

## LEPTIN RESPONSE TO TWO HOURS OF ROWING IN COLLEGE-LEVEL FEMALE ROWERS

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## LEPTINSKI ODZIV NA DVOURNO VESLANJE PRI VESLAČICAH »COLLEGE« KAKOVOSTI

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### Abstract

The plasma leptin response to a single endurance rowing training session in 10 female college level single scull rowers was investigated. All venous blood samples were obtained during the follicular phase of the menstrual cycle. At first, resting blood samples were obtained in the morning after an overnight fast twice with 48 h between sampling (leptin concentration:  $11.0 \pm 8.1$  vs  $11.6 \pm 9.4$  ng.ml<sup>-1</sup>;  $p=0.97$ ). Venous blood samples were also obtained after on-water rowing lasting about 2 h ( $7518 \pm 293$  s; distance covered  $18.9 \pm 1.4$  km; heart rate  $150 \pm 7$  beats.min<sup>-1</sup>). Blood lactate concentration did not change significantly during training session (from  $1.6 \pm 0.4$  to  $1.9 \pm 0.5$  mmol.l<sup>-1</sup>) indicating that training was performed at moderate intensities. Leptin values were significantly reduced after prolonged rowing by a mean 44%. Insulin and glucose values were also decreased after prolonged rowing. Plasma leptin concentration after an endurance rowing training session was related ( $r=0.64$ ;  $p<0.05$ ) to the distance covered. No such relation was observed for other measured blood biochemical parameters. Regression analyses demonstrated a positive relationship between total body fat mass and plasma leptin ( $R^2>0.70$ ;  $p<0.001$ ) regardless of sampling time in female rowers. In conclusion, our findings indicate that a prolonged low-intensity training session results in an energy deficit beyond the threshold that is necessary to reduce plasma leptin concentration without changing body fat mass in female rowers. It was suggested that plasma leptin could be regarded as a key signal for metabolic adaptation to endurance rowing training session in female endurance athletes.

*Key words: leptin, prolonged rowing, female athletes*

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### Izvleček

Članek proučuje odziv leptina v plazmi (leptina) na enkratno vzdržljivostno veslaško vadbo desetih veslačic »college« kvalitete v enojcu. Vsi venski krvni vzorci so bili pridobljeni med folikularno fazo menstrualnega ciklusa. Na začetku so bili krvni vzorci v mirovanju pridobljeni zjutraj, po nočnem gladovanju, dvakrat z 48-timi urami med vzorčenjema (koncentracija leptina:  $11.0 \pm 8.1$  proti  $11.6 \pm 9.4$  ng.ml<sup>-1</sup>;  $p=0.97$ ). Vzorci venske krvi so bili pridobljeni tudi po približno dvournem veslanju na vodi ( $7518 \pm 293$  s; opravljena razdalja  $18.9 \pm 1.4$  km; srčni utrip  $150 \pm 7$  u.min<sup>-1</sup>). Koncentracija laktata v krvi se v toku veslanja ni bistveno spremenila (od  $1.6 \pm 0.4$  do  $1.9 \pm 0.5$  mmol.l<sup>-1</sup>), kar kaže, da je bila vadba opravljena pri zmerni intenzivnosti. Vrednosti leptina so bile značilno zmanjšane po veslanju, v povprečju za 44%, znižane so bile tudi vrednosti insulina in glukoze. Koncentracija leptina po veslanju je bila povezana ( $r=-0.64$ ;  $p<0.05$ ) s preveslano razdaljo, za ostale izmerjene krvne biokemične parametre povezava ni bila dobljena. Regresijske analize so prikazale pozitivno povezavo med skupno količino telesne maščobe in leptinom ( $R^2>0.70$ ;  $p<0.001$ ), ne glede na trenutek vzorčenja. Torej naše ugotovitve kažejo, da daljša vadba nižje intenzivnosti povzroči energijski deficit pod ravni, ki je potrebna, da se zmanjša koncentracija leptina, ne da bi hkrati bila spremenjena količina maščobne mase pri veslačicah. Tako predlagamo, da je leptin lahko uporabljen kot ključni signal za metabolično prilagoditev na vzdržljivostno veslaško vadbo pri vzdržljivostnih športnicah.

*Ključne besede: leptin, veslanje, vzdržljivost, športnice*

## INTRODUCTION

Leptin, the product of the *ob* gene, is involved in the regulation of body weight and energy expenditure (Flier, 1998; Frühbeck, Jebb, & Prentice, 1998; Hickey, & Calsbeck, 2001). In humans, fasting leptin concentration is reduced with weight loss (Hickey, & Calsbeck, 2001) and increased with overfeeding (Kolaczynski, Nyce, & Considine, 1996). Furthermore, fasting leptin is positively linked with fat mass in both lean and obese individuals (Flier, 1998; Frühbeck, Jebb, & Prentice, 1998). In trained subjects, circulating leptin is low, and even at biological extreme low levels of body fat, leptin concentration is related to fat content in female (Laughlin, & Yen, 1997) and male (Sudi et al., 2001) athletes. Exercise studies have generally suggested that fasting leptin concentration is not altered independently of changes in fat mass (Hickey, & Calsbeck, 2001). These observations together suggest that leptin may be responsive to a disruption in energy balance.

