ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol., Technol. Aliment. 9(4) 2010, 469-476
ISSN 1644-0730 (print) ISSN 1889-9594 (online)

THE EFFECT OF NUTRITIONAL STATUS, SELECTED SEX HORMONES AND SHBG ON PLASMA LEPTIN LEVELS IN YOUNG FEMALE ATHLETES WITH MENSTRUAL DISORDERS*

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Background. This study was focused on investigations of secondary regulators of plasma leptin levels such as prolactin, testosterone, sex hormone binding globulin (SHBG) and nutritional status, in young female athletes with menstrual disorders.
Material and methods. Thirty four female professional rowers with menstrual disorders (*amenorrhoea* and *oligomenorrhoea*), aged 18.1 ±2.0, with a training period of 4.3 ±2.1 and BMI of 21.0 ±2.1 kg/m^2, with too high (IL) or too low plasma leptin levels (DL) participated in the study. The nutritional status was evaluated based on the analysis of body composition using the BIA method – percentage of adipose tissue (FM) and fat free mass (FFM) and skinfold thickness (ST) using a Harpenden skinfold caliper. Moreover, serum levels of leptin, prolactin, testosterone and SHBG were estimated using RIA kits.
Results. Values of BMI, ST, FM were significantly ($p < 0.05$) higher in IL athletes, while FFM was significantly ($p < 0.05$) lower compared to DL rowers (BMI: IL 22.3 ±2.2 kg/m^2, DL 20.2 ±1.4 kg/m^2, ST: IL 12.4 ±4.0 mm, DL 9.5 ±2.1 mm, FM: IL: 23.2 ±4.9%, DL: 19.3 ±3.3%, FFM: IL 76.8 ±4.9%, DL 80.7 ±3.4%). The results of plasma leptin level correlated ($p < 0.05$) with anthropometric parameters (age: $r = –0.38$, body mass: $r = 0.46$, BMI: $r = 0.59$, ST: $r = 0.40$), body composition (FM%: $r = 0.48$, FM kg: $r = 0.55$, FFM%: $r = –0.48$), prolactin ($r = 0.72$) and testosterone levels ($r = 0.43$).
Conclusions. The results confirmed the strong influence of body mass and fat mass on serum leptin levels. However, high prolactin and testosterone levels may also favourably increased plasma leptin levels in athletes and also affect menstrual disorders.

Key words: leptin, testosterone, prolactin, SHBG, *amenorrhoea*, *oligomenorrhoea*

*The project was financed by Ministry of Science and Higher Education under a number N N312 239738.

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INTRODUCTION

In recent years the number of women professional athletes has considerably increased. Unfortunately, it has been repeatedly confirmed that physical exercise of high intensity, which the organisms of female athletes are exposed to, has an effect on their health. It is estimated that from 20-48% female athletes suffer from hypothalamic-pituitary menstrual disorders, which is most commonly caused by the maintenance of a negative energy balance, inadequate nutritional status and excessive physical exercise – frequently initiated before athletes enter puberty [Beals 2002, Nichols et al. 2007]. The highest percentage of menstrual disorders is recorded in case of specific sports disciplines, such as endurance and esthetic sports, which requires a slim figure and low body weight [Dusek 2001, Lutosławska 2002].

In female athletes suffering from menstrual disorders, disturbance of secretion of gonadoliberin (GnRH pulsatility) is observed resulting in a reduced level of the luteinizing hormone (LH), as well as estrogen (hypoestrogenism). Moreover, these athletes frequently exhibit low levels of leptin, as well as hypoglycemia, hypoinsulinemia, hypercortisolemia and hyperandrogenemia, hypthyroidemia [Thong et al. 2000]. Leptin has been suggested as a link between amenorrheic athletes, energy status and reproductive system [Miles 2001]. Some studies have reported that leptin enters the brain to stimulate GnRH neurons directly [Hilton 2000] but other suggests that this stimulatory effect may not be directly mediated by leptin [Miles 2001]. Furthermore, leptin has been perceived as a critical intermediary in the path from energy deficiency to menstrual dysfunction and disorders in secretion of GnRH in female athletes [Miles 2001]. The studies conducted with the participation of professional athletes with low body weight and percentage of adipose tissue showed a positive correlation between serum leptin and estradiol levels [Horlick 2000, Roemmich 1998, Wolińska-Witort 2007]. However, specific serum leptin and gonadotropic level hormones may be just one of the necessary factors in the maintenance of functioning of the hypothalamus-pituitary-gonad axis. Apart from that, secretions of other hormones are necessary to activate reproductive axis, but their relationship with leptin has not been thoroughly clarified.

