

## Acute circuit-resistance exercise increases expression of lymphocyte agouti-related protein in young women

Abbass Ghanbari-Niaki<sup>1</sup>, Marziyeh Saghebjo<sup>2</sup>, Amir Rashid-Lamir<sup>3</sup>, Rozita Fathi<sup>1</sup> and Robert R Kraemer<sup>4</sup>

<sup>1</sup>Exercise Biochemistry Division, Faculty of Physical Education and Sports Sciences, University of Mazandaran, Babolsar, Mazandaran; <sup>2</sup>Department of Physical Education & Sports Science, Birjand University, Birjand-Khorasan; <sup>3</sup>Faculty of Physical Education and Sports Sciences, Ferdowsi University of Mashhad, Mashhad, Khorasan Razavi, Iran; <sup>4</sup>Department of Kinesiology and Health Studies, SLU10845, Southeastern Louisiana University, Hammond, LA, USA

Corresponding authors: Dr Robert R Kraemer, Department of Kinesiology and Health Studies, Southeastern Louisiana University, SLU10845, Hammond, LA 70402, USA. Email: robert.kraemer@selu.edu or Dr. Abbass Ghanbari-Niaki, Exercise Biochemistry Division, Faculty of Physical Education and Sports Sciences, University of Mazandaran, Babolsar, Mazandaran, Iran. Emails: ghanbara@yahoo.ca, ghanbara@umz.ac.ir

### Abstract

Exercise-induced leukocytosis and lymphocytosis is accompanied by up-regulation and down-regulation of hundreds of genes in white blood cells (WBCs). Agouti-related protein (AgRP) is an orexigenic peptide secreted predominantly from the arcuate nucleus in the hypothalamus. AgRP affects feeding behavior and plays a role in energy and glucose homeostasis and adiposity. The purpose of the study was to determine effects of circuit resistance exercise (CRE) (9 exercises, 25 s per exercise) at different intensities on peripheral blood lymphocyte (PBL) AgRP mRNA expression and its concentrations in lymphocytes and plasma. Twenty-five young female college students were randomly divided into five groups: control, 40% 1-repetition maximum (1-RM), 60% 1-RM, 80% 1-RM and combined (40 + 60 + 80% 1-RM) loads. Peripheral blood mononuclear cells were isolated by a lymphocyte density gradient centrifugation method for AgRP mRNA expression. Lymphocyte ATP, glycogen, AgRP, growth hormone (GH), and plasma AgRP, GH and glucose concentrations were measured. CRE increased AgRP mRNA lymphocyte expression significantly ( $P < 0.0001$ ) at all intensities. A higher and significant ( $P < 0.01$ ) increase was found in the 60% 1-RM group when compared with the other groups. The CRE-induced lymphocyte AgRP expression was accompanied by elevations in plasma AgRP, glucose and GH levels as well as higher WBCs, lymphocytes and neutrophil counts. Lymphocyte AgRP and GH concentrations were significantly reduced ( $P < 0.05$ ). Lymphocyte ATP content was unchanged and glycogen was reduced in the combined group but not in the other groups. Data indicate that AgRP mRNA is expressed in PBLs and that CRE increases its expression. Data also reveal that the expression of AgRP was accompanied with higher plasma AgRP and GH concentrations. Findings suggest that AgRP may provide an important signal in the immune environment and that the lymphocyte may be considered as an extra-hypothalamic source of plasma AgRP following exercise stress.

**Keywords:** orexigenic peptide, leukocyte, human, weight training, AgRP expression

*Experimental Biology and Medicine* 2010; 235: 326–334. DOI: 10.1258/ebm.2009.009209

### Introduction

Circulating white blood cells (WBCs) in humans rapidly increase with sufficient physical activity. Lymphocytosis is produced in response to endurance exercise,<sup>1,2</sup> short-term cycling exercise<sup>3</sup> and resistance exercise.<sup>4,5</sup> For example, running a marathon has been shown to increase WBC number by as much as 140%.<sup>1</sup> Moreover, acute and chronic effects of exercise on neutrophil and lymphocyte gene expression have been

reported.<sup>6–11</sup> Thus, exercise is a potent stimulus for increasing lymphocyte number and gene expression.

Natelson *et al.*<sup>11</sup> determined the effects of an acute exhaustive maximal treadmill test on peripheral blood leukocyte cytokine gene expression. They reported a 47% reduction in tumor necrosis factor (TNF)- $\alpha$  gene expression, but the expression of interleukins 1a, 1b, 2, 4, 6 and 10 was unchanged. Büttner *et al.*<sup>6</sup> demonstrated that acute

strenuous and moderate treadmill exercise produced significant increases in total WBC, lymphocyte and granulocyte counts immediately after the treadmill tests. They also found that following the strenuous treadmill exercise tests, 450 and 150 genes were differentially up- and down-regulated, respectively, with exercise intensity affecting the degree of specific gene expression. Changes were found for genes that code for stress proteins as well as genes involved in inflammatory responses, energy metabolism, potassium homeostasis and anti-inflammatory responses. Zieker *et al.*<sup>12,13</sup> reported an increase in leukocytes, monocytes, and a reduction in lymphocytes counts immediately after a one-half marathon, which was accompanied with up-regulation of several genes and down-regulation of others. Collectively, these studies demonstrate that acute exercise has a pronounced effect on WBC gene expression.

Ghrelin is an orexigenic (appetite stimulating) peptide produced mainly in the stomach.<sup>14</sup> There is evidence that this peptide is expressed in human T-cells, B-cells and neutrophils,<sup>15</sup> suggesting a necessary signaling role of this appetite hormone in the immune system. Mager *et al.*<sup>16</sup> reported the expression of ghrelin and its receptor in peripheral blood mononuclear cells (PBMCs) of subjects with metabolic syndrome and found ghrelin expression positively correlated with PBMC expression of TNF- $\alpha$  and IL1- $\beta$ . They concluded that ghrelin PBMC expression suggests that ghrelin plays an autocrine role in the immune system. Agouti-related protein (AgRP) is another orexigenic peptide that is primarily secreted from hypothalamic-NPY-containing neurons of the arcuate nucleus (ARC).<sup>17,18</sup> AgRP is also expressed by extra-hypothalamic tissues<sup>17-19</sup> including skeletal muscle,<sup>20</sup> adrenal gland, testis, kidney and lung<sup>18</sup>; however, to our knowledge, no studies have investigated its expression in PBMCs. Analogous to ghrelin, AgRP acts as a signaling peptide that affects feeding behavior, energy homeostasis and adiposity; it is up-regulated by fasting, insulin-induced hypoglycemia and leptin administration.<sup>21</sup> Similar to ghrelin, AgRP may also play a role in immune function. There is evidence that AgRP is a major neuroendocrine regulator of inflammation after exposure of an organism to pathogens or other stressors.<sup>22</sup> Additionally, it has been reported that central injection of AgRP enhances the hypothalamic-pituitary-adrenal response to the inflammatory cytokine, IL-1 in the primates.<sup>23</sup> Moreover, since the active isoform of human AgRP crosses the blood-brain barrier (even though it is slow to do so),<sup>12</sup> expression of AgRP in the periphery could have an effect on melanocortin receptors and thus hunger. Finally, *in vitro* evidence demonstrates that AgRP inhibits leptin expression in adipocytes via inhibition of  $\alpha$ -melanocyte-stimulating hormone (MSH).<sup>24</sup> Given the ties that  $\alpha$ -MSH has both with adipocyte and central nervous system (CNS) satiety signaling as well as immune function,<sup>25</sup> and coupled with the emerging concept that obesity is associated with an inflammatory diseased state, it seems plausible that in addition to its known orexigenic effects, AgRP may also play a role in neuroendocrine regulation and specifically that AgRP may interact with  $\alpha$ -MSH to modulate neuroendocrine responses to inflammation.

