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ORIGINAL ARTICLE

The effect of 4-week training period on plasma neuropeptide Y, leptin and ghrelin responses in male rowers

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Abstract The aim was to investigate the effect of high-volume low intensity resistance training protocol combined with endurance training on plasma neuropeptide Y (NPY) concentration in rowers. Additionally, leptin and ghrelin, as markers for body energy balance concentrations, were monitored. 12 highly trained national and international level male rowers participated in this study. The participants were tested three times—after reference week (T1), after 2 weeks of high-volume training (T2) and after a recovery week (T3) for aerobic performance, energy intake and expenditure, and blood biochemical parameters. The submaximal rowing performance decreased significantly ($P = 0.019$) at T2. Fasting leptin decreased significantly (from 2.05 ± 0.88 to 1.28 ± 0.53 ng/mL; $P = 0.009$) at T2 and increased significantly (from 1.28 ± 0.53 to 1.79 ± 0.79 ng/mL; $P = 0.002$) at T3. Fasting ghrelin decreased significantly (from 980 ± 300.2 to 873.35 ± 198.6 pg/mL; $P = 0.036$) at T3 compared to T2, while no changes were found in fasting NPY. Significant decreases in exercise-induced leptin were observed at T2 (from 1.13 ± 0.5 to 1.08 ± 0.5 ng/mL; $P = 0.012$), PRE and POST test leptin values at T2 were significantly decreased compared to T1(1.40 ± 0.9 to 1.13 ± 0.5 and 1.44 ± 0.8 to 1.08 ± 0.5, respectively). Acute exercise-induced increases in NPY were found at T2 (from 128.1 ± 23.2 to 155.1 ± 28.9 pmol/L; $P = 0.002$) and at T3 (from 131.3 ± 20.5 to 159.7 ± 32.8 pmol/L, $P = 0.004$). In conclusion, the combination of high-volume training protocol and energy imbalance induces significant post-exercise changes in NPY, leptin, and ghrelin concentrations and decreases fasting leptin.

Keywords Energy balance · Strength endurance · Rowing · Overreaching · Neuropeptide Y

Introduction

To obtain improvements in performance, athletes use stressful training periods followed by recovery periods in order to allow supercompensation and regain performance. For endurance type of sports those hard training periods are mainly performed at relatively low intensity, but for longer duration (Mäestu et al. 2005). Low intensity, high-volume training protocols are known to affect appetite, energy balance and body weight by altering several key hormones that modulate energy balance (Karamouzis et al. 2002; Simsch et al. 2002; Jürimäe et al. 2003). However, negative energy balance might be an additional stressor for organism that could lead to increases in excessive fatigue accumulation and consequently decreases in performance. (Rämson et al. 2008).

Neuropeptide Y (NPY) is a 36-amino acid peptide neurotransmitter and is concentrated in the hypothalamus, an area for appetite regulation. In humans, it has been shown that NPY is released into the circulation in response to sympathetic activation by a number of stimuli including hypoglycemia, exercise, and acute stress (Morgan et al. 2002) and is thought to facilitate the containment of negative consequences following exposure to stress (Fletcher et al. 2010; Heilig 2004). NPY is also a potent long-lasting vasoconstrictor, which exerts direct action and modulates the

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effects of other mediators such as norepinephrine, serotonin or angiotensin II (Joannic et al. 1998). NPY plays a leading role in the regulation of eating behavior and energy expenditure by decreasing physical activity (Kokot and Ficek 1999; Shin et al. 2003) and therefore may affect training potential. In contrast, central NPY exerts inhibitory actions causing lowering of sympathetic activity, motor activity, heart rate and blood pressure (Kuo and Zukowska 2007).