This study was focused on investigations of secondary regulators of plasma leptin levels, such as prolactin, testosterone, SHBG and nutritional status, in young female athletes.

MATERIAL AND METHODS

Thirty four female professional rowers from different sports clubs in Poznań, aged 18.1 ±2.1, participated in this study. Athletes were characterised by a minimum 3-year training period, average 4.3 ±2.1. The inclusion criterion for the study was too high (IL) or too low plasma leptin levels (DL) [Pasco 2001] and menstrual disorders. A questionnaire was used to estimate type of disorders in menstrual cycle. Questions asked for data on the characteristics of the menstrual cycle: age at menarche, duration of menstrual cycle, duration of menstrual bleeding, non-appearance of menstruation, and painful menstruation. Secondary amenorrhea was diagnosed when there were no regular menstrual cycles in a 3-month period if previous menstruation had been regular, or in a 6-month period if previous menstruation had been irregular. The appearance of menstrual bleeding in a period of 35 days to 6 months was defined as oligomenorrhoea [Skałba 2008].

All the hormones (prolactin, testosterone, sex hormone binding globulin (SHBG), leptin), were estimated using RIA kits in plasma which was deeply (–20°C) frozen prior to the procedure [Kumru 2005, Milewicz 2005]. Blood samples were taken in the morning (6.00-9.00), before the first meal. The subjects were instructed to abstain from caffeine and alcohol for 24 h before blood sampling and to refrain from performing strenuous exercise on the day of blood sampling. None of the patients was on any medication which may change the levels of evaluated parameters.

The nutritional status was evaluated based on anthropometrical indices: (height, weight), using an anthropometer, coupled with a WPT 200 OC verified medical scale (Rad Wag) and BMI (kg/m^2) calculation. Participants were dressed in minimal clothing during the measurements, which were recorded to the nearest 0.5 kg and 0.5 cm, respectively. Biceps, subscapular and suprailiac skinfold measurements (ST) were performed on the right side of the body, as described by Heyward and Wagner [2003] accurate to 0.1 mm, using a Harpenden skinfold caliper. The mean of three measurements was used as the representative value. Analysis of body fat mass (FM) and fat-free mass (FFM) was performed by BODYSTAT 1500, according to Heyward and Wagner [2003], with the subjects lying in a supine position, after an overnight fast. The measurements were conducted in the morning hours.

Before the study, girls and their parents were informed of all aspects of the study and they gave their written consent in each case. The study was approved by the Poznań Medical Ethics Committee (no. 334/09).

Comparisons between groups were carried out using a one-way ANOVA. In addition, to examine the relationship between serum concentration of leptin and those of other parameters, Spearman correlation coefficients were calculated. Statistical analyses were performed using STATISTICA program, version 8.0 by StatSoft (2008). A P value of less than 0.05 was considered significant.

RESULTS

Depending on the results of serum leptin level in female athletes were assigned to two groups. The first group included athletes which leptin level was higher than the standard [Pasco 2001], while the other comprised women with a reduced level of this hormone in blood serum (IL 15.3 ±6.9 ng/ml, DL 3.6 ±1.5 ng/ml, $p < 0.05$). Moreover, differences in the serum leptin level in relation to fat mass was also significant (serum leptin/FM: IL 1.1 ±0.5 ng/ml/kg, DL 0.3 ±0.1 ng/ml/kg, $p < 0.05$).