A few studies have investigated the effects of physical exercise on tissue AgRP mRNA expression and plasma

concentrations in humans and rats.<sup>26-28</sup> Although there is some evidence that resistance exercise affects lymphocyte gene expression,<sup>29</sup> there are no published data regarding the effects of exercise on AgRP mRNA expression and its concentrations in the human peripheral blood lymphocytes (PBLs). The present study was conducted to investigate the effect of a single session of circuit-resistance exercise (CRE) at different intensities on lymphocyte AgRP mRNA expression and its concentrations in the lymphocyte and plasma. Activation of hypothalamic-pituitary axis in response to resistance exercise results in increases in growth hormone (GH) as well as other endocrine responses.<sup>30</sup> GH is important in the regulation of immune function; it is expressed in the spleen and in peripheral blood and it stimulates T- and B-cell proliferation, modulates cytokine response, and GH receptors are found in different forms of leukocytes.<sup>31</sup> Resistance exercise can also alter blood glucose levels,<sup>32</sup> which may affect immune function as well as AgRP.<sup>33,34</sup> Given the effects of GH and glucose, a second aim of the study was to determine whether changes in plasma and lymphocyte AgRP are accompanied by changes in lymphocyte GH, glycogen and ATP levels as well as changes in plasma insulin, GH and glucose. It was hypothesized that acute exercise (circuit training) would (1) increase lymphocyte expression of AgRP; (2) that its expression would be accompanied by increases in plasma GH and glucose and reductions in glycogen and ATP concentrations in lymphocytes; (3) that exercise would increase lymphocyte and plasma AgRP levels; and (4) that increased exercise intensity would enhance these responses.

## Materials and methods

### Subjects and research design

The study was approved by the ethic committee of the School of Medical Sciences of Tarbiat Modares University and conducted in accordance with the policy statement of the Declaration of the Iranian Ministry of Health. Written consent was obtained from 25 female physical education students (age 20–30 y, height 150–171, body weight 50–65 kg and body mass index [BMI] 18–24.5 kg/m<sup>2</sup>) without weight circuit-resistance training experiences (Table 1). Subjects were randomly assigned to five groups with five subjects in each group: (1) control (no exercise); (2) 40% of a one-repetition maximum (1-RM); (3) 60% of a 1-RM; (4) 80% of a 1-RM; and (5) combined loads (40 + 60 + 80%) 1-RM. Subjects completed a medical examination and a medical questionnaire to ensure that they were not taking any medication, were free of disease and were not using steroids. All groups were completely familiarized with experimental procedures and had their 1-RM determined for each of the nine exercises used in the CRE protocol. Subjects were all tested in the luteal phase of their menstrual cycle.

### Exercise testing procedures

Before the main trial, participants were taken to the weight room three times. In the first and second visits, all of the participants performed strength tests to determine their

**Table 1** (a) Physical characteristics of participants. (b) The one-repetition maximum (1-RM) values in kg of nine exercises for 40%, 60%, 80% and combined (40, 60, 80)% 1-RM groups

| (a)                      |                  |                  |                  |                        |             |       |
|--------------------------|------------------|------------------|------------------|------------------------|-------------|-------|
| Variables                | Control          | 40% 1-RM         | 60% 1-RM         | 80% 1-RM               | Mixed% 1-RM |       |
| Age (y)                  | 22.8 ± 1.1       | 21.2 ± 1.0       | 22.2 ± 0.3       | 23.2 ± 0.3             | 24.6 ± 1.7  |       |
| Height (cm)              | 162 ± 1.3        | 161.2 ± 3.6      | 159.0 ± 2.4      | 167.0 ± 3.7            | 162.0 ± 2.1 |       |
| Weight (kg)              | 54.0 ± 1.7       | 52.6 ± 2.0       | 50.0 ± 1.0       | 55.0 ± 3.0             | 54.0 ± 2.7  |       |
| BMI (kg/m <sup>2</sup> ) | 20.4 ± 0.42      | 20.2 ± 1.33      | 19.6 ± 0.7       | 21.3 ± 0.6             | 20.6 ± 0.5  |       |
| (b)                      |                  |                  |                  |                        |             |       |
| Variables                | 40% 1-RM (N = 5) | 60% 1-RM (N = 5) | 80% 1-RM (N = 5) | Combined% 1-RM (N = 5) | F           | P     |
| Exercise 1 (kg)          | 17.0 ± 1.27      | 18.7 ± 1.98      | 17.7 ± 1.0       | 18.8 ± 1.6             | 0.336       | 0.800 |
| Exercise 2 (kg)          | 148.5 ± 7.7      | 152 ± 16.5       | 163 ± 9.0        | 142.0 ± 11.86          | 0.71        | 0.558 |
| Eeercise 3 (kg)          | 28.3 ± 1.0       | 28.0 ± 1.45      | 26.7 ± 0.6       | 25.10 ± 1.0            | 2.21        | 0.132 |
| Exercise 4 (kg)          | 42.6 ± 2.5       | 39.4 ± 2.17      | 44.4 ± 1.8       | 38.0 ± 2.37            | 1.89        | 0.178 |
| Exercise 5 (kg)          | 21.7 ± 2.16      | 22.62 ± 1.1      | 20.60 ± 1.47     | 1.92 ± 24.8            | 1.171       | 0.356 |
| Exercise 6 (kg)          | 28.87 ± 3.14     | 28.75 ± 1.6      | 29.9 ± 1.64      | 25.0 ± 2.37            | 1.053       | 0.400 |
| Exercise 7 (kg)          | 31.12 ± 1.37     | 30.50 ± 2.82     | 30.7 ± 1.90      | 29.90 ± 1.10           | 0.077       | 0.971 |
| Exercise 8 (kg)          | 61.50 ± 6.62     | 63.9 ± 5.50      | 74.40 ± 5.43     | 64.0 ± 1.60            | 1.471       | 0.266 |
| Exercise 9 (kg)          | 60.12 ± 2.7      | 68.26 ± 6.64     | 64.8 ± 2.7       | 74.60 ± 4.0            | 2.24        | 0.128 |

BMI, body mass index; 1-RM, 1-repetition maximum  
 N = 5 subjects per group. Values are mean ± SEM (kg)

1-RM for each of the nine free-weight resistance exercises (arm curl, triceps extension, back extension, squat 90°, leg curl, bench press, overhead press, dead lift, seated row). The procedure employed was the same as reported previously.<sup>26,35</sup> The resistance exercise sessions were held in the morning between 08:30 and 11:00 h to avoid effects of circadian rhythms. The duration of the whole program was 26 min. Subjects were instructed to follow normal lifestyle habits, avoid medications, refrain from exercise three days prior, and report to the lab for testing in a fasted (12 h) state.