However to date, limited evidence exists concerning NPY responses to exercise. Chen et al. (2007) found that the content of NPY in hypothalamic nuclei continued to increase at 0, 30, and 180 min post-exercise compared with pre-exercise control trial ($P < 0.01$), during an acute and chronic high-intensity treadmill exercise in rats. Karamouzis et al. (2002) found an 81% increase and almost a 100% decrease after 25-km marathon swimming in plasma NPY and leptin concentrations, respectively. Furthermore, a significant negative correlation between the values of leptin and NPY was found and authors concluded that changes in leptin and NPY took place during marathon swimming in order to compensate for the negative energy balance due to prolonged effort (Karamouzis et al. 2002). Significantly higher plasma NPY concentrations were also observed in soldiers subjected to the "uncontrollable stress" of interrogation, which produced extreme subjective psychological distress and clinically significant dissociation (Morgan et al. 2002). Irwin (2008) suggested that increased NPY levels serve as a tonic measure of sympathetic activity. Increased sympathietic activity on the other side has been proposed as a possible marker for overtraining (Urhausen and Kindermann 2002). Thus, it could be speculated that decreases in performance that are accompanied by high-volume training may induce the increase in plasma NPY concentration. Recently, Fletcher et al. (2010) found increased plasma NPY in subjects with chronic fatigue syndrome and showed significant correlations with Profile of Mood State stress scales.

There is evidence that leptin depresses NPY expression and NPY is increased in starvation when leptin is low (Steinacker et al. 2004). Increased NPY leads to increased neuronal activity and depresses the hypothalamo–pituitary–adrenocortical axis (HPA). However, according to longitudinal studies investigating leptin, it could be speculated that changes in fasting leptin concentration could be too sensitive to investigate training stress in order to avoid overtraining, since a decrease in leptin concentration has not been linked to a decrease in performance in several studies (Jürimäe et al. 2003; Rämson et al. 2008). No studies have yet been conducted to study exercise-induced changes in NPY during different training regimen in athletes. An inclusion of NPY to that path could be more helpful since decrease in leptin stimulates the production of NPY. Therefore, it was of interest to investigate whether long-term stressors (high-volume training) changed fasting or exercise-induced changes in plasma NPY to provide evidence that NPY is a marker of excessive stress in athletes.

Ghrelin provides an endocrine link between stomach and central circuits involved in the regulation of energy intake; it transfers information from the stomach to the hypothalamus (Van der Lely et al. 2004). Ghrelin's most powerful physiological function is to signal the brain when energy must be consumed and stored (Chowdhury et al. 2004). Ghrelin concentrations increase while fasting and decrease after caloric intake, thus it may play a role in hunger (Cummings et al. 2004). It has been reported that 6 weeks of exercise training at a low to moderate exercise intensity resulted in reduced total ghrelin levels in rat plasma and soleus muscle (Ghanbari-Niaki et al. 2009). Moreover, there are some data that suggest under certain conditions, exercise may suppress circulating ghrelin levels which could decrease feeding behavior (Kraemer et al. 2004).

The aim of this study was to investigate the effect of high-volume training on plasma NPY concentration in rowers in conditions of increased training stress and high energy expenditure. Additionally, leptin and ghrelin as markers for body energy balance concentrations were monitored. It has been demonstrated that ghrelin levels increase in long-term exercise intervention in overweight participants (Foster-Schubert et al. 2005) but fasting levels remain unchanged in athletes after high-volume training period. We hypothesized that NPY concentrations would increase during the period of low intensity high volume endurance trainings.

Methods

Participants

The participants of this study were 12 highly trained national and international level male rowers (age 22.2 ± 3.4 years, height 183.9 ± 4.6 cm, body mass 82.8 ± 9.5 kg and training experience 7.5 ± 2.2 years). None of the participants had an unsatisfactory medical history or were taking any medication during the study. The study was approved by the Ethical Committee of University of Tartu and was carried out in accordance with the Helsinki Declaration of 1975. All participants were informed about the study procedures, possible risks and the purpose of the investigation before they signed a written consent.

Study design

The study was conducted during the beginning of the preparatory period (i.e. October and November). just 2 or 3 weeks after the subjects started with preparatory period.