Significant differences between anthropometric parameters in groups were observed (age: IL 17.1 ±1.3, DL 18.9 ±2.0, body mass: IL 65.1 ±9.5 kg, DL 58.0 ±5.6 kg, BMI: IL 22.3 ±2.2 kg/m^2, DL 20.2 ±1.4 kg/m^2 and ST: IL 12.4 ±4.0 mm, DL 9.5 ±2.1 mm, $p < 0.05$) . Furthermore, body fat mass were significantly lower (FM: IL 23.2 ±4.9%, DL 19.3 ±3.3%, FM: IL 14.9 ±5.1 kg, DL 11.1 ±1.7 kg, $p < 0.05$) but fat free mass (IL 76.8 ±4.9%, 80.7 ±3.4%, $p < 0.05$) were significantly higher in female athletes with decreased serum leptin levels.

In case of hormones and SHBG no significant differences were found between groups. However, some trends were observed, although non-significant for lower prolactin and testosterone levels, as well as higher SHBG levels in female athletes with a decreased serum leptin levels (Table 1).

Table 1. Characteristics of athletes

Parameters	All n = 34	Increased serum leptin level (IL) n = 14	Decreased serum leptin level (DL) N = 20
Serum leptin level			
Serum leptin, 6.8-8.20 μg/ml	8.4 ±7.3	15.3 ±6.9[b]	3.6 ±1.5[a]
Serum leptin/FM, μg/ml/kg	0.6 ±0.5	1.1 ±0.5[b]	0.3 ±0.1[a]
Anthropometric characteristics			
Age, year	18.1 ±2.0	17.1 ±1.3[a]	18.9 ±2.0[b]
Body mass, kg	60.9 ±8.2	65.1 ±9.5[b]	58.0 ±5.6[a]
Body height, cm	169.8 ±7.1	170.8 ±7.9	169.2 ±6.6
BMI, kg/m^2	21.0 ±2.1	22.3 ±2.2[b]	20.3 ±1.5[a]
ST, mm	10.7 ±3.3	12.4 ±4.0[b]	9.5 ±2.1[a]
Body composition			
FM, %	20.9 ±4.5	23.2 ±4.9[b]	19.3 ±3.3[a]
FM, kg	12.6 ±4.0	14.9 ±5.1[b]	11.1 ±1.7[a]
FFM, %	79.1 ±4.5	76.8 ±4.9[a]	80.7 ±3.4[b]
FFM, kg	48.4 ±6.2	50.4 ±6.1	46.9 ±6.0
Hormones and globulin			
Prolactin, 3.00-20.0 ng/ml	13.8 ±10.8	19.3 ±14.6	11.3 ±8.0
Testosterone, 15.0-84.0 ng/dl	32.4 ±20.6	34.6 ±7.2	31.9 ±24.4
SHBG, 18.0-114.0 nmol/l	66.8 ±45.4	50.0 ±26.7	74.4 ±50.4

*Different letter superscripts indicate significant differences ($p < 0.05$).

Moreover, serum leptin levels were positively correlated with body mass ($r = 0.46$, $p < 0.05$), BMI ($r = 0.59$, $p < 0.05$), skinfold thickness ($r = 0.40$, $p < 0.05$) and fat mass (FM%: $r = 0.48$, FM kg: $r = 0.55$, $p < 0.05$), while negatively correlated with the fat free mass ($r = –0.48$, $p < 0.05$). It also turned out that the higher leptin level was connected with higher levels of testosterone ($r = 0.43$, $p < 0.05$) and prolactin ($r = 0.72$, $p < 0.05$).

When leptin levels was expressed in relation to fat mass a positive correlation with BMI ($r = 0.38$, $p < 0.05$) and prolactin level ($r = 0.68$, $p < 0.05$) was shown (Table 2).