### Blood collection and lymphocyte preparation

Blood samples were obtained from an antecubital vein immediately after the end of CRE protocols. Venous blood samples were collected from the subjects into heparinized (10–20 U/mL) tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ, USA). Plasma was separated by centrifugation within 15 min of collection and divided into three aliquots. The aliquots were frozen and stored at –80°C for subsequent analyses (within 2–3 weeks). Peripheral blood mononuclear cells were isolated by lymphocyte (Cedarlane Laboratories Limited, Burlington, ON, Canada) density gradient centrifugation at 900 g, according to the manufacturer's instructions and the pellet containing lymphocytes were used for further analyses.

### PBLs AgRP mRNA expression

Freshly prepared blood lymphocytes ( $5 \times 10^5$  cells per sample) were powdered with cold mortar and pestle, and used for the isolation of RNA. Total RNA was extracted by the guanidine thiocyanate method<sup>36</sup> and mRNA was purified using a mRNA isolation kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instruction. Two hundred nanograms of mRNA

were used for the synthesis of the first-strand cDNA in a 20 µL volume by using oligo (dT) primer in the first-strand synthesis kit (Fermentas GmbH, St Leon-Rot, Germany). Relative expression levels of AgRP mRNA in the lymphocytes were determined using a semi-quantitative polymerase chain reaction (PCR) method. The following primers were used to amplify lymphocyte AgRP and  $\beta$ -actin cDNA (as an internal control): AgRP-forward: 5' AGA AGA CAG CAG CAG ACC GAG 3'; AgRP-reverse: 5' TGA AGA AGC GGC AGT AGC AC 3';  $\beta$ -actin-forward: 5' TCC TGT GGC ATC CAT GAA ACT 3';  $\beta$ -actin-reverse: 5' ATC GTG CAC CGC AAA TGC TTC 3'. AgRP cDNA were amplified giving a 186-bp product. PCR was formed for 35 cycles of denaturation at 94°C for 30 s, annealing of 60°C for 30 s and extension at 72°C for 50 s. Reactions were set up using a two-fold serial dilution of template cDNA to assess the best dilution of template in PCR. Template cDNA was standardized by amplification of a 315-bp internal control of  $\beta$ -actin, a housekeeping gene and a protein known to be expressed in the tissues. All the reactions were repeated a minimum of three times to ensure repeatability. All PCR products were electrophoresed on an agarose gel and bands visualized by ethidium bromide staining and quantified by computer integrated densitometry (Eastman Kodak Co, New Haven, CT, USA). Levels of mRNA were expressed as a ratio of signal intensity for the  $\beta$ -actin gene.

### Biochemical analyses

The plasma samples were analyzed for glucose, GH and AgRP. Glucose was determined using an enzymatic method (glucose oxidase and peroxidase, Man Co., Tehran, Iran). Plasma and lymphocyte GH were determined by ELISA (Diagnostic Biochem Canada Inc, London, Ontario, Canada; intra-assay coefficient of variation

(CV%: 5.95, sensitivity 2 ng/mL). Plasma and lymphocyte AgRP were measured using a commercial kit (Human AgRP Immunoassay, R&D Systems GmbH, Wiesbaden-Nordenstadt, Germany; intra-assay CV%: 3.4, sensitivity: 0.68 pg/mL). Lymphocyte ATP was measured using a commercial ATP determination kit (bioluminescence method, sensitive assay; Biaffin GmbH & Co KG, Kassel, Germany). Glycogen contents in lymphocytes were obtained using a glycogen colorimetric kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

## Statistics

The data were analyzed using an SPSS package (version 13) by personal computer. The obtained data for lymphocyte AgRP, GH, ATP, glycogen, and plasma glucose, GH and AgRP concentrations were analyzed using a one-way analysis of variance. Significant effects were followed by appropriate planned comparison. Statistical significance was accepted at  $P < 0.05$ .

## Results

The descriptive characteristics of the subjects are highlighted in Table 1. There were no significant differences between groups for age, height, body weight or BMI. Analysis using a semi-quantitative reverse-transcription-PCR technique showed that AgRP was expressed in PBLs in all given exercise intensities (Figure 1). A higher and significant ( $P < 0.001$ ) AgRP mRNA expression was found in the 60% 1-RM group than in the control and other exercise groups (Figure 1). The WBC, lymphocyte and neutrophil counts were significantly increased in all exercise groups, but the counts of lymphocyte were higher in the 60% ( $P < 0.027$ ) and 80% 1-RM ( $P < 0.012$ ) groups than in the other groups (Figure 2). Lymphocyte AgRP and GH concentrations were significantly lower in all exercise groups ( $P < 0.028$  and  $P < 0.05$ , respectively), but the 60% 1-RM group had lower levels of lymphocyte AgRP and GH (Figure 3). Lymphocyte ATP contents remained unchanged and lower glycogen levels were found in the combined group compared with the control group but no other group, although higher lymphocyte glycogen content was observed in the 60% group when compared with the 40% and combined loaded groups (Figure 4). In contrast to the lymphocyte content, plasma AgRP and GH concentrations were significantly higher in the 60%, 80% and combined loaded groups than in the control group (Figures 3 and 5). However, the 80% 1-RM and combined loaded groups had higher plasma GH concentrations than other groups (Figure 5). Plasma glucose concentrations were significantly ( $P < 0.001$ ) higher in all exercise groups compared with the control group, and the levels of plasma glucose were higher in the 40% and 60% groups than in the other groups (Figure 5).

