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# Zależność stężenia leptyny od wieku, poziomu insuliny, SHBG i hormonów płciowych u kobiet

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## Streszczenie

**Cel pracy.** Znany jest związek pomiędzy tłuszczową masą ciała a poziomem leptyny. Celem przedstawianej pracy jest poszukiwanie innych czynników wpływających na poziom leptyny jak wiek, stężenie insuliny, SHBG, hormony płciowe.

**Materiał.** 86 kobiet (średni wiek 47.0 ±14.3 lat, estradiol 50.0±60.6 ng/l, FSH 52.4±42.9 IU/l; BMI 26.9±5.9) podzielono na trzy grupy według BMI. Grupę A stanowiło 39 kobiet o prawidłowej masie ciała (średni wiek 44.4±16.0 lat; estradiol 69.6±79.8 ng/l; FSH 50.4±47.7 IU/l; BMI 22.9±1.3), grupę B 27 kobiet z nadwagą (średni wiek 55.0±6.4 lat; estradiol 25.1±17.2 ng/l; FSH 75.6±26.3 IU/l; BMI 27.7±1.6) a grupę C 21 kobiet otyłych (średni wiek: 48.7±12.2 lat; estradiol 36.9±44.0 ng/l; FSH 42.3±36.6 IU/l i BMI 34.6±4.9).

**Metody.** Standardowa ocena kliniczna i pomiar poziomu hormonów wykonywane były w warunkach podstawowych :LH, FSH, prolaktyna, estradiol, leptyna, IGF-I, hormon wzrostu, IGFBP-3, insulina, DHEAS, SHBG, testosteron. Poziom hormonów oceniano metodą RIA. W analizie statystycznej zastosowano testy Shapiro – Wilk, Manna – Whitneya oraz test korelacji rang Spearmana.

**Wyniki.** Biorąc pod uwagę wszystkie badane kobiety (n=86) stwierdzono korelację poziomu leptyny z wiekiem (r=0.32; p<0.02), masą ciała (r=0.60; p<0.001), BMI (r=0.71; p<0.001) a także odwrotną korelację ze stężeniem estradiolu (r=-0.21; p<0.05), IGF-I (r=-0.24; p<0.05), SHBG (r=-0.34; p<0.01) i DHEAS (r=-0.30; p<0.01).

Jedynie w grupie B stosunek stężenia leptyny do wieku zależał od BMI (r=0.40; p<0.05). W grupie tej wykazano związek stężenia leptyny ze stężeniem DHEAS (r=-0.40; p<0.05) i PRL (r=0.51; p<0.05). W grupie C wykazano zależność poziomu leptyny od SHBG (r=-0.56; p<0.02) oraz związek stężenia insuliny i leptyny (r=0.48; p<0.05).

Zależność masy ciała, BMI i wieku występuje w całej grupie 86 badanych kobiet (r=0.30; p<0.002: r=0.36; p<0.001 odp.). Zależność ta zanika w poszczególnych grupach lub staje się korelacją odwrotną.

Analizując zależność wieku i poziomu leptyny u wszystkich kobiet stwierdzono prostą korelację między tymi parametrami dla kobiet przed menopauzą (n=29; r=0.43; p<0.02) i odwrotną u kobiet po menopauzie (n=38; r=-0.32; p<0.05).

Poziom leptyny w surowicy krwi był najwyższy (p< 0.001) w grupie C (23.2±10.4 µg/l) a najniższy w grupie A (8.9±4.1 µg/l). To koresponduje z różnicami średnich wskaźników masy ciała w badanych grupach.

Analiza regresji wieloczynnikowej ujawniła, że wskaźnik masy ciała odpowiada w 31% (p<0.01) a poziom SHBG w surowicy w 17,7% (p<0.02) za zmienność poziomu leptyny w surowicy u wszystkich badanych kobiet. W grupie A masa ciała i wiek łącznie odpowiadały za 61% (p<0.01) oraz estradiol za 44 % (p,0.02) zmienności stężeń leptyny w surowicy. W grupie B insulina odpowiadała za 39% (p<0.05) a łącznie z testosteronem za 46% (p< 0.05) zmienności stężeń leptyny w surowicy. W grupie kobiet otyłych żaden z badanych parametrów w sposób istotny statystycznie nie oddziaływał na zmienność stężeń leptyny w surowicy.