Table 2. A relationship between serum leptin levels and anthropometric parameters, body composition, levels of selected hormones and SHBG

Parametr	Leptin, μg/ml	Leptin/FM, μg/ml/kg
Anthropometric characteristics		
Age, year	–0.38 ($p < 0.05$)	–0.31 ($p < 0.05$)
Body mass, kg	0.46 ($p < 0.05$)	0.18 (NS)
Body height, cm	0.06 (NS)	0.00 (NS)
BMI, kg/m^2	0.59 ($p < 0.05$)	0.38 ($p < 0.05$)
ST, mm	0.40 ($p < 0.05$)	0.11 (NS)
Body composition		
FM, %	0.48 ($p < 0.05$)	0.16 (NS)
FM, kg	0.55 ($p < 0.05$)	0.12 (NS)
FFM, %	–0.48 ($p < 0.05$)	– 0.15 (NS)
FFM, kg	0.26 (NS)	0.18 (NS)
Hormones and globulin		
Prolactin, ng/ml	0.72 ($p < 0.05$)	0.68 ($p < 0.05$)
Testosterone, ng/dl	0.43 ($p < 0.05$)	0.22 (NS)
SHBG, nmol/l	–0.11 (NS)	–0.11 (NS)

DISCUSSION

The results again confirmed the strong influence of body mass and fat mass on serum leptin levels. A higher serum leptin levels was observed in women with higher BMI, higher mean of skinfolds and higher body fat mass. Similar results were observed by Horlick et al. [2000], Lonnerdal and Havel [2000], Roemmich [1998] and Ruhl et al. [2007].

The study was not limited only to the presentation of a relationship between leptin concentration and anthropometric parameters or body composition, but it was also focused on a relation between serum leptin and prolactin, testosterone and SHBG levels. In other studies positive correlation between the serum lepitin level, luteinizing hormone (LH) and the follicle stimulating hormone (FSH) were confirmed [Schwarz and Seeley 1997]. In turn, there is a limited data in young females athletes concerning the relationship between serum leptin and prolactin, testosterone or SHBG level, i.e. hormones playing a similarly significant role in the functioning of the hypothalamus-pituitary-ovary axis. The studies conducted on these relationships concerned mostly post-menopausal women [Milewicz at al. 2005].

Prolactin is a hormone secreted by the interior pituitary gland [Skałba 2008]. Its action affecting the reproductive system first of all leads to an increased weight of mammary glands during pregnancy, initiation of milk secretion and maintenance of lactation [Skałba 2008]. In terms of the effect on ovaries it was found that an excess of this hormone causes a deficient activity of the corpus luteum [Skałba 2008]. Moreover, hyperprolactinemia, corresponding to the concentration of prolactin of more than 20 ng/ml, may also result in the incidence of amenorrhoea secundaria. In this study a positive correlation between leptin and the level of prolactin was recorded [Skałba 2008]. What is more, in the group of women characterised by an higher leptin level, a higher prolactin level was also found, although these differences were not significant. It may be supposed that not only too low serum leptin level but also higher serum leptin combination with higher prolactin level may disordering the menstrual cycle such amenorrhoea and oligomenorrhoea in female athletes.

Controversial results were obtained for the relation between serum leptin and testosterone level. It was found that the level of testosterone increased with an increase in serum leptin level. Theoretically, hyperandrogenism should correspond to low leptin concentrations [Milewicz 2005, Skałba 2008]. However, reports on the effect of high concentrations of testosterone and other androgens on leptin level are still ambiguous and require further studies, particularly in case of female athletes suffering from menstrual dysfunction. In this case, the occurrence of a positive correlation between an increase in testosterone level and leptin may be related to an increase in adipose tissue content. Also Mantzoros et al. [1997] observed a significantly higher level of testosterone in women characterised by higher leptin concentrations and higher percentage of adipose tissue.

In this study no direct relationship between SHBG and leptin was found. However, in other studies a positive correlation between SHBG and the serum leptin level was indicated but only for post-menopausal women [Filip 2005].