## Discussion

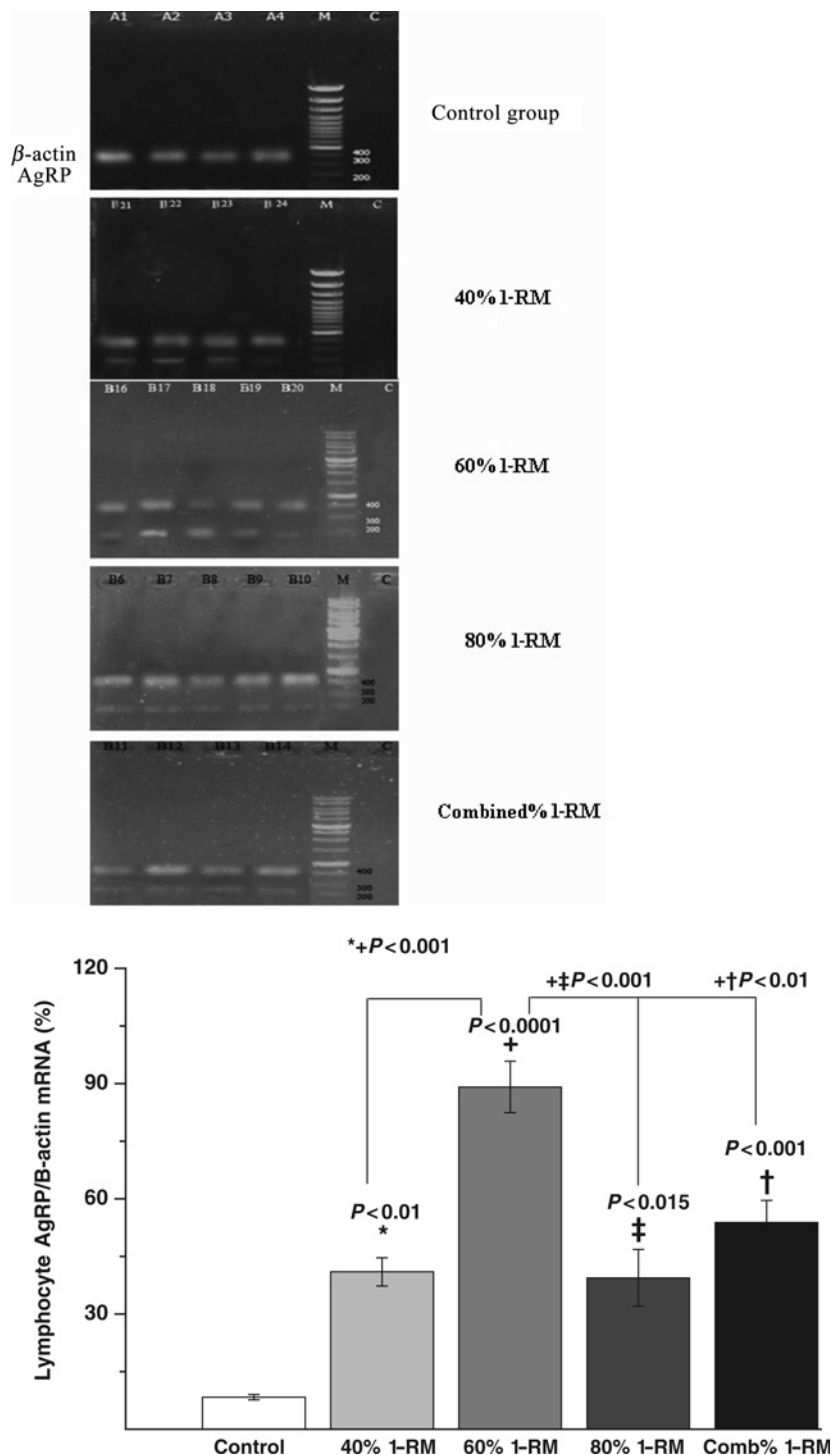
To our knowledge, this is the first study to demonstrate that AgRP is expressed by PBLs. It is also the first investigation

to show that exercise increases AgRP expression in these cells. Another major new finding is that plasma AgRP increased with greater resistance exercise loads. In addition, there were reduced AgRP and GH concentrations in lymphocytes following the CRE protocols, but this reduction was more pronounced in the 60% 1-RM group.

As demonstrated in previous studies,<sup>6,9,16,37</sup> physical exercise enhanced white blood cell number and gene expression in the present study. Connolly *et al.*<sup>8</sup> investigated the effects of 30 min of constant rate of cycle ergometry (80%  $\text{VO}_2$  peak) on PBMC gene expression. They reported that the number of total WBCs, lymphocytes and monocytes were significantly elevated immediately after exercise. They also observed that 311 genes were differentially regulated between pre- and post-exercise and 552 genes were differentially regulated between post-exercise and recovery periods (90 min). Most of the genes were categorized as immune, inflammatory, stress, cell growth, metabolism, signal transduction and transcription factors. Studies such as these reveal the potent effect of exercise on PBL gene expression.

In the present study we found that AgRP mRNA is expressed in PBLs, which is the first time this has been reported. This discovery is analogous to findings regarding the PBL expression of two other appetite hormones, leptin<sup>38</sup> and ghrelin.<sup>15</sup> In addition, since immune cells release inflammatory cytokines that can affect food intake and energy balance,<sup>39</sup> the expression of leptin and ghrelin and AgRP in PBLs would be consistent with these physiological responses. Indeed, it has been reported that with reduction in ghrelin derived from T-cells there is an increase in expression of proinflammatory cytokines.<sup>39</sup> Additionally, it has been shown that leptin affects immune responses in part through action on an isoform of the leptin receptor in T-cells.<sup>40</sup> From these data and those presented in the present study, it appears that PBL signaling via appetite peptides may provide an important role for immune system communication and possibly CNS tissues regarding the state of energy homeostasis. This may be especially important due to the energy requirements for effective immune system function.

The effects of physical exercise on  $\text{TNF-}\alpha$ , interleukin 1 and 6,<sup>41</sup> and ghrelin<sup>16</sup> in human lymphocytes have been investigated, but AgRP has not. Mager *et al.*<sup>16</sup> determined the effects of an incremental aerobic exercise (30 min at 55–65% of  $\text{VO}_{2\text{max}}$ , 4 times/week) and resistance training (4 sessions/week at 70% 1-RM,  $2 \times 10$ –12 repetitions) on ghrelin gene expression and its plasma concentrations in peripheral mononuclear cells of overweight or obese women. Using realtime PCR they reported both ghrelin and GHSR 1b mRNA were expressed in PBLs of all study subjects. They also found that the expression of ghrelin in aerobic and resistance groups was not accompanied by significant changes in plasma ghrelin concentrations. In the present study we used resistance exercise, a stressor known to affect the immune system,<sup>42</sup> on human PBL expression and plasma levels of AgRP. With consideration of the effects of physical exercise on PBL gene expression and with regard to AgRP mRNA expression in other tissues, our results are in agreement with previous studies. Moreover, our data showed that higher AgRP mRNA