**Wnioski.** Wyniki prezentowanej pracy potwierdzają silny wpływ masy ciała na stężenie leptyny we krwi. Jednak także insulina, SHBG, hormony płciowe podobnie jak wiek mają wpływ na stężenie leptyny w surowicy w określonych grupach kobiet.

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***Słowa kluczowe:*** *leptyna, wiek, insulina, SHBG, sterydy płciowe*

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# Age, insulin, SHBG and sex steroids exert secondary influence on plasma leptin level in women

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## Abstract

**Aim.** As the link between body fat and leptin is well known, the aim of the study was to seek for secondary regulators of plasma leptin level.

**Patients.** 86 women (mean: age 47.0±14.3 years; estradiol 50.0±60.6 ng/l; FSH 52.4±42.9 IU/l; BMI 26.9±5.9) divided into three groups according to their BMI. Group A: 39 normal weight women (mean: age 44.4±16.0 years; estradiol 69.6±79.8 ng/l; FSH 50.4±47.7 IU/l; BMI 22.9±1.3). Group B: 27 overweighted women (mean: age 55.0±6.4 years; estradiol 25.1±17.2 ng/l; FSH 75.6±26.3 IU/l; BMI 27.7±1.6). Group C: 21 obese women with mean: age 48.7±12.2 years; estradiol 36.9±44.0 ng/l; FSH 42.3±36.6 IU/l and BMI 34.6±4.9.

**Methods.** Standard clinical evaluation and hormone evaluation (LH, FSH, prolactin, estradiol, leptin, insulin-like growth factor-I (IGF-I), human growth hormone (hGH), insulin-like growth factor binding protein-3 (IGFBP-3), insulin, dihydroepiandrosterone sulphate (DHEAS), sex hormone binding globin (SHBG) and testosterone were done in basic condition which levels of were measured by RIA kits.

**Statistical analysis.** Shapiro-Wilk test, Mann-Whitney-Wilcoxon u test, Spearman rank correlation coefficient and stepwise multiple regression: p values of 0.05 or less were considered as significant.

**Results.** Taking all women into account (n=86) the plasma leptin level correlated directly with age (r=0.32; p<0.02), body mass (r=0.60; p<0.001), BMI (r=0.71; p<0.001) as well as inversely with estradiol (r=-0.21; p<0.05), IGF-I (r=-0.24; p<0.05), SHBG (r=-0.34; p<0.01) and DHEAS (r=-0.30; p<0.01). However only in the group B leptin/age relation remained (r=0.40; p<0.05) after the division according to BMI. In the group B the leptin /DHEAS (r=-0.40; p<0.05) and leptin/PRL (r=0.51; p<0.05) links were also present. In the group C the leptin/SHGB relation (r=-0.56; p<0.02) only remained and an association between insulin and leptin was found (r=0.48; p<0.05). The body mass and BMI relation to age were again present only in all 86 women (r=0.30; p<0.002: r=0.36; p<0.001 resp.). Having split the women into groups, these links either disappeared or became inverse ($r_C$=-0.39; p<0.05). Taking into consideration age/leptin relation in all women, the division according to the menopausal status revealed the direct relation in premenopausal women (n=29; r=0.43; p<0.02) and a reverse one in postmenopausal women (n=38; r=-0.32; p<0.05)

The plasma leptin level was the highest (p<0.001) in group C (23.2±10.4 μg/l) and the lowest was found in the group A (8.9±4.1 μg/l). That corresponded with the differences in mean body mass index and mean body mass.

The stepwise multiple regression revealed that body mass index accounted for 31% (p<0.001) and plasma SHBG level accounted for 17.7% (p<0.02) of plasma leptin variance in all women. In the group A body mass and age together accounted for 61% (p<0.01) and estradiol alone accounted for 44% (p<0.02) of plasma leptin variance. In the group B insulin alone accounted for 39% (p<0.05) and together with testosterone accounted for 46% (p<0.05) of plasma leptin variance. Finally in obese women none of the evaluated parameters significantly accounted for leptin variance.

