone health (7). In this study, we found no diet-induced differences in bone strength. These findings are in contrast to those of other recent studies investigating the effects of dietary iron on bone health using rats exhibiting iron deficiency anemia (13–15). However, our findings are similar to others that have investigated the effects of moderate iron deficiency on bone strength where no effect of iron deficiency on bone breakage in femur was observed when the experimental intervention did not induce frank anemia (21).

Significant changes in the mineral concentrations of bones from moderately ID rats were identified in this study. Levels of calcium and zinc were elevated in ID rats, suggesting that the added deposition of these minerals in bone may compensate for diminished iron. Other recent studies have described altered levels of zinc and calcium in bones from ID rats (21), although BMD and femur breaking strength were significantly diminished in these rats, which were fed diets containing <8.0 mg/kg of iron. Taken in parallel with our data, it appears that increased calcium and zinc in the bones of ID rats may confer protection against functional defects; however, when dietary iron falls below the levels provided in this study (~9 mg/kg), that protection is lost. A number of potential mechanisms for added deposition of zinc and calcium in the bones of ID animals have been proposed, including mineral-mineral interactions at the site of absorption (32–35). As increased calcium absorption has been observed in ID rats (35), it is possible that the increased calcium deposition in the bones of the ID rats from this study is due to changes in absorption. Future studies should aim to determine whether this interaction affects the dietary requirement for zinc and calcium and whether moderate iron deficiency affects mineral levels in other tissues that may participate in the storage and metabolism of nutritionally essential micronutrients, including liver.

In summary, the major findings of this study indicate that moderate iron deficiency results in the preferential accretion of body fat in a rat model. The increased FM is accompanied by corresponding reductions in LBM. Changes in body composition were affected by PA, suggesting that PA could be an effective countermeasure against some functional outcomes of moderate iron deficiency. These novel findings suggest that maintaining adequate iron nutrition, coupled with PA, may be critical for the prevention of body fat accretion. Furthermore, the increased deposition of calcium and zinc in bones from moderately ID rats could confer protection against functional defects. Future work should focus on the mechanism whereby dietary iron, coupled with PA, affects body composition and bone health.

Acknowledgments

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### Literature Cited