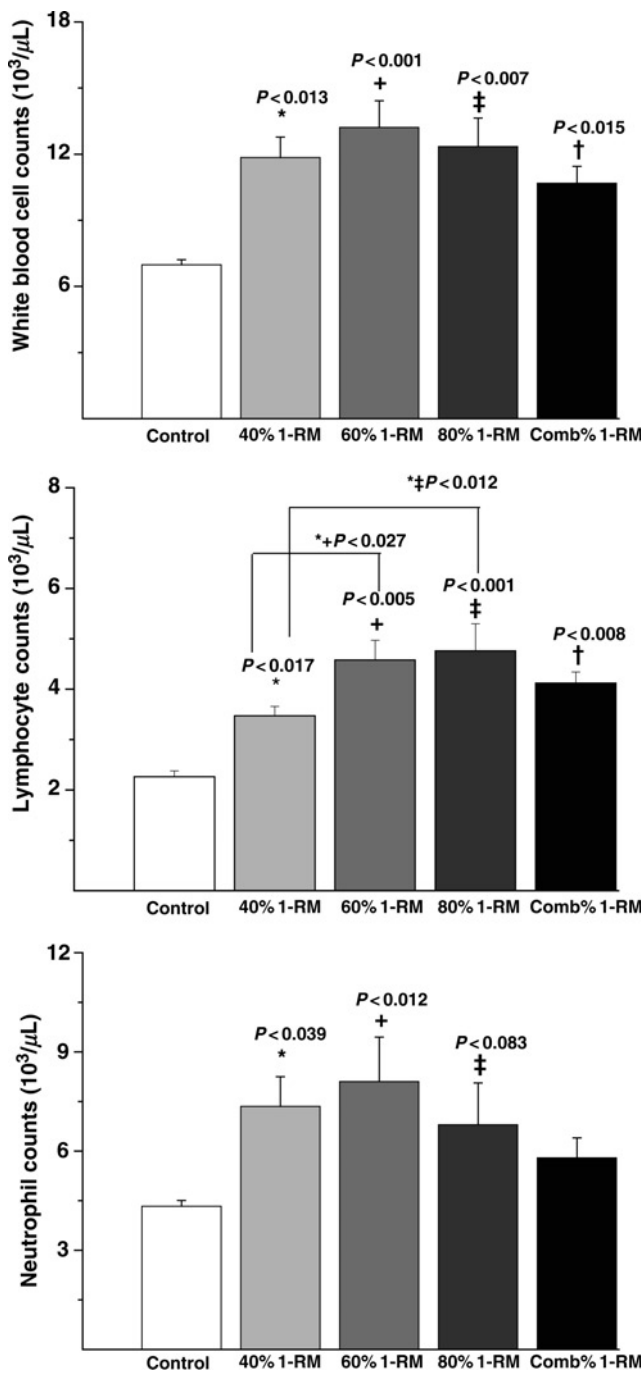


**Figure 1** Semi-quantitative reverse-transcription PCR (RT-PCR) and lymphocyte AgRP/ $\beta$ -actin of mRNA expression (%) in peripheral blood lymphocytes of control ( $N = 4$ ), 40% ( $N = 4$ ), 60% ( $N = 5$ ), 80% ( $N = 5$ ) and combined (40, 60, 80)% of 1-RM loaded groups ( $N = 4$ ). Values are mean  $\pm$  SEM. \*40% versus control,  $\dagger$ 60% versus control,  $\ddagger$ 80% versus control,  $\dagger$ combined % load versus control,  $\ast\dagger$ 60% versus 40%,  $\ast\dagger$ 60% 1-RM versus 80%,  $\ast\dagger$ 60% versus combined % load group. 1-RM, 1-repetition maximum; AgRP, agouti-related protein

expression was accompanied with decreased and increased lymphocyte and plasma AgRP concentrations, respectively. This suggests a possible role of AgRP for paracrine and endocrine signaling. Future research is needed to further elucidate these findings.

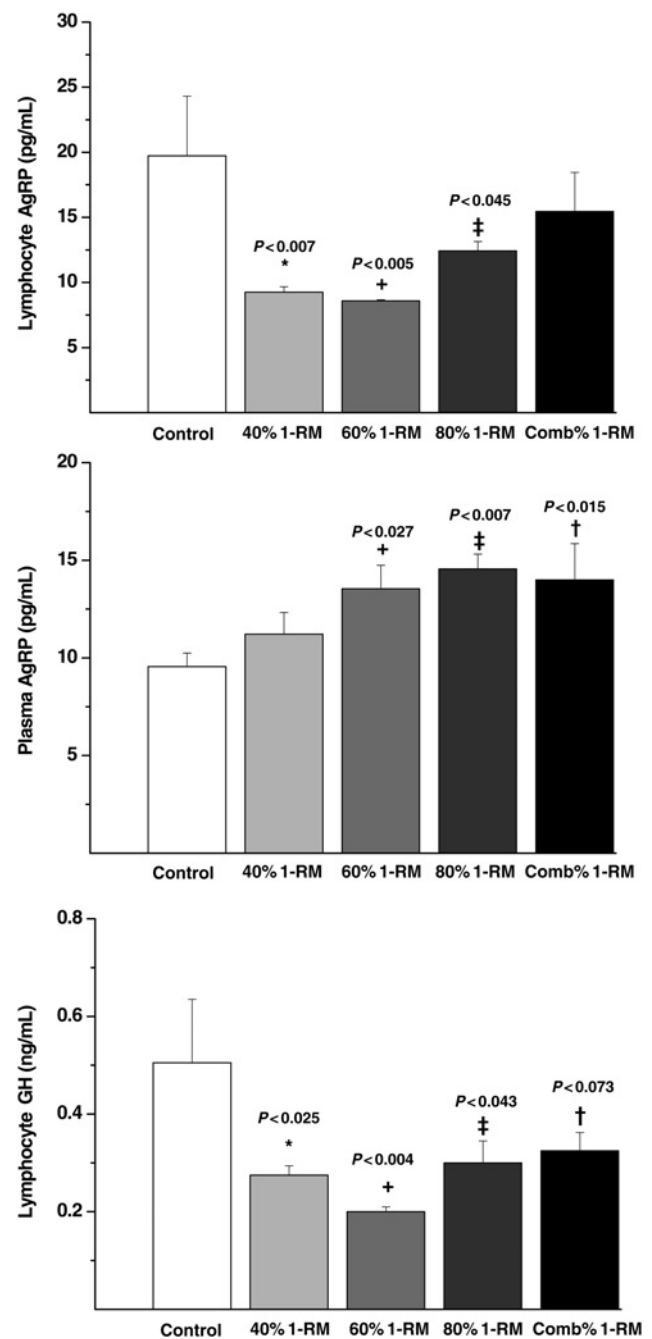
There was a greater lymphocyte AgRP mRNA expression in the 60% group compared to the 40%, 80% and combined

workload groups. Intuitively, these findings would call into question the use of different subjects for the different exercise intensities and intersubject variability. However, plasma concentrations of GH typically increase with increases in resistance exercise muscle loading and GH in the present study followed this expected pattern of increase. This also held true for the combined group that used lower