4-week (Weeks 1–4) training period (Rämson et al. 2008) was designed with increases in training volume at weeks 2 and 3. The week before Week 1 was aimed to execute pre-training baseline measurements and serves as a reference week. During Week 1, the training volume was about 10 h (similar to the week before Week 1) in order to execute the baseline measures for each subject (Desgorces et al. 2004; Jürimäe et al. 2003; Rämson et al. 2008; Simsch et al. 2002). Therefore, the control group was not required (Jürimäe et al. 2003). During Week 2, training volume was increased to 15 h and during Week 3 training volume was increased up to 20 h. Week 4 was meant for recovery and training volume was decreased to about the same level as Week 1. About 50% of the trainings were low-intensity resistance trainings in the gym and 50% was low-intensity endurance rowing training, cycling or running in the range of their aerobic threshold intensity. The resistance training consisted of 40–50% RM, 40–50 repetitions and 6 different exercises which were performed in circuit method. This type of training cycle is very common in rowers to develop muscular endurance in combination of aerobic capacity on dry land. During the study period, the athletes trained 6 days a week and 1 day, Monday, was meant for recovery each week. All the testing sessions (fasting blood, 2 h rowing performance) were carried out on the same day and time (i.e. on Tuesday) each week (Fig. 1).

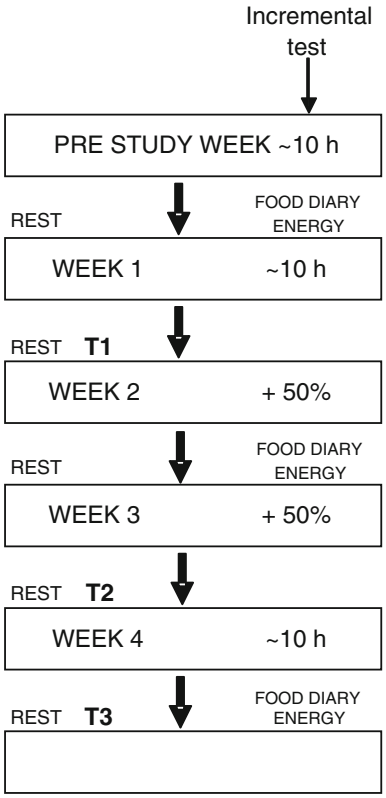

Fig. 1 The schematic view of the study. Pre study week was with an average training volume of 10 h. After week 1 training volume was increased by 50% and then further 50% on week 3. Week 4 was for recovery with training load approximately 10 h. *REST* indicates rest day. *T1*, *T2*, *T3* indicate testing day (Tuesday) for fasting blood and performance test. *ENERGY* energy expenditure calculation

Test procedures

Body mass and height of the participants were measured using a Martin metal anthropometer and medical balance scales (A&D Instruments Ltd., UK). Body composition parameters (fat mass and fat free mass) were measured with the Lunar DPX-L total body scanner (Lunar Corporation, Madison, WI, USA), which was operated in the medium scan mode (~20 min). The scanner was calibrated daily as suggested by the manufacturer.

Incremental rowing ergometer test

Training intensities for the study period were individually calculated for each subject based on a stepwise incremental test results on the Concept II rowing ergometer (Morrisville, VT, USA) as described previously (Rämson et al. 2008, 2009) at the end of the week, preceding Week 1 using the heart rate turn point method (HRTP) (Hofmann et al. 2007). To calculate HRTP, two regression lines were calculated iteratively by the special hardware and the intersection point between both optimized regression lines was defined as the heart rate turn point. The heart rate turn point method has been shown to be reliable in determining training intensities for rowing (Hofmann et al. 2007; Mikulic et al. 2011). However for trainings, 20% lower intensity of the determined heart rate turn point was taken as the highest intensity to use during the trainings of the 4-week training period to execute training intensities around 2 mmol/L of blood lactate (Rämson et al. 2008). Athlete's heart rate was monitored during each training session according to their personal 80% HRTP values (approximately the range of aerobic threshold) using a Polar S625X HR monitor throughout the study period (Polar Electro, Finland).

Aerobic performance test

The aerobic performance of the participants was measured with a 2 h low intensity rowing (2HR) on rowing ergometer (Rämson et al. 2008, 2009). Target heart rate was set at the level obtained during the incremental test using a practical set ±3 beats/min of the 80% HRTP. All aerobic performance tests were done after a day of full rest (i.e. on Tuesday, at 15.00—17.00 PM) at the beginning of Week 1 (T1), after the high-volume period (T2) and after the recovery period (T3).