**Conclusion.** The results presented in this paper confirmed the strong influence of body fat mass on serum leptin concentration. However insulin, SHBG, sex steroids as well as age may also exert secondary influence on plasma leptin level in certain groups of women.

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***Key words:*** *leptin, age, insulin, SHBG, sex steroids*

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## Introduction

Leptin is a 16kD peptide hormone encoded by OB gene located at the 7q31.3. It is synthesised mainly by adipocytes and secreted in a pulsatile manner with mean periodicity of roughly 30 minutes (6,16,29). Diurnal changes of serum leptin level have been reported with highest levels between midnight and early morning and lowest in early afternoon (21).

Body mass index (BMI) and fat cell volume strongly, directly correlate with leptin secretion rate, although there is a considerable interindividual variability in OB gene expression and serum leptin concentration at each BMI level (14). Increase in body fat mass results in elevation of plasma leptin concentration (17). Leptin secretion rate from adipose tissue correlates in turn with plasma leptin concentration (15). Not only BMI which reflects body fat mass but also the pattern of body fat distribution relates independently to circulating leptin level (28). Trunk fat mass explains more of the variance in circulating leptin concentration than leg fat, suggesting that propensity to leptin resistance may be increased in women with central adiposity (25).

The leptin level variability among subjects with the same fat stores suggests that these stores are unlikely to be the only factor that regulates leptin concentration (41). Geisthovel et al. suggested the insulin as the second (beside body fat mass) leptin concentration regulating factor (30). Serum leptin level is higher in normally menstruating females than in males (2,22,26). Gender differences in plasma leptin levels have been ascribed to a variable degree in amount of body fat as well as to differences in body fat distribution pattern (20,26). Independent of body fat mass and distribution in turn, no differences in leptin concentration between females and males were observed (20). However a possible role of sex hormones cannot be rule out (19). There are conflicting data in regard to the role of sex steroids in regulation of serum leptin level. Puberty is associated with serum leptin concentration increase regardless the children body mass (26). Leptin was found in follicular fluid and its administration in vitro increases the number of maturing oocytes in ovarian tissue. It is also thought as a link between energy stores and reproductive axis passing the message about body nutritional status permitting the reproduction to go forward if sufficient metabolic resources are available and blocking it if resources are low or metabolic system is stressed. On the other hand the estrogen administration either in form of ethinyl derivatives or native 17-β estradiol was not able to increase the serum leptin level (19). Roemmich et al. showed by means of stepwise multiple regression that combined influence of estradiol, free testosterone and subcutaneous fat depot was responsible for 74% of leptin level changes (31). Janssen et al. suggested a role of DHEAS in gender specific differences in leptin levels (37).

The data on role of age in regulation of leptin plasma level is also contradictory. Ryan and Nicklas found the increase in intraabdominal adipose tissue and subcutaneous fat with age while Kumar et al. observed in rats the age related increment in adiposity, leptin mRNA and serum leptin level (38,39). De Silva et al. in a cohort of 359 women and Sumner et al. in a group od 101 African American found no link between serum leptin level and age whereas Jorgensen et al. observed higher leptin levels in elderly people (3,5,23). Ostlund et al. found an inverse relation between age and leptin which plasma level was reduced by 53% in subjects over age of 60 years (41). Sinha and Caro found a leptin/age correlation ($r=0.26$; $p<0.001$) in a group of 275 women (20). Koistinen et al. suggested in turn no apparent effect of aging on serum leptin level (40).

## Aim of the study

Therefore it seemed interesting to seek for secondary regulators of plasma leptin level such as age, sex steroids growth hormone axis and insulin during consecutive stages of female life.

## Patients

86 women were divided according to their BMI into three groups. 38 normal weight women (mean: age 44.4±16.0 years; estradiol 69.6±79.8 ng/l; FSH 50.4±47.7 IU/l; BMI 22.9±1.3) formed group A. Group B consisted of 27 overweighted women (mean: age 55.0±6.4 years; estradiol 25.1±17.2 ng/l; FSH 75.6±26.3 IU/l; BMI 27.7±1.6). Group C embraced 21 obese women with mean: age 48.7±12.2 years; estradiol 36.9±44.0 ng/l; FSH 42.3±36.6 IU/l; BMI 34.6±4.9.

