Hematological Response of Acute Exercise in Obese Mice: The Obesity Attenuation Effect on Leukocytes Response

Iberê Machado Kostrycki¹,², Matias Nunes Frizzo¹,³, Guilherme Wildner¹, Yohanna Hannah Donato¹, Analú Bender dos Santos¹,³, Cláudia Ramos Rhoden², Mirna Stela Ludwig¹,³, Thiago Gomes Heck¹,³

¹Research Group in Physiology, Department of Life Sciences, Regional University of Northwestern Rio Grande do Sul State, Ijuí, RS, Brazil, ²Postgraduate Program in Health Sciences, Porto Alegre, RS, Brazil, ³Postgraduate Program in Integral Attention to Health, Ijuí, RS, Brazil

ABSTRACT

Kostrycki IM, Frizzo MN, Wildner G, Donato YH, dos Santos AB, Rhoden CR, Ludwig MS, Heck TG. Hematological Response of Acute Exercise in Obese Mice: The Obesity Attenuation Effect on Leukocytes Response. JEPonline 2016;19(6):85-93. The purpose of this study was to investigate the effects of a single exercise session in obese (high fat diet-HFD treated) mice on hematological parameters. The mice were randomly separated into two groups that received either the standard diet or the high-fat diet for 16 wks. The mice were submitted to 20 min of high intensity swimming exercise. Control groups (Lean and HFD groups) were maintained at rest. The data indicate that the high intensity exercise increased leukocyte count (Exercise group). The Obese mice had higher basal levels of leukocyte (HFD group) and showed no increase in leukocyte count induced by exercise (HFD+Exercise group). The findings indicate that obesity may cause attenuation in the immune response induced by high intensity exercise.

Key Words: Exercise, Obesity, Leukocytes, White Blood Cell
INTRODUCTION

Obesity is a multifactorial disease that is strongly associated with diverse comorbidities (35), and it is considered an important public health problem in different cultures with increasing prevalence (28). Currently, nearly 30% of the adult global population is not physically active. In fact, their predisposition to sedentary habits is largely responsible for the increase in the prevalence of obesity (12). The metabolic alterations that result from a sedentary lifestyle is highly correlated with the increase in abdominal adiposity, which is a major risk factor for type 2 diabetes (T2DM) development (5,28). In the physiopathology of T2DM is the immunomodulation unbalance, and the increase in adipose tissue may be consider as a central organ in low grade chronic inflammation linked to T2DM (16). These conditions regarding human obesity are represented by experimental models with high-fat diet (HFD) treatments (7,11,20).

In contrast, to prevent and treat many chronic diseases and the related comorbidities, it is important to engage in regular exercise (6). Besides the well-known benefits of regular exercise training, the acutely performed physical activity may also trigger immune responses. That is, depending on the intensity of the challenge, the activity is likely to produce either positive or negative immune responses (33,34). In fact, it is well known that the increase in white blood cell count depends on exercise intensity and duration (27). Thus, by regulating intensity and/or duration, the presence and the magnitude of the physiological response to the acute exercise session can be observed as immunoinflammatory parameters of health (17).

The stressor stimuli such as obesity (8) and exercise (26) may cause alterations in the blood cell count. Since acute exercise sessions can release physiological danger signals from immune cells for all systems (15), the measure of hematological parameters may represent an important biomarker for immunoinflammatory state after exercise (10). Therefore, the purpose of this study was to investigate the effects of a single session of high intensity swimming exercise on hematological parameters in HFD treated mice.

METHODS

Animals
Twenty-eight male B6129SF2 (B6) mice (30 days-old) from Jackson Laboratory (Bar Harbor, ME, USA) were reproduced in the Regional University of Northwestern State's Rio Grande do Sul, Life Sciences Department, Animal Care Facility, and maintained at a controlled temperature (23 ± 1°C) in a 12/12 hr light/dark cycle (lights on at 7 a.m.). The mice were housed in plastic cages (40 x 33 x 17 cm; 3 to 5 animals per cage). Throughout the experiments, the animals had free access to water and fed ad libitum. All the procedures were approved by the Regional University of Northwestern State's Rio Grande do Sul Committee of Animal Welfare (CEUA-UNIJÚ, protocol # 011/2013).