Blood sampling

The 10 mL fasting blood sample was drawn in the morning at 7.30–8.00 after the day of full rest at the base line week (T1), after high-volume training period (T2) and after recovery period (T3) with the subject in the upright position. On the same day, in the afternoon, the 2HR test was held. Venous blood samples were drawn also before 2HR (PRE), 5 min after (POST) and 30 min after the test (POST 30) (Rämson et al. 2008). Plasma was separated and frozen at −20°C for subsequent analysis. Post-exercise blood samples were corrected to the shifts in the plasma volume according to Dill and Costill (1974). Samples from one individual were run in the same assays. Leptin was determined in duplicate by radioimmunoassay (RIA) (Mediagnost GMBH, Germany) with a detection limit of 0.01 ng/mL, and the intra-assay and inter-assay coefficients of variation (CV) were <5 and <7.5%, respectively (Mediagnost GMBH, Germany). Ghrelin was determined using a commercially available RIA kits (Linco Research, USA) with a sensitivity of 93 pg/L, and the intra- and inter-assay CV were <10 and 14.7%, respectively. Insulin was determined utilizing IMMULITE 2000 (DPC, Los Angeles, CA, USA). The intra- and inter-assay CV were 4.8 and 12.0%, respectively. NPY was determined using commercially available RIA kit (Euro-Diagnostica AB, Malmö, SWE) with intra- and inter-assay coefficients CV were <5.0 and <9.2%, respectively.

Blood samples in the amount of 20 µl were also collected from fingertip for lactate analysis and were analysed using the photo enzymatic method by Lange (Lange, Germany). Fingertip blood samples were obtained before the 2HR, during the 2HR (after 60 min) and 5 min after completing the 2HR.

Energy expenditure and energy intake

At the end of the reference week (Week 1), at the end of the high-volume period (Week3) and at the end of the recovery week (Week4), the participants filled out the eating diaries of the consecutive 3 days of two working and one weekend day. They had to note all the consumed food, drinks and food supplements in order to measure their caloric intake. The caloric intake was then calculated as the average of the 3 days. The calculations were done using Micro-Nutrica 2.0 program. The participants were allowed to eat as much as they subjectively felt (Rämson et al. 2008). However, their daily food intake consisted of a high carbohydrate diet and the composition remained stable throughout the study. The daily energy expenditure for the same days when caloric intake was measured was calculated according to the method of (Bouchard et al. 1983) and has been previously used in athletes of different disciplines (Mäestu et al. 2008; Rämson et al. 2008).

Table 1 Changes of the anthropometric parameters and energy intake and expenditure during the 4-week training period at three different testing sessions

	T1	T2	T3
Weight (kg)	82.80 ± 9.5	83.49 ± 9.0	81.66 ± 8.2
BMI (kg m^{-2})	23.95 ± 2.4	24.07 ± 2.3	23.46 ± 2.3
Body fat (%)	13.87 ± 6.6	13.30 ± 6.1[1]	12.56 ± 5.9[2]
FM (kg)	11.4 ± 6.2	11.1 ± 5.8	9.3 ± 5.5
FFM (kg)	71.4 ± 8.3	72.8 ± 7.9	73.0 ± 7.8
Energy intake (kcal)	3,981.3 ± 577.1	4,473.5 ± 513.3[1]	3,914.7 ± 649.2[2]
Energy expenditure (kcal)	4,136.8 ± 774.1	4,881.8 ± 813.0[1]	4,102.4 ± 689.1[2]

Numbers indicate significant differences ($P < 0.05$) from the pointed testing session

Statistical analysis

Mean and standard deviations (SD) were calculated. Friedman analyses of variance by ranks were used to examine the changes in the dependent variables. The Wilcoxon matched-pairs signed-ranks test was used to assess the differences between the measured variables as data were not normally distributed. Kendall rank correlation coefficients were used to evaluate associations among different variables of interest. The level of significance was set at $P < 0.05$.

Results

The average training volume increased significantly at Week 2 ($P = 0.002$) and 3 ($P = 0.002$) compared to Week 1 (Week 1 626.4 ± 22.1 min, Week 2 866.7 ± 34.7 min, Week 3 1,137.9 ± 72.4 min, respectively) and decreased significantly at Week 4 (to 657.8 ± 16.6 min; $P = 0.002$) compared to Week 3. Approximately 50% of all the training sessions during the study were low intensity strength endurance trainings in the gym with a resistance of 40–50% 1RM, while the rest was low intensity rowing, running and cycling at blood lactate concentration in the range of 2 mmol/L. The caloric intake and energy expenditure increased significantly ($P = 0.003$ and $P = 0.003$, respectively) as a result of high-volume training period at T2 (Table 1).