## Methods

Standard clinical evaluation (general and gynaecological evaluation) was carried out in each woman. Body mass index was calculated as body mass (kg) divided by height squared ($m^2$).

Hormonal assessment (routine - FSH, testosterone, estradiol as well as leptin, insulin-like growth factor-I (IGF-I), human growth hormone (hGH), insulin-like growth factor binding protein-3 (IGFBP-3), insulin, sex hormone binding globulin (SHBG), dihydroepiandrosterone sulphate (DHEAS) was done in basic conditions (blood for serum collection was obtained at $8^{00}$ am after12 hours of fasting, none of the patients was on any medication which may change the levels of evaluated parameters) in each patient. Basic levels of hormones were measured by RIA kits in plasma which was deeply (-20°C) frozen prior to the procedure.

## Statistical analysis

Due to lack of normal distribution of data (confirmed by W.Shapiro-Wilk test) nonparametric tests were used. To evaluate the significance of mean values differences Mann-Whitney U test was applied and mutual relations between parameters were estimated by means of Spearman rang correlation coefficient as well as stepwise multiple regression; p values of 0.05 or less were considered as significant.

## Results

The mean age of evaluated women (n=86) was 47.0±14.3 years. Mean body mass was 70.7±16.6 kg and mean BMI was 26.9±5.9. Mean plasma leptin level was 15.8±9.5 ng/ml The clinical characteristics of the total group is shown in table 1.

Women with normal range of BMI (20-25) (group A) were the youngest (44.4±16.0 years) compared to overweighted women from group B (55.0±6.4 years) and to obese ones (48.7±12.2 years; NS). Needless to say that mean body mass and BMI were the lowest in group A (61.4±5.2 kg; 22.9±1.3 resp.; p<0.001) and the highest in group C (89.9±15.8 kg; 34.6±4.9 resp.). Mean leptin level mimic the differences in mean BMI and body mass. The highest was in group C (23.2±6.8 ng/ml) and the lowest (p<0.001) in group A (8.9±4.1ng/ml). Mean FSH level was the highest in the oldest group B (75.6±26.3U/l) Gonadotropin level was the lowest in the group C (42.3±36.6 U/l). Mean estradiol levels was the highest in group C and the lowest in group B however due to large standard deviations the differences did not reach statistical significance. Despite the differences in mean BMI, the mean plasma insulin levels were similar. The highest mean testosterone level was found in obese women (group C – 0.54±0.19 ng/ml) and the lowest in group A (0.41±0.18 ng/ml; p<0.01).

The highest mean plasma hGH and IGF-I levels were found in the youngest group A (5.3±8.1 ng/ml; 247.0±104.4 ng/ml resp.; p<0.01) and the lowest in the oldest women (group B) (1.6±1.7 ng/ml; 185.0±56.7 ng/ml resp.). Mean SHBG level was the highest in group A (74.1±59.1 ng/ml) and the lowest in the obese group C (39.4±19.5 ng/ml)(table 2).

There were no statistically significant differences in mean DHEAS and IGFBP-3 levels between groups (table 2).

In all women leptin level correlated to body mass and BMI (r=0.60; r=0.71; p<0.001 resp.) as well as with age (r=0.32; p<0.02). Body mass and BMI were related to age in the same women (r=0.30; p<0.002; r=0.36; p<0.001 resp.). Age was inversely linked to estradiol (r=-0.57; p<0.001), IGF-I (r=-0.60; p<0.001) and DHEAS (r=-0.51; p<0.001).

Plasma leptin correlated inversely to estradiol (r=-0.21; p<0.05), IGF-I (r=-0.24; p<0.05), SHBG (r=-0.34; p<0.02) and DHEAS (r=-0.30; p<0.02). BMI in turn was related to plasma estradiol (r=-0.25; p<0.02), IGF-I (r=-0.32; p<0.002) and SHBG (r=-0.34; p<0.005). Estradiol in turn, was associated with IGF-I (r=-0.37; p<0.001) and with FSH (r=-0.57; p<0.001) levels.

In group of women with normal weight (A) the relations between leptin and other evaluated parameters disappeared as well as those between body mass and BMI which were present in all women. In the group the estradiol/age, DHEAS/age and IGF-I/age relations were still present (r=-0.58; p<0.001; r=-0.46; p<0.01; r=-0.71; p<0.001 resp.). The estradiol/IGF-I and estradiol/FSH links were also present (r=0.51; r=-0.70; p<0.001 resp.).