CONCLUSION

The results confirmed the strong influence of age, body mass and fat mass on serum leptin levels. However, high prolactin and testosterone levels may also favourably increase plasma leptin level in female athletes and also affect menstrual disorders, such amenorrhoea and oligomenorrhoea. But further analysis must be carried out to explain the meaning of leptin as a factor taking part in regulation of menstrual cycle in athletes.

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WPŁYW STANU ODŻYWIENIA, WYBRANYCH HORMONÓW PŁCIOWYCH ORAZ SHBG NA POZIOM LEPTYNY W SUROWICY KRWI MŁODYCH SPORTSMENEK CIERPIĄCYCH NA ZABURZENIA MIESIĄCZKOWANIA

Cel badań. Celem pracy była ocena zależności pomiędzy stężeniem leptyny w surowicy krwi a poziomem prolaktyny, testosteronu, globuliny wiążącej hormony płciowe (SHBG), z uwzględnieniem stanu odżywienia sportsmenek cierpiących na zaburzenia miesiączkowania.

Materiał i metody. Do badań włączono 34 wioślarki cierpiące na zaburzenia miesiączkowania (*amenorrhoea* i *oligomenorrhoea*), w wieku 18,1 ±2,0, ze średnim stażem treningowym 4,3 ±2,1 i BMI 21,0 ±2,1 kg/m^2, charakteryzujące się zbyt dużym (IL) lub zbyt małym (DL) stężeniem leptyny w surowicy krwi. Ocena stanu odżywienia obejmowała analizę składu ciała (tkanka tłuszczowa (FM), beztłuszczowa masa ciała (FFM)), wykonaną z zastosowaniem metody bioimpedancji elektrycznej. Średnią grubość fałdów skórno-tłuszczowych (ST) zmierzono, wykorzystując cyrkiel Harpendena. Prócz tego oznaczono poziom leptyny, prolaktyny, testosteronu oraz SHBG z wykorzystaniem kitów RIA.

Wyniki. Wartość BMI, ST, FM okazała się istotnie większa ($p < 0,05$) u zawodniczek IL, z kolei wartość FFM znamiennie mniejsza ($p < 0.05$) w porównaniu z wioślarkami z grupy DL (BMI: IL 22,3 ±2,2 kg/m^2, DL 20,2 ±1,4 kg/m^2; ST: IL 12,4 ±4,0 mm, DL 9,5 ±2,1 mm; FM: IL 23,2 ±4,9%, DL 19,3 ±3,3%; FFM: IL 76,8 ±4,9%, DL 80,7 ±3,4%). Poziom leptyny korelował ($p < 0,05$) z parametrami antropometrycznymi zawodniczek (wiek: $r = –0,38$, masa ciała: $r = 0,46$, BMI: $r = 0,59$, ST: $r = 0,40$), składem ciała (FM%: $r = 0,48$, FM kg: $r = 0,55$, FFM%: $r = –0,48$), poziomem prolaktyny ($r = 0,72$) i testosteronu ($r = 0,43$).

Wnioski. Otrzymane wyniki potwierdziły duży związek masy ciała oraz ilości tkanki tłuszczowej z poziomem leptyny w surowicy krwi. Ponadto stwierdzono, iż wysoki poziom prolaktyny oraz testosteronu może wpływać na wzrost leptyny w surowicy krwi i przyczyniać się do zaburzeń miesiączkowania u sportsmenek.

Słowa kluczowe: leptyna, testosteron, prolaktyna, SHBG, *amenorrhoea*, *oligomenorrhoea*

Accepted for print – Zaakceptowano do druku: 28.08.2010

For citation – Do cytowania: Łagowska K., Jeszka J., 2010. The effect of nutritional status, selected sex hormones and SHBG on plasma leptin levels in young female athletes with menstrual disorders. Acta Sci. Pol., Technol. Aliment. 9(4), 469-476.