The distance of the 2 h low intensity rowing ergometer test decreased significantly ($p = 0.019$) after high-volume training period (Table 2) and remained unaltered after the recovery week.

Fasting leptin decreased significantly ($P = 0.009$) after high-volume training and fasting ghrelin decreased

Table 2 The descriptive parameters of the three performance tests during the 4-week training period

	T1	T2	T3
Distance (m)	27,153.8 ± 1,124.9	26,493.0 ± 1,211.6[1]	26,755.9 ± 1,361.7
HR (beats/min)	134.8 ± 4.5	133.6 ± 2.1	133.8 ± 2.9
La Pre (mmol/L)	2.2 ± 0.8	2.3 ± 0.6	2.3 ± 0.9
La 60′ (mmol/L)	2.6 ± 0.7	2.4 ± 0.8	2.9 ± 0.9
La Post (mmol/L)	2.4 ± 1.0	2.0 ± 0.4	2.8 ± 0.7

Numbers indicate significant differences ($P < 0.05$) from the pointed testing session

Table 3 Changes in the fasting biochemical parameters during the 4-week training period at three different testing sessions

	T1	T2	T3
Leptin (ng/mL)	2.05 ± 0.88	1.28 ± 0.53[1]	1.79 ± 0.79[1, 2]
Insulin (μIU/min)	3.62 ± 1.3	3.81 ± 1.6	4.45 ± 2.0
NPY (pmol/L)	125.2 ± 25.9	138.0 ± 35.9	120.7 ± 22.4
Ghrelin (pg/mL)	973.46 ± 183.4	980 ± 300.2	873.35 ± 198.6[1]

Numbers indicate the significant differences ($P < 0.05$) from the pointed testing session

significantly ($P = 0.002$) after the recovery week (Table 3). No further significant changes were observed in fasting insulin and NPY values during the study period.

Exercise-induced NPY values were significantly higher at Post and Post 30 tests compared to Pre test values at T2 (accordingly $P = 0.002$ and $P = 0.004$) and T3 (accordingly $P = 0.004$ and $P = 0.003$). Exercise-induced leptin concentration decreased ($P = 0.012$) at Post test compared to Pre test value at T2. Leptin Pre and Post concentrations decreased significantly (accordingly $P = 0.048$ and $P = 0.049$) at T2 compared to T1. Leptin Pre and Post concentrations increased significantly (accordingly $P = 0.017$ and $P = 0.017$) at T3 compared to T2. Insulin concentrations were significantly decreased at Post and Post 30 tests compared to Pre test values at T1, T2 and T3 ($P < 0.036$ for all tests). Insulin concentration increased ($P = 0.046$) at T3 Post test compared to T2 Post value. During T1, Post and Post 30 test values of ghrelin increased (accordingly $P = 0.028$ and $P = 0.034$) compared to Pre test value. No more exercise-induced changes in ghrelin concentration were found.

Leptin and NPY were negatively related during the fasting conditions at Week 1 ($R = -0.644$; $P = 0.005$). There were no further significant correlations between leptin and NPY values during fasting or during exercise-induced changes ($P > 0.05$) nor there were significant correlations between ghrelin and NPY concentrations in either fasting or exercise-induced conditions ($P < 0.05$; data not shown). Also, we did not find any significant correlations between energy expenditure or intake and biochemical parameters ($P > 0.05$).

Discussion

This study is the first to investigate longitudinally the influence of high training volume, low intensity, concurrent endurance and resistance training period (about half of the trainings were done in a resistance mode) on acute and chronic changes in the plasma NPY concentration and two other markers of body energy reserves. The 2-week high-volume training period decreased the performance of the participants (Table 1). The most important finding of the present study was that the post-exercise concentrations of NPY increased significantly and post-exercise concentrations of leptin decreased significantly after high-volume training protocol.