Endurance athletes expend considerable amounts of energy during training and it is hypothesised that leptin may be useful for monitoring relative training stress in these athletes. Information regarding the response of plasma leptin to prolonged endurance training session is limited. In this study, female rowers were investigated as a large amount of muscle mass is involved in rowing (Jürimäe, Jürimäe, & Purge, 2001; Steinacker, 1993) and the fat free mass also influences serum leptin concentration (Fernandez-Real, Vayreda, Casamitjana, Gonzalez-Huix, & Ricart, 2000). Furthermore, rowers are characterised with a relatively high amount of body fat compared with other endurance athletes (Nieman et al., 1999; Steinacker, 1993). Prolonged extensive rowing on water is the major component of training programmes of rowers (Jürimäe, Jürimäe, & Purge, 2001; Nieman et al., 1999). The purposes of this study were to: 1) determine fasting levels of leptin on resting conditions after an overnight fast, twice with 48 h between sampling in female rowers; and 2) observe leptin response to a single extensive endurance rowing training session in female rowers.

## METHODS

Ten female college level rowers volunteered to participate in the study. They had trained regularly for

the last  $4.3 \pm 0.6$  years. The study was conducted during pre-competition in May. The rowers were familiarised with the procedures before providing their written consent to participate in the experiment as approved by the Medical Ethics Committee of the University of Tartu. All subjects were normally cycling and had menstrual cycle duration of 26-35 days. They were required to have at least three months of documented menstrual cycles, and were not using the oral contraceptive pill for at least six months preceding the study. Resting blood samples were obtained in the morning after an overnight fast, twice with 48 h between sampling. They were not allowed to exercise between sampling. All venous blood samples were obtained during the follicular phase of the menstrual cycle (Thong et al., 2000).

The height (Martin metal anthropometer) and body mass (A&D Instruments Ltd, UK) of the participants were measured to the nearest 0.1 cm and 0.05 kg, respectively. Body composition was measured using dual-energy X-ray absorptiometry. Scans of the whole body were performed on each of the subjects using a Lunar DPX-IQ scanner (Lunar Corporation, Madison, USA).

The rowers were asked not to participate in any physical activity in the 24 h before the training session. The training session consisted of an on-water prolonged sculling. Subjects reported to the training site and the first blood samples were collected from the athletes at 08.00 h. Subjects then had their traditional light breakfast. Single sculls rowing at an intensity equal to approximately blood lactate level of  $2 \text{ mmol.l}^{-1}$  was performed for about 2 h on water at a temperature of 20-22 °C, humidity of 40-45% and non-windy conditions (Jürimäe, Jürimäe, & Purge, 2001). Training started at 10.00 h after warm-up that included stretching and jogging for 15 min. The participants then rowed for an average of 2 h 5 min ( $7518 \pm 293$  s) over a distance of  $18.9 \pm 1.4$  km. Heart rate was  $150 \pm 7$   $\text{beats.min}^{-1}$  (range  $144$ - $170$   $\text{beats.min}^{-1}$ ). The participants were re-weighed and another venous blood sample taken after exercise. Before exercise, the blood lactate concentration was  $1.6 \pm 0.4$   $\text{mmol.l}^{-1}$ , which did not change significantly during training ( $1.9 \pm 0.5$   $\text{mmol.l}^{-1}$ ). The participants were not allowed a drink during exercise or in the first 2 h of recovery (Jürimäe, Jürimäe, & Purge, 2001).

A 10 ml blood sample was obtained from an antecubital vein with the participant in the upright position. The plasma was separated and frozen at

-20 °C for later analysis. Leptin was determined in duplicate by radioimmunoassays (Mediagnost, Tübingen, Germany). This assay has a detection limit of 0.01 ng.ml<sup>-1</sup>, and intra-assay and inter-assay coefficient of variation (CV) was <5% and <7.5%, respectively. Insulin was determined by means of an immunoradiometric assay (Biosource Europe S.A., Nivelles, Belgium) with an intra- and inter-assay CV of 4.5% and 12.2% at an insulin concentration of 6.6 µU.ml<sup>-1</sup>, respectively. Samples from one individual were run on the same assay. Glucose (mmol.l<sup>-1</sup>) was measured by means of the hexokinase/glucose 6-phosphate-dehydrogenase method by using a commercial kit (Boehringer Mannheim, Mannheim, Germany).

Means and standard deviations were determined. Leptin concentrations were log transformed to normalise the distribution (Thong, McLean, & Graham, 2000). T-test for dependent samples was used to assess training induced changes in measured variables. Regression and Pearson product moment correlation analyses were used to evaluate associations among different variables. An alpha level of 0.05 was adopted.