Experimental design
The 28 mice were randomly separated into two groups that received either the standard diet or the high-fat diet for 16 wks. Then, the mice were submitted to either 20 min of high intensity swimming exercise or maintained at rest, which resulted in the following groups: Lean (N = 5); Lean+Exercise (N = 8); Obese (N = 7); and Obese+Exercise (N = 8). Immediately after the exercise or rest sessions the animals were killed for blood sample and white adipose tissue analysis.
Diet
The animals received for 16 wks the pelleted standard diet consisted of crude protein, mineral material, fibrous matter and minerals (Nuvilab CR-1, commercially obtained from Nuvital Nutrientes SA.; total metabolizable energy: 16.6 MJ/kg, being 11.4 % as fats, 62.8% as carbohydrates, and 25.8% as proteins) or a lard-based diet (HFD) (37.4% w/w; total metabolizable energy: 22.8 MJ/kg, being 58.3% as fats, 24.5% as carbohydrates, and 17.2% as proteins) (4,11).

Exercise
For swimming familiarization, each mouse swam 10 min in a glass tank filled with 20 cm of water at 30 ± 1°C, by two consecutive days during the 16th-wk of diet treatment. After 2 days of familiarization, the animals remained 48 hrs without any manipulation. Thus, swimming exercise (Exercise and HFD+Exercise groups) was performed one time (acute exercise) at high intensity by adding load coupled to the base of the tail with adhesive tape (8% of the animal body weight). The animals swam in 20 cm deep water at 30 ± 1°C for 20 min. Animals from controls groups (Lean and HFD groups) stayed the same time in shallow water (2 ± 1 cm deep) at the same temperature. The time to exhaustion (6 sec of mouse submersion during swim session) was analyzed. After the exercise session, blood was collected from the distal part of the tail (~25 µL) to measure lactate by specific strips and lactimeter (Accutrend® Lactate, Roche) (14).

Hematological and White Adipose Tissue Analyses
After decapitation, blood was then immediately collected into heparinized (30 IU·mL⁻¹ final volume) tubes (for metabolite measurements) or in disodium EDTA (2 mg·mL⁻¹ final volume) treated tubes. Hematological parameters were investigated in EDTA-samples in a Horiba ABX Micros 60 Hematology Analyzer (for quantitative cell analyses) (3,25). Soon after blood harvesting, the white adipose tissue of each animal was surgically excised and weighed.

Statistical Analyses
Kolmogorov-Smirnov normality test was applied before all analysis. One-way analysis of variance (ANOVA) followed by post-hoc Tukey’s test were performed to compare the groups upon haematological variables. Blood lactate concentration and time to exhaustion were compared by the Student t test. Significance level was set at P<0.05. The data were analysed using Graph Pad Prism 6.0 software. Values are presented as mean ± standard deviation.

RESULTS
As expected, the 16 wks of HFD intake promoted an increase in adiposity compared to standard chow (white adipose tissue weight: Lean = 0.29 ± 0.09; Lean+Exercise = 0.40 ± 0.23; Obese = 1.16 ± 0.57; Obese+Exercise = 0.89 ± 0.37 gm, P = 0.004). Also, the efforts performed by Lean and Obese mice were similar in terms of intensity as showed by blood lactate concentration measured after the exercise session (Lean+Exercise = 5.40 ± 0.16 vs. Obese+Exercise = 5.30 ± 0.61 mmol·L⁻¹, P=0.832). But, the time to exhaustion was earlier in the obese mice (Lean+Exercise = 15.8 ± 5.7 vs. Obese+Exercise = 7.50 ± 6.25 min, P=0.014).

The acute exercise induced an increase in leukocytes (WBC) in bloodstream in Lean mice. However, the HFD treatment per se also increased WBC in non-exercised mice (Obese vs. Lean) (Table 2). However, the obese mice showed no significant increase in WBC induced by exercise (Obese+Exercise compared to Obese group) (Table 2). Also, Mean Corpuscular Hemoglobin (MCH) was lower after the exercise session in the Obese than in the Lean mice (Obese+Exercise compared to Lean+Exercise group). All other hematological variables were not different among the groups (Table 2).
Table 2. Hematological Response of Acute Exercise in Obese Mice.