Plasma concentrations of NPY in response to exercise are not much studied in athletes. To our best knowledge, only Karamouzis et al. (2002) have investigated the NPY response to acute 25-km marathon swimming. They indicated a remarkable 47% increase in NPY, while leptin was decreased (51%). Studies have also reported that physical exercise under normal conditions may induce hyperphagia by increasing energy intake in humans (Verger et al. 1994) and stimulates NPY activity in the rat hypothalamus (Chen et al. 2007). However, leptin and NPY probably change because of the negative energy balance and not the exercise per se (Hagobian et al.2008; Jürimäe et al. 2009). NPY levels also increase during the high-intensity sympathetic nerve stimulation and to other heavy and prolonged stressors such as cold pressure test and intense physical exercise (Zukowska-Grojec and Wahlestedtc 1993). Lighter stressors, as the isometric handgrip and orthostatic tests, have failed to increase the plasma NPY.

However, we were not able to demonstrate any changes in fasting NPY values after 2-week high-volume training (T2) and after a recovery period (T3), despite increasing negative energy balance at T2 (Table 2). However, fasting leptin values decreased significantly after high-volume trainings (T2) compared to baseline measurements (T3). Change in fasting leptin has been classified as one of the potential marker to monitor excessive training stress during periods of training sessions in endurance sports (Simsch et al. 2002; Mäestu et al. 2003; Desgorces et al. 2004; Rämson et al. 2008). The link between leptin and NPY has

also been demonstrated in athletes (Karamouzis et al. 2002) that was similar to our study in fasting conditions at T1 (i.e. in the conditions of relative rest) where NPY and leptin concentrations were significantly related ($R = -0.644$; $P = 0.005$). It was interesting to find however, that leptin and NPY were not correlated after high-volume trainings (T2) and after recovery period (T3). This finding is difficult to explain. Of course, it is tempting to speculate that the condition of the athletes after stressful trainings was high enough to induce a change in leptin but too small for an increase in NPY. It can also be speculated that there is a lag period for a change in NPY compared to leptin in conditions of small but chronic negative energy balance, resulting in the loss of the correlation between NPY and leptin (as found in T2 and T3). Future studies are therefore warranted to answer that question.

Another interesting finding was that plasma NPY was not increased after 2HR during the baseline measurements, but increased significantly after high-volume training period at T2 and after recovery period at T3 (Table 4). Exercise-induced changes in blood biochemical parameters have been suggested as more sensitive markers to use in the diagnosis of overtraining (i.e. excessive training stress) in athletes (Urhausen and Kindermann 2002; Jürimäe et al. 2003; Mäestu et al. 2004; Rämson et al. 2008). The results of our study confirm those findings as different NPY response to exercise can be seen compared to fasting NPY concentrations during the study period. Similar results have also been obtained on rats, where NPY values increased after chronic (total 7 weeks) and acute treadmill exercise and continued to increase 30 and 180 min after the test (Chen et al. 2007). Also, after marathon swimming NPY concentration increased significantly by 43% (Karamouzis et al. 2002). Although the intensity was similar during the three performance tests in current study, only the second test that was performed after high-volume trainings induced a significant decrease in leptin. This was probably due to the increasing negative energy balance in the organism as discussed before (Mäestu et al. 2008) and seems to confirm the previous findings that the energy balance is the main factor for changes in leptin during the exercise (Karamouzis et al. 2002; Hagobian et al. 2008).

The finding that plasma concentrations of NPY were significantly increased post-exercise even after recovery period (T3) without changes in leptin is difficult to answer. It is likely that the neuropeptidergic system exerts some degree of control on food intake and energy expenditure. It has been suggested that leptin levels do not change during exercise unless there is excessive energy expenditure (Kraemer et al. 2004) or the athletes are already in an overreaching state (Jürimäe et al. 2003). Similar to our previous study (Rämson et al. 2008), in the present study we found significant reduction in post-exercise leptin concentration after a high-volume training period. However, we were able to measure more precisely the performance of the subjects in the present study. Although exercise-induced changes in leptin concentration were recovered at T3, the performance in the 2 h was not. Therefore, it is possible that previous high negative energy balance at T2 was not fully compensated with the 1-week recovery despite increased pre-exercise and fasting leptin levels, which indeed depend more on acute energy intake. This is somewhat supported by no correlation found between leptin and NPY at T3 ($r = -0.109$; $p = 0.64$) and unchanged rowing performance at T2 compared to T3. Moreover, it has also been found that long-term physical activity may enhance the ability to synthesize NPY under the conditions of stress (Levenson and Moore 1998) and exercise would also contribute to the stimulation of brain NPY neurons by reducing energy stores (Richard 1995). Since NPY was still increased post-exercise at T3 and the performance was not restored compared to the decrease at T2 we suggest that exercise-induced NPY concentrations in plasma may be used to discriminate the recovery potential of the athletes during low intensity trainings. We will wait for more data concerning NPY and athletes' training status to clarify this suggestion.