## RESULTS

Table 1 summarizes subject characteristics for the 10 college level female rowers. Fasting leptin (11.0±8.1 vs 11.6±9.4 ng.ml<sup>-1</sup>; p=0.97), insulin (8.1±1.9 vs 8.1±2.5 µU.ml<sup>-1</sup>; p=0.89) and glucose (5.0±0.4 vs 4.9±0.4 mmol.l<sup>-1</sup>; p=0.80) concentrations measured twice with 48 h between sampling in the follicular phase of the menstrual cycle were not different. Body mass was reduced after training (Table 2). Leptin, insulin and glucose values were also decreased after prolonged rowing.

Leptin concentration after an endurance rowing training session was related to the distance covered

Table 1. Subject characteristics for female rowers

Variable	Mean ± S.D.
Age (years)	19.4±1.6
Height (cm)	173.4±5.1
Body mass (kg)	67.7±10.4
% Fat mass	28.9±8.5
Fat mass (kg)	19.7±9.2
Lean mass (kg)	46.1±3.8

**Table 2.** Body mass, leptin, insulin and glucose before (PRE) and after (POST) an endurance rowing training session (Mean ± S.D.).

Variable	PRE	POST	t	p-level
Body mass (kg)	67.7±10.4	67.0±10.4*	8.434	0.001
Leptin (ng.ml <sup>-1</sup> )	11.6±9.4	6.5±7.1*	5.612	0.001
Insulin (µU.ml <sup>-1</sup> )	10.7±2.8	8.1±2.5*	-2.556	0.031
Glucose (mmol.l <sup>-1</sup> )	4.9±0.4	4.5±0.5*	4.892	0.001

\* Significantly different from PRE; P<0.05.

(r=-0.64; p<0.05). No such relation was observed for body mass, insulin or glucose parameters. In addition, leptin correlated with insulin measured at every time points (r>0.64; p<0.05). Regression analyses showed that total body fat mass was related to plasma leptin at every time point measured (R<sup>2</sup>>0.70; p<0.001).

## DISCUSSION

In this study, the effects of a prolonged endurance rowing training session, in which there was no elevation in blood lactate concentration, on plasma leptin were assessed in college-level female rowers at the follicular phase of the menstrual cycle. To our knowledge, no studies have yet investigated the influence of a prolonged endurance training session on plasma leptin concentration in a relatively homogeneous group of female athletes with relatively high body mass values. Furthermore, it has to be considered that approximately 70% of the whole muscle mass is involved in rowing and rowers are characterised by relatively high body fat values compared with other endurance athletes (Jürimäe, Jürimäe, & Purge, 2001; Steinacker, 1993). In the present study, plasma leptin concentration was significantly reduced as a result of training session and related to the distance covered (r=-0.64; p<0.05). This demonstrates that leptin reflected well the metabolic state of athletes after the exercise session which demanded a high energy expenditure.

All rowers of this study were tested at the follicular phase of the menstrual cycle as there is a considerable evidence that leptin may be intimately involved in the regulation of reproductive function in women athletes (Laughlin, & Yen, 1997; Thong, McLean, & Graham, 2000). A significant rise in plasma leptin during the late follicular and luteal phases of the menstrual cycle has been reported

in healthy women (Riad-Gabriel, Jinagouda, Sharma, Boyadjian, & Saad, 1998). Fasting plasma leptin ( $11.0 \pm 8.1$  vs  $11.6 \pm 9.4$  ng·ml<sup>-1</sup>;  $p=0.97$ ), measured twice with 48 h between sampling in the follicular phase of the menstrual cycle in female rowers was not different and was at the same range as in other endurance-trained women athletes (Noland et al., 2001; Thong, McLean, & Graham, 2000).

The main finding of the present study was a mean 44% decrease in plasma leptin after an average of 2 h 5 min sculling over a distance of  $18.9 \pm 1.4$  km in college-level female rowers. In comparison, a 34% reduction in baseline leptin concentrations was measured in 14 men after a 2 h treadmill exercise at an intensity of 75% of their individual maximal oxygen consumption (Tuominen et al., 1997). The results of both these studies suggest that a high energy deficit caused by a prolonged exercise contributes to the decrease in leptin levels. In agreement with this, Leal-Cerro et al. (1998) reported a significant decrease in leptin concentration after a marathon run with a net energy expenditure of over 2800 Cal.

Leptin concentrations measured after an endurance rowing training session was related to the distance covered ( $r=-0.64$ ;  $p<0.05$ ). This suggests that the leptin response depends on the total amount of energy deficit. In accordance with our results, recent review article has also proposed that if plasma leptin concentrations are to be altered, an undefined threshold for total energy deficit as a result of either exercise training or reduced caloric intake probably exists (Hickey, & Calsbeck, 2001). The results of the present study suggest that as training in rowing is mainly performed beyond the threshold of energy deficit, leptin could be regarded as an important signal for metabolic adaptation to training and following recovery in female rowers.

In summary, high energy deficit caused by a prolonged low-intensity rowing training session reduced plasma leptin levels in female endurance athletes with relatively high body fat values. Furthermore, circulating plasma leptin levels were significantly related to the distance covered in college-

level female rowers. This suggests that plasma leptin could be regarded as an important signal for metabolic adaptation to endurance rowing training session in female rowers.

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