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>Lean + Exercise</th>
<th>Obese</th>
<th>Obese + Exercise</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (10^3/mm^3)</td>
<td>5.82 ± 0.6</td>
<td>8.4 ± 1.8*</td>
<td>8.48 ± 2.1*</td>
<td>9.30 ± 3.1*</td>
<td>0.048</td>
</tr>
<tr>
<td>RBC (10^6/mm^3)</td>
<td>8.4 ± 0.5</td>
<td>8.4 ± 0.7</td>
<td>8.3 ± 1.0</td>
<td>8.0 ± 1.2</td>
<td>0.841</td>
</tr>
<tr>
<td>HGB (g/dL)</td>
<td>13.8 ± 1.5</td>
<td>13.8 ± 1.1</td>
<td>13.2 ± 1.3</td>
<td>12.3 ± 2.1</td>
<td>0.233</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>37.0 ± 3.2</td>
<td>37.2 ± 3.2</td>
<td>36.6 ± 4.5</td>
<td>35.1 ± 5.9</td>
<td>0.791</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>44.0 ± 2.3</td>
<td>44.3 ± 1.2</td>
<td>43.8 ± 1.2</td>
<td>43.1 ± 1.8</td>
<td>0.781</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>16.5 ± 1.0</td>
<td>16.8 ± 1.1</td>
<td>16.0 ± 0.8</td>
<td>15.2 ± 1.1†</td>
<td>0.027</td>
</tr>
<tr>
<td>CMCH (%)</td>
<td>37.5 ± 1.6</td>
<td>37.3 ± 1.5</td>
<td>36.3 ± 1.8</td>
<td>35.5 ± 1.5</td>
<td>0.094</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>14.6 ± 0.3</td>
<td>14.4 ± 0.6</td>
<td>15.4 ± 0.8</td>
<td>15.0 ± 0.6</td>
<td>0.054</td>
</tr>
<tr>
<td>PLT (10^3/mm^3)</td>
<td>958 ± 120</td>
<td>889 ± 191</td>
<td>896 ± 256</td>
<td>820 ± 283</td>
<td>0.763</td>
</tr>
<tr>
<td>Neutr (%)</td>
<td>4.8 ± 1.3</td>
<td>4.4 ± 1.6</td>
<td>5.2 ± 1.5</td>
<td>4.9 ± 2.0</td>
<td>0.825</td>
</tr>
<tr>
<td>Monoc (%)</td>
<td>4.2 ± 1.1</td>
<td>4.1 ± 0.6</td>
<td>4.2 ± 1.0</td>
<td>3.9 ± 0.7</td>
<td>0.890</td>
</tr>
<tr>
<td>Lymph (%)</td>
<td>90.8 ± 1.8</td>
<td>91.5 ± 1.9</td>
<td>90.7 ± 2.3</td>
<td>91.3 ± 2.6</td>
<td>0.884</td>
</tr>
</tbody>
</table>

Data expressed as mean ± standard deviation. **RBC** = Red blood cells, **HGB** = Hemoglobin, **HCT** = Hematocrit, **MCV** = Mean corpuscular volume, **MCH** = Mean corpuscular hemoglobin, **MCHC** = Mean corpuscular hemoglobin concentration, **RDW** = Red blood cell distribution width, **PLT** = Platelet total count, **Neutr** = Neutrophil, **Monoc** = Monocyte, **Lymph** = Lymphocyte. * vs. Lean. † vs. Lean+Exercise.

DISCUSSION

The purpose of this study was to demonstrate if obesity could affect the hematological response after a single acute bout of exercise. The major finding was that the increase in WBC concentration induced by high intensity exercise was blunted in obese mice. Approximately 15 min of fatigable exercise is able to induce a 44% increase in WBC concentration in bloodstream of lean mice. This level of WBC was observed in resting obese mice in our study and thus, the increasing in WBC concentration induced by exercise are attenuated in obese mice. These results may be considered an impaired immune and/or stress response of leukocytes in obesity against high intensity exercise challenge.