We did not find any changes in fasting ghrelin concentration after high-volume training that is similar to that has previously been published (Rämson et al. 2008). However, different training regimens were used. In the current study about 50% of the trainings were low resistance exercise

Table 4 Changes in exercise-induced blood biochemical values during the 4 week training period at three different testing sessions

	T1	T2	T3
Leptin (ng/mL)			
Pre	1.40 ± 0.9	1.13 ± 0.5[1]	1.42 ± 0.9[2]
Post	1.44 ± 0.8	1.08 ± 0.5*[1]	1.43 ± 0.9[2]
Post 30`	1.38 ± 0.8	1.18 ± 0.6	1.37 ± 0.9
Insulin (μIU/min)			
Pre	13.14 ± 8.1	10.60 ± 7.2	12.33 ± 6.3
Post	2.90 ± 1.4*	2.31 ± 1.1*	3.86 ± 2.2*[2]
Post 30`	3.61 ± 2.7*	3.11 ± 2.9*	2.78 ± 1.2*
NPY (pmol/L)			
Pre	137.3 ± 38.2	128.1 ± 23.2	131.3 ± 20.5
Post	150.2 ± 26.8	155.1 ± 28.9*	159.7 ± 32.8*
Post 30	141.9 ± 20.5	146.5 ± 27.1*	151.1 ± 23.5*
Ghrelin (pg/mL)			
Pre	751.60 ± 173.5	818.29 ± 221.7	817.7 ± 305.83
Post	890.46 ± 297.6*	932.14 ± 379.9	870.51 ± 277.2
Post 30`	881.01 ± 263.2*	890.29 ± 291.1	879.90 ± 256.9

Numbers indicate the significant differences ($P < 0.05$) from the pointed testing session

* Significantly different from pre test value ($P < 0.05$)

trainings but in Rämson et al. (2008) 90% of the trainings were aerobic (rowing, running or cycling) and only 10% resistance type of exercise. A decrease in ghrelin concentration was observed after the recovery week ($P = 0.004$; Table 4). It has further been found that submaximal aerobic exercise is not associated with alterations in circulating plasma ghrelin concentrations (Kraemer et al. 2004; Burns et al. 2007). Contrary, prolonged energy restriction has been shown to increase ghrelin concentration in obese (Foster-Schubert et al. 2005), or in athletes using high amount of body muscles (Jürimäe et al. 2007, 2009) and even in athletes with initially low body fat mass (Mäestu et al. 2008). The finding that ghrelin response to exercise may disappear in stressful energy restricted period has also been reported before in the literature (Rämson et al. 2008) however, the mechanism is not yet known. It is very likely that nutrition might have had a possible exercise-induced effect due to the different body energy reserve status at three different performance tests with more energy restricted state at T2 where the participants were negative for about 400 kcal/day (Table 1).

One of the limitations of the study was that there was no measure of central NPY concentration; only peripheral NYP concentration was measured. Wang et al. (2008) demonstrated that plasma changes do not necessarily mirror NPY changes in the hypothalamus after 8 weeks of intensive training in rats. These investigators also demonstrated an increase in hypothalamic NPY, similar to Chen et al. (2007), but plasma levels of NPY were reduced.

In conclusion, the combination of high-volume trainings and energy imbalance induces significant post-exercise changes in NPY, leptin, and ghrelin concentrations and decreases fasting leptin. We suggest that post-exercises concentrations of NPY can be used in monitoring as the recovery marker of the high volume low intensity trainings.

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