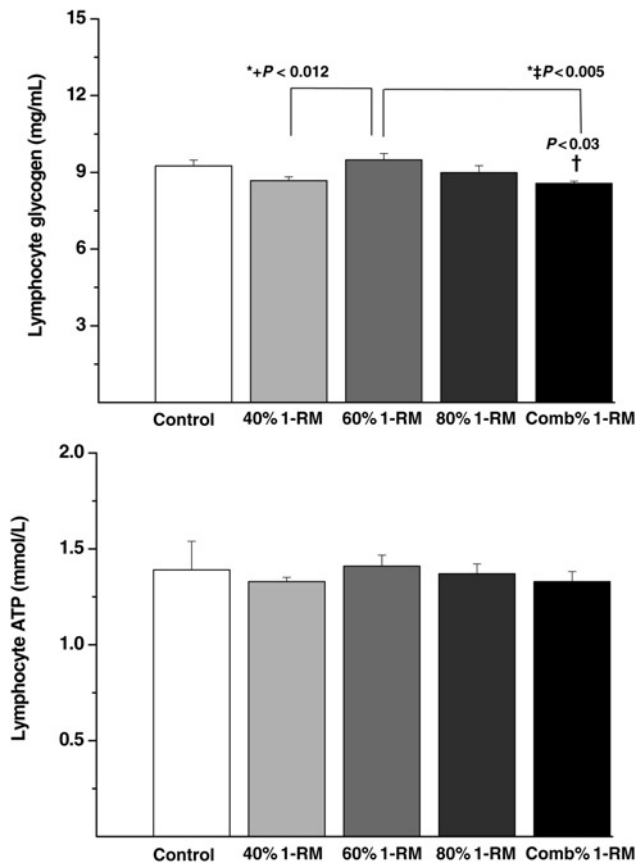
**Figure 2** Total WBCs, lymphocyte and neutrophil counts in control, 40%, 60%, 80% and combined (40, 60, 80)% loaded groups. Five subjects in each group. Values are mean  $\pm$  SEM. \*40% versus control,  $^+60\%$  versus control,  $^\ddagger80\%$  versus control combined % load versus control and for WBC and lymphocyte counts. The counts of lymphocyte were significantly higher in the 60% and 80% groups than in the 40% group.  $^{*+}60\%$  versus 40% group,  $^{*+}80\%$  versus 40% group. 1-RM, 1-repetition maximum; WBC, white blood cell

(40% 1-RM), moderate (60% 1-RM) and higher (80% 1-RM) workloads. This suggests that the physiological responses to different workloads were not due to subject variation within groups with regard to exercise load. Thus, it remains to be determined why greater AgRP mRNA expression occurred with the 60% 1-RM load versus the other loads.



**Figure 3** Lymphocytes AgRP and GH and plasma AgRP concentrations in control, 40%, 60%, 80% and combined (40, 60, 80)% load groups. Five subjects per group. Values are mean  $\pm$  SEM. The levels of lymphocyte AgRP were significantly lower in 40%, 60% and 80% groups, but the reduction of AgRP was more pronounced in the 60% group. The same pattern was observed in lymphocyte GH levels. \*40% 1-RM versus control,  $^+60\%$  1-RM versus control,  $^\ddagger80\%$  versus control,  $^\ddagger$ combined % load versus control. 1-RM, 1-repetition maximum; AgRP, agouti-related protein; GH, growth hormone

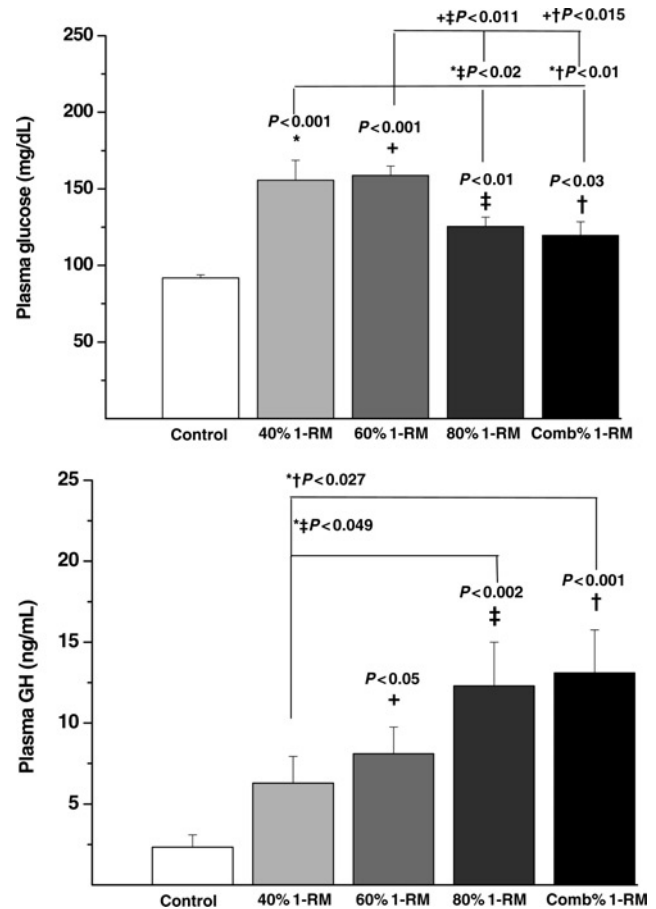
Lymphocyte AgRP content was considerably higher at the 80% compared with the 60% 1-RM load; thus, it is possible that at the 80% 1-RM load the higher AgRP lymphocyte content had already supplied ample feedback within the lymphocyte to reduce AgRP mRNA expression, although this cannot be determined from our research protocol.



**Figure 4** Lymphocyte glycogen and ATP contents in control, 40%, 60%, 80% and combined (40, 60, 80)% load groups. Five subjects per group. Values are mean  $\pm$  SEM. A higher level glycogen content was observed in the 60% group when compared with the 40% and combined % load groups. †Combined % load versus control, \*\*60% versus 40% group, ††60% versus combined % load group. 1-RM, 1-repetition maximum

What is clear is that exercise stimulated increased AgRP mRNA expression since all exercise trials were associated with greater AgRP mRNA levels than resting values.

The effects of physical exercise on plasma AgRP concentrations and brain AgRP mRNA expression have been examined in only a few existing studies. Ghanbari-Niaki *et al.*<sup>26</sup> reported that a single session of CRE at 35% 1-RM increased plasma AgRP concentrations in 20 young men immediately after the exercise bout. Levin and Dunn-Meynell<sup>27</sup> reported no significant effect of exercise on AgRP expression in the ARC of wheel-running and food-restricted rats. In their study, the combination of exercise and caloric restriction had no additive effect on ARC AgRP expression. Another study demonstrated that the AgRP mRNA expression in the ARC was significantly increased in sedentary, wheel-running and food-restricted rats as compared with freely fed rats.<sup>28</sup> This effect was stronger in the running animals, in which AgRP was increased 4.8-fold versus 2.3-fold in the sedentary group. In the present study, there was a pattern of increase in plasma AgRP concentration with increasing workload, similar to GH responses. Some of the increase could have been due to greater lymphocyte expression of AgRP, although clearly other peripheral sources of AgRP likely contributed to higher plasma levels. It is not clear



**Figure 5** Plasma glucose and GH concentrations in control, 40%, 60%, 80% and combined (40, 60, 80)% of load groups. Five subjects per group. Values are mean  $\pm$  SEM. Plasma glucose were higher in exercise protocols. A higher and significant plasma glucose concentration was observed in the 60% and 40% groups when compared with the other groups. \*40% versus control, †60% versus control, †80% versus control, †combined % load versus control, ††60% versus 40%, ††60% 1-RM versus 80%, ††60% versus combined % load group. 1-RM, 1-repetition maximum; GH, growth hormone

why there was a varied workload pattern associated with lymphocyte AgRP content. Future studies are required to elucidate these findings.