In overweighted women (group B) despite the lack of BMI/age and body mass/age as well as leptin/BMI and leptin/body mass links, the leptin/age relation was present (r=0.40; p<0.05). In the group B leptin was also related to DHEAS (r=-0.40; p<0.05) and to PRL (r=0.51; p<0.05). DHEAS was linked to age(r=-0.67; p<0.01) and estradiol (r=0.51; p<0.02) in group B. The age/estradiol, age/IGF-I

***Table 1.*** *The clinical characteristics of normal weighted (group A) and overweighted (group B) patients as well as obese women (group C).*

| Group<br>Parameter | A<br>n=38 | B<br>n=27 | C<br>n=21 | P |
|---|---|---|---|---|
| Age (years)<br>(range) | 44.4 ± 16.0<br>(19– 48) | 55.0 ± 6.4<br>(50-54.0) | 48.7 ± 12.2<br>(25.0 – 60.0) | NS |
| Body mass (kg)<br>(range) | 61.4 ± 5.2<br>(43 - 78) | 71.8 ± 12.4<br>(55 – 101.9) | 89.9 ± 15.8<br>(57-130.2) | A/C<0.001; NS |
| BMI<br>(range) | 22.9 ± 1.3<br>(16.6-24.3) | 28.5 ± 1.4<br>(25.2 – 29.9) | 34.6 ± 4.9<br>(30.1 – 39.0) | <0.01 |
| FSH (IU/l)<br>(range) | 55.2 ± 22.4<br>(2.9 - 79.8) | 75.6 ± 23.6<br>(32.1-143.5) | 42.3 ± 36.6<br>(33.1 – 150) | B/C<0.05; NS |
| Testosterone (ug/l)<br>(range) | 0.41 ± 0.18<br>(0.1 – 0.9) | 0.48 ± 0.15<br>(0.3 – 0.8) | 0.54 ± 0.19<br>(0.1 – 0.8) | A/C<0.01; NS |
| Estradiol (ng/l)<br>(range) | 20.8± 24.6<br>(5.0 - 284) | 18.6 ± 23.7<br>(9.9 – 47.7) | 89.1 ± 74.9<br>(3.6- 249) | NS |

***Table 2.*** *The mean plasma levels of leptin, insulin, DHEAS, SHBG and somatropic axis in normal weighted patients (group A) and overweighted (group B) patients as well as obese women (group C).*

| Group<br>Parameter | A<br>n=38 | B<br>n=27 | C<br>n=21 | p |
|---|---|---|---|---|
| leptin (μg/l)<br>(range) | 8.9± 4.1<br>(2.6-28.5) | 18.0± 9.0<br>(5.4-32.4) | 23.2 ± 6.8<br>(3.0 - 36.8) | A/C<0.001;NS |
| IGF-I (μg/l)<br>(range) | 247.0± 104.4<br>(116.1-688) | 185.0± 56.7<br>(83.8-264.3) | 206.3 ± 56.5<br>(71.3 – 346.1) | A/B < 0.01;NS |
| hGH (μg/l)<br>(range) | 5.3± 8.1<br>(0.1-28.5) | 1.6± 1.7<br>(0.2-6.8) | 1.9 ± 1.7<br>(0.1 - 7.3) | A/B < 0.01; NS |
| IGFBP-3 (μg/l)<br>(range) | 4.0± 1.3<br>(2.0 – 7.8) | 3.4 ± 1.1<br>(2.0 - 5.5) | 3.9± 1.2<br>(1.1 - 5.7) | NS |
| insulin (IU/l)<br>(range) | 15.1± 6.7<br>(4.1 - 27.4) | 13.4 ± 4.4<br>(4.6 - 21.2) | 13,0± 8.7<br>(2.9 - 45.3) | NS |
| DHEAS (μg/l)<br>(range) | 194.2± 103.0<br>(5.5 - 352.5) | 108.3 ± 59.4<br>(33.9 - 326.9) | 149.2 ± 81.3<br>(31.7 - 340.0) | NS |
| SHBG (μg/l)<br>(range) | 74.1±59.1<br>(17.9-193.9) | 40.8±62.2<br>(7.6—172.1) | 39.4±19.5<br>(8.9-112.9) | A/C<0.05; NS |

and estradiol/IGF-I links vanished in the group B.