Acute exercise can alter the number and function of circulating cells of the immune system depending on the duration and intensity (38). It is generally supposed that the immune response to exercise effort is correlated with exercise workload (time-power binomium). Thus, one exercise session can promote immunostimulatory or immunosuppressive effects (15,33) The point of exercise intensity that divides benefits or impairment of the immune function can be more critical in chronic conditions such as obesity. Thus, although exercise is frequently consider as “exercise medicine” with indisputable health benefits, it is not clear as to the short-term exercise effects on the immune response in the chronic low grade inflammatory state of obesity (21).

Although the exercise induced leukocytosis is a well known physiological outcome, the subject’s hemoconcentration and fluid loss from the plasma to the working muscle influence the increase in WBC concentration after exhaustive exercise. Also, the increase in cardiac output results in demargination of leukocytes by shear stress and the increase in lymphatic blood flow appears to contribute to the elevated lymphocyte concentration; all effects mediated by catecholamines. At the molecular level, the down regulation of surface adhesion molecules contributes to demargination of WBC during exercise (10). In the present study, the differential leukocyte count indicates that the
exercise induced leukocytosis did not modify the proportion of each cell in mice circulation. However, the obese mice showed a different magnitude of increasing WBC that may represent an impairment of one or more mechanism listed above to promote leukocytosis during the exercise challenge.

Higher basal levels of leukocytes, lymphocytes, neutrophils, and platelets, and lower levels of hemoglobin can be observed in obese subjects compared to lean subjects. Furthermore, a positive correlation can be found between adiposity and leukocytes levels (8). Similarly, young obese subjects have a higher WBC count associated with neutrophils and monocytes overpopulation compared to young lean subjects. This immunologic status was correlated to cardiorespiratory performance, independently of body mass index (36). These human conditions were well represented in our experimental model since adiposity increased ~300% and the performance decreased ~50% in the HFD treated mice.

The impaired performance observed in the sedentary obese mice in the present study can be related to an immune fragility of the organism. The increase in physical capacity by regular training (e.g., increased oxygen consumption peak – VO$_2$ peak) is related to the decrease in inflammatory biomarkers. Hence, in young obese subjects of either gender the VO$_2$ peak is negatively correlated with inflammatory status (24,36). On the other hand, the inflammatory effect of adiposity is observed by the correlation between obesity and leukocyte count. The white adipose tissue works as an endocrine organ releasing pro-inflammatory signals to the entire body, as adipokynes, which are related with insulin resistance and cardiovascular disease development.

Additionally, the higher levels of reactive C protein and interleukin-6 observed in obese subjects can promote the production and release of leukocytes to bloodstream (29). Thus, the inflammatory state of obesity, that can be observed by leukocyte count and its correlation with inflammatory markers (9), is an important subclinical process in diabetes development and its complications (13,22). Similarly, in this regards, hyperglycemia and dyslipidemia are associated with a higher basal leukocyte count and inflammation (1,2,23,37). Thus, the increased levels of leukocytes observed in our obese mice represent a human situation of metabolic dysfunction and can be considered a biomarker of cardiovascular risk and a prognostic biomarker for metabolic syndrome (18,30,31). But, interestingly, one high intensity exercise session can modulate immune cells and the inflammatory state, promoting short pro-inflammatory challenges that culminate in beneficial anti-inflammatory responses (15,19,22). The difference in the leukocytes at basal levels and after the exercise challenge indicates an augmented risk in obesity against immune-inflammatory challenges.

**CONCLUSIONS**

One session of high intensity exercise increased leukocyte count in lean mice, while the obese mice had higher basal levels of leukocyte count and showed no increase in leukocyte count induced by exercise. The data indicate that obese mice were under a pro-inflammatory state induced by higher adiposity. They were unable to show a classical immune stress response to exercise. Thus, the bottom line is that obesity may cause attenuation in immune response induced by high intensity exercise and that obesity represents fragility against immune-inflammatory challenges.
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Address for correspondence: Thiago Gomes Heck, PhD, Research Group in Physiology, Department of Life Sciences, Regional University of Northwestern Rio Grande do Sul State (UNIJUI), Rua do Comércio, 3000 – Bairro Universitário, Ijuí, RS, Brazil, CEP: 98700-000. Email: gomesheck@yahoo.com

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