This is the first report in relation to levels of GH in PBLs after exercise, particularly following the CRE. Thus, with regard to the discrepancies between plasma and lymphocyte content of AgRP and GH, we can only speculate that a higher plasma AgRP and GH in contrast to the AgRP and GH PBL content is the result of AgRP and GH release or depletion/degradation from different sources among PBLs. This suggests that the lymphocyte might be a minor source of AgRP and GH release into circulation.

In our study, lymphocyte ATP content remained unchanged, but the levels of glycogen were significantly lower in the combined group than in the control group, and significantly lower in the 40% and combined groups compared with the 60% group. Together these findings suggest that lymphocyte AgRP expression is not affected by lymphocyte ATP and glycogen concentrations following exercise.

Limited data exist regarding effects of resistance exercise load on leukocytosis. In the present study, neutrophil

counts were lower (although not significantly so) after the 80% 1-RM trial versus the 60% 1-RM trial, whereas the mean value for lymphocytes at 80% 1-RM was greater (although not significantly so) than at 60% 1-RM loads. WBC count was slightly lower for 80% 1-RM than for 60% 1-RM, but significantly greater than 40% 1-RM. It appears that there are variable responses of different kinds of leukocytes to different exercise loads. For instance, Mayhew et al.<sup>43</sup> investigated effects of resistance exercise rest intervals (1 versus 3 min between sets) on leukocytosis and reported increased lymphocyte number to a greater degree in response to a one-minute rest interval protocol compared with resistance exercise protocol using three-minute rest intervals between sets, which was similar in direction to our findings. However, there was actually a higher mean neutrophil level in the three-minute versus the one-minute protocol. This is also consistent with the direction of our findings with mean neutrophil counts being greater (but not significantly so) in response to the 80% 1-RM versus 60% 1-RM trial.

In summary, this is the first study demonstrating that acute exercise increases PBL AgRP mRNA expression. The study also provides the first evidence that larger resistance exercise loads elicit greater increases in plasma AgRP concentrations and that loading affects lymphocyte AgRP content. Findings indicate that PBLs should be considered as a possible extra-hypothalamic source for plasma AgRP levels following acute exercise. These data are consistent with the general concept of intra- and inter-tissue appetite signaling in that immune system responses to acute exercise require greater energy expenditure and that orexigenic signaling via AgRP could be important in response to immune activity elicited by exercise. Further study is needed to determine the role of AgRP mRNA expression in the exercise environment.

#### ACKNOWLEDGEMENTS

This work was supported by the Research secretary and Faculty of Physical Education and Sports Science of University of Mazandaran. We wish to thank Miss Marziyeh Saghebjo, Dr Fatemeh Rahbarizadeh, Dr Mehdi Hedayati and Dr Hamid Ayati for their kind help and sincere cooperation. We also thank all female college students for their participation in this study.

#### REFERENCES

- Kraemer RR, Brown BS. Alterations in plasma-volume-corrected blood components of marathon runners and concomitant relationship to performance. *Eur J Appl Physiol* 1986;**55**:579–84
- Moorthy AV, Zimmerman SW. Human leukocyte response to an endurance race. *Eur J Appl Physiol Occup Physiol* 1978;**38**:271–6
- Kendall A, Hoffman-Goetz L, Houston M, MacNeil B, Arumugam Y. Exercise and blood lymphocyte subset responses: intensity, duration, and subject fitness effects. *J Appl Physiol* 1990;**69**:251–60
- Mayhew DL, Thyfault JP, Koch AJ. Rest-interval length affects leukocyte levels during heavy resistance exercise. *J Strength Cond Res* 2005;**19**:16–22
- Simonson SR, Jackson CG. Leukocytosis occurs in response to resistance exercise in men. *J Strength Cond Res* 2004;**18**:266–71
- Büttner P, Mosig S, Lechtermann A, Funke H, Mooren FC. Exercise affects the gene expression profiles of human white blood cells. *J Appl Physiol* 2007;**102**:26–36
- Cases N, Sureda A, Maestre I, Tauler P, Aguiló A, Córdova A, Roche E, Tur JA, Pons A. Response of antioxidant defences to oxidative stress induced by prolonged exercise: antioxidant enzyme gene expression in lymphocytes. *Eur J Appl Physiol* 2006;**98**:263–9
- Connolly PH, Caiozzo VJ, Zaldivar F, Nemet D, Larson J, Hung SP, Heck JD, Hatfield GW, Cooper DM. Effects of exercise on gene expression in human peripheral blood mononuclear cells. *J Appl Physiol* 2004;**97**:1461–9
- Fehrenbach E. Multifarious microarray-based gene expression patterns in response to exercise. *J Appl Physiol* 2007;**102**:7–8
- Fehrenbach E, Niess AM, Schlotz E, Passet F, Dickhuth HH, Northoff H. Transcriptional and translational regulation of heat shock proteins in leukocytes of endurance runners. *J Appl Physiol* 2000;**89**:704–10
- Natelson BH, Zhou X, Ottenweller JE, Bergen MT, Sisto SA, Drastal S, Tapp WN, Gause WL. Effect of acute exhausting exercise on cytokine gene expression in men. *Int J Sports Med* 1996;**17**:299–302
- Zieker D, Fehrenbach E, Dietzsch J, Fliegner J, Waidmann M, Nieselt K, Gebicke-Haerter P, Spanagel R, Simon P, Niess AM, Northoff H. cDNA microarray analysis reveals novel candidate genes expressed in human peripheral blood following exhaustive exercise. *Physiol Genomics* 2005;**23**:287–94
- Zieker D, Zieker J, Dietzsch J, Burnet M, Northoff H, Fehrenbach E. CDNA-microarray analysis as a research tool for expression profiling in human peripheral blood following exercise. *Exerc Immunol Rev* 2005;**11**:86–96
- Kraemer RR, Castracane VD. Exercise and humoral mediators of peripheral energy balance: ghrelin and adiponectin. *Exp Biol Med* 2007;**232**:184–94
- Hattori N, Saito T, Yagyu T, Jiang BH, Kitagawa K, Inagaki C. GH, GH receptor, GH secretagogue receptor, and ghrelin expression in human T cells, B cells, and neutrophils. *J Clin Endocrinol Metab* 2001;**86**:4284–91
- Mager U, Kolehmainen M, de Mello VD, Schwab U, Laaksonen DE, Rauramaa R, Gylling H, Atalay M, Pulkkinen L, Uusitupa M. Expression of ghrelin gene in peripheral blood mononuclear cells and plasma ghrelin. *Eur J Endocrinol* 2008;**158**:499–510
- Ollmann MM, Wilson BD, Yang YK, Kerns JA, Chen Y, Gantz I, Barsh GS. Antagonism of central melanocortin receptors *in vitro* and *in vivo* by agouti-related protein. *Science* 1997;**278**:135–8
- Shutter JR, Graham M, Kinsey AC, Scully S, Lüthy R, Stark KL. Hypothalamic expression of ART, a novel gene related to agouti, is up-regulated in obese and diabetic mutant mice. *Genes Dev* 1997;**11**:593–602
- Takeuchi S, Teshigawara K, Takahashi S. Widespread expression of Agouti-related protein (AGRP) in the chicken: a possible involvement of AGRP in regulating peripheral melanocortin systems in the chicken. *Biochim Biophys Acta* 2000;**1496**:261–9
- Ghanbari-Niaki A, Abednazari H, Tayebi SM, Hossaini-Kakhak A, Kraemer RR. Treadmill training enhances rat agouti-related protein in plasma and reduces ghrelin levels in plasma and soleus muscle. *Metabolism*. 2009;**58**:1747–52
- Breen TL, Conwell IM, Warlaw SL. Effects of fasting, leptin and insulin on AgRP, and POMC peptide release in hypothalamus. *Brain Res* 2005;**1032**:141–8
- Catania A. The melanocortin system in leukocyte biology. *J Leukoc Biol* 2007;**81**:383–92
- Xiao E, Xia-Zhang L, Vulliémoz NR, Ferin M, Wardlaw SL. Agouti-related protein stimulates the hypothalamic-pituitary-adrenal (HPA) axis and enhances the HPA response to interleukin-1 in the primate. *Endocrinology*. 2003;**144**:1736–41
- Hoggard N, Rayner DV, Johnston SL, Speakman JR. Peripherally administered [Nle<sup>4</sup>,D-Phe<sup>7</sup>]-alpha-melanocyte stimulating hormone increases resting metabolic rate, while peripheral agouti-related protein has no effect, in wild type C57BL/6 and ob/ob mice. *J Mol Endocrinol* 2004;**33**:693–703
- Gustafson B, Gogg S, Hedjazifar S, Jenndahl L, Hammarstedt A, Smith U. Inflammation and impaired adipogenesis in hypertrophic obesity in man. *Am J Physiol Endocrinol Metab*. 2009;**297**:E999–E1003