In obese women (group C) body mass age relation became inverse (r=-0.39; p<0.05). Again the leptin/ body mass and leptin/BMI as well as leptin/age links were not found. However leptin correlated with SHBG (r=-0.56; p<0.02) and insulin (r-0.48; p<0.05) levels. Age was related to IGF-I (r=-0.60; p<0.001) and estradiol (r=-0.52; p<0.01) plasma levels.

The results of stepwise multiple regression showed that body mass index accounted for 31% ($r^2$=0.31; p<0.001) and SHBG for 17.7% ($r^2$=0.177; p<0.02) of plasma leptin variance in all women. In group A body mass alone accounted for 34% (p<0.05) whereas together with age accounted for 61% (p<0.01) of plasma leptin variance. In group B insulin alone accounted for 39% and together with testosterone for 46 % (p<0.05) of plasma leptin variance. In obese women (group C) none of the evaluated parameters was included in a significant way into the mathematical model of stepwise multiple regression.

The leptin increases with age in the youngest group (n=29)(r=0.43; p<0.02). There is no link between leptin and age in early menopausal women (r=0.11; NS). In post menopausal women the leptin/age relation became again significant but in an inverse way (r=-0.32; p<0.05).

## Discussion

Our results confirmed once again a well known direct correlation between body fat mass reflected by BMI and serum leptin level, which is a hormonal product of the adipose tissue and mediates the satiety signal from peripheral fat stores to the central nervous system (2,3,16,20,25-27,29). The highest mean plasma leptin level was found in women with the greatest fat stores (BMI>30; group C). However the statistically significant plasma leptin with BMI and body mass/leptin relations disappeared when the BMI range became shorter (after the division for groups A, B, C). That astonishing could result from either narrower BMI values range in each of groups than in the total material or the necessity of nonparametric statistics use due the non-gausian distribution of data. The correlation coefficients and p values were close to significance (r=0.29-0.31; p=0.06-0.08) only in the group A, whereas in other groups the coefficients were low (r=0.04-0.1; p=0.6-0.8). Moreover body mass index is an easy but not exact tool to measure body fat stores and that may also explain that disappearance of the known, leptin/BMI relation. Unfortunately we did not measure the actual fat stores of the patients. Moller et al. in group of 70 healthy subjects, observed lack of correlation (r=0.2; p=0.4) between leptin and relative body fat content in middle aged (53.0±1.0 years) and elderly (70.0±1.0 years) males and females, whereas in young (25.0±1.0 years) normally menstruating (i.e. normoestrogenic) women this correlation was very strong (r=0.71; p=0.009) (18). That disrupted link in those groups may contribute to the obesity occurring with age and also to the observed in postmenopausal women visceral fat mass increase what is reflected by the gain in WHR (18,27).

Age, sex hormones, insulin and SHBG may affect plasma leptin level (1,3,5,12,23,25). That was also confirmed by stepwise multiple regression analysis (19). In our study the application of stepwise multiple regression analysis revealed that BMI, age, insulin, testosterone, estradiol and SHBG accounted for certain percentage of plasma leptin variance in investigated groups.

There is much controversy upon age influence on serum leptin concentration. Normally menstruating women show higher leptin level than men and postmenopausal ones (23,26). That level in both normally weighted and obese women may up to 3 times higher than in men (41). Sumnar et al. in a cohort of 101 African Americans with nearly twice

as high mean serum leptin level as observed in our groups showed hardly any differences in mean serum leptin levels between pre- and postmenopausal women (33.9±17.3 ng/ml; 31.4±22.3 ng/ml resp.) (23). De Silva et al., Taylor et al. as well as Ahren et al. found no relation between leptin level and age in women (1,3,25). Castracane et al. found no differences in mean serum leptin level between pre- and postmenopausal women receiving either estrogen-progestin formulations (oral contraceptives or hormonal replacement therapy resp.) (2). Contrary to that, studies on animals showed the increase of serum leptin and leptin mRNA concentrations with age and suggest the development of leptin resistancy in ageing animals (13). Similar data regards healthy adults. Singha and Caro in a group of 275 patients observed a direct correlation between age and serum leptin level ($r=0.26$; $p<0.001$) (20). In our study the age/leptin relation in 86 patients was also found ($r=0.32$; $p<0.002$). It was also observed in overweighted women (group B)($r=0.40$; $p<0.05$), whereas vanished both in women with normal weight and in obese ones. Ostlund et al. found an inverse link between age and plasma leptin level in postmenopausal women over 60 years of age, but the correlation coefficient was weaker ($r=-0.175$; $p=0.012$)(41). In younger subjects (mean age 29 years in normal-weight group and 37 years in obese group) Considine et al found that age did not have an independent relation to plasma leptin after adjustment for body fat (42). In our study, according to Ostlund et al. suggestion we introduced another division of the material – according to age. The age/leptin relation was found as a direct one in premenopausal women ($r=0.43$; $p<0.02$) and as an inverse in postmenopausal ones ($r=-0.32$; $p<0.05$). The mean age of our postmenopausal group was similar to that in Ostlund's study. Another explanation of the leptin/age link may be taken from the presence of the age related body mass and BMI increase observed in total material.

Sztefko et al. found significantly lower mean leptin level in young women with hypothalamic amenorrhoea compared with normally menstruating women and postmenopausal one and Jorgensen et al. in turn observed higher leptin levels in elderly people (5,24). Koistinen et al. observed a significant difference in mean leptin level between males of 30 years of age and those with mean age around 75 years. The observed age-related increase in mean plasma leptin level could also be explained by the influence of pituitary and gonadal hormones as well as insulin (40).

Pituitary as well as gonadal hormones may also affect the plasma leptin level. Similar to age/leptin relation, there is much controversy on that effect. Low concentration of leptin in vitro stimulate independently hypothalamic LHRH release and LH, FSH release from pituitary. That action is to be mediated via neuropeptide Y in central nervous system. (26). The patterns of LH and leptin release are similar. Leptin, LH and estradiol show parallel type of fluctuactions during menstrual cycle (26). Krassas et al. observed an inverse relation ($r=-0.60$) between leptin and gonadotropins levels in women with polycystic ovary syndrome (PCOS), although due to small number (n=8) of subjects it was not statistically significant (7). Krzyżanowska et al. showed in a group of 11 obese women the inverse relation between gonadotropins and serum leptin levels ($r_{LH}=-0.70$; $p=0.03$; $r_{FSH}=-0.65$; $p=0.05$) which disappeared in normal-weighted subjects (12). Contrary to that, Teirmaa et al. and Sztefko et.al. observed (in cohorts of 8 and 61 resp.) a direct relation between serum LH and leptin levels ($r_T=0.40$; $p<0.01$; $r_{Sz}=0.70$; $p<0.01$ resp.) (24,26). Śmiarowska et al. in a group of 7 women with anorexia nervosa also found a direct correlation between LH and serum leptin ($r=0.82$; $p=0.04$), which vanished in normally weighted, normally menstruating women (11). Kulik-Rechtberger and Rechtberger found links between leptin and FSH ($r=0.23$; $p<0.01$) as well as leptin and estradiol ($r=0.33$; $p<0.001$) in pubertal girls (43). In our study, the stepwise regression analysis revealed that FSH together with body mass and age accounted for 74% of plasma leptin variance in the group A. In all 86 women we observed an inverse relation between leptin and estradiol ($r=-0.21$; $p<0.05$).

Adipocytes' leptin mRNA was reduced by 60% after adding of DHEAS to the culture (119,129). In our previous study we found a link between serum leptin and DHEAS levels in young women with low estradiol level ($r=-0.32$; $p<0.05$)(33). Janssen et al. also found an inverse relation between DHEAS and leptin (37). In our present study we also found an inverse relation between plasma leptin and DHEAS levels in all women and that relation was also present in overweighted women.

Jorgensen found that hGH increases, independently of body composition, the resting metabolic rate, which in turn correlates strongly ($r=0.56$; $p<0.001$) with leptin (5). In our study we observed a reverse link between plasma leptin and IGF-I levels ($r=-0.24$; $p<0.05$).

Paolisso et al. found