- 26 Ghanbari-Niaki A, Nabatchian S, Hedayati M. Plasma agouti-related protein (AgRP), growth hormone, insulin responses to a single circuit-resistance exercise in male college students. *Peptides* 2007;**28**:1035–9
- 27 Levin BE, Dunn-Meynell AA. Chronic exercise lowers the defended body weight gain and adiposity in diet-induced obese rats. *Am J Physiol* 2004;**286**:R771–R778
- 28 de Rijke CE, Hillebrand JJ, Verhagen LA, Roeling TA, Adan RA. Hypothalamic neuropeptide expression following chronic food restriction in sedentary and wheel-running rats. *J Mol Endocrinol* 2005;**35**:381–90
- 29 Dennis RA, Trappe TA, Simpson P, Carroll C, Huang BE, Nagarajan R, Bearden E, Gurley C, Duff GW, Evans WJ, Kornman K, Peterson CA. Interleukin-1 polymorphisms are associated with the inflammatory response in human muscle to acute resistance exercise. *J Physiol* 2004;**560**(Pt 3):617–26
- 30 Kraemer RR, Kilgore JL, Kraemer GR, Castracane VD. Growth hormone, IGF-I, and testosterone responses to resistive exercise. *Med Sci Sports Exerc* 1992;**24**:1346–52
- 31 Meazza C, Pagani S, Travaglini P, Bozzola M. Effect of growth hormone (GH) on the immune system. *Pediatr Endocrinol Rev* 2004;**1** (Suppl. 3):490–5
- 32 Kraemer RR, Durand RJ, Hollander DB, Tryniecki JL, Hebert EP, Castracane VD. Ghrelin and other glucoregulatory hormone responses to eccentric and concentric muscle contractions. *Endocrine* 2004;**24**:93–8
- 33 Maciver NJ, Jacobs SR, Wieman HL, Wofford JA, Coloff JL, Rathmell JC. Glucose metabolism in lymphocytes is a regulated process with significant effects on immune cell function and survival. *J Leukoc Biol* 2008;**84**:949–57
- 34 Tamura H, Kamegai J, Shimizu T, Ishii S, Sugihara H, Oikawa S. The effect of agouti-related protein on growth hormone secretion in adult male rats. *Regul Pept* 2005;**125**:145–49
- 35 Ghanbari-Niaki A, Saghebjo M, Rahbarizadeh F, Hedayati M, Rajabi H. A single circuit-resistance exercise has no effect on plasma obestatin levels in female college students. *Peptides* 2008;**29**:487–90
- 36 Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidiniumthiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987;**162**:156–9
- 37 Radom-Aizik S, Zaldivar F Jr, Leu SY, Galassetti P, Cooper DM. Effects of 30 min of aerobic exercise on gene expression in human neutrophils. *J Appl Physiol* 2008;**104**:236–43
- 38 Samara A, Marie B, Pfister M, Visvikis-Siest S. Leptin expression in peripheral blood mononuclear cells (PBMCs) is related with blood pressure variability. *Clin Chim Acta* 2008;**395**:47–50
- 39 Dixit VD, Schaffer EM, Pyle RS, Collins GD, Sakthivel SK, Palaniappan R, Lillard JW Jr, Taub DD. Ghrelin inhibits leptin- and activation-induced proinflammatory cytokine expression by human monocytes and T cells. *J Clin Invest* 2004;**114**:57–66
- 40 Siegmund B, Sennello JA, Jones-Carson J, Gamboni-Robertson F, Lehr HA, Batra A, Fedke I, Zeitz M, Fantuzzi G. Leptin receptor expression on T lymphocytes modulates chronic intestinal inflammation in mice. *Gut* 2004;**53**:965–72
- 41 Moldoveanu AI, Shephard RJ, Shek PN. Exercise elevates plasma levels but not gene expression of IL-1beta, IL-6, and TNF-alpha in blood mononuclear cells. *J Appl Physiol* 2000;**89**:1499–504
- 42 Dennis RA, Trappe TA, Simpson P, Carroll C, Huang BE, Nagarajan R, Bearden E, Gurley C, Duff GW, Evans WJ, Kornman K, Peterson CA. Interleukin-1 polymorphisms are associated with the inflammatory response in human muscle to acute resistance exercise. *J Physiol* 2004;**560**(Pt 3):617–26
- 43 Mayhew DL, Thyfault JP, Koch AJ. Rest-interval length affects leukocyte levels during heavy resistance exercise. *J Strength Cond Res* 2005;**19**:16–22

(Received July 14, 2009, Accepted September 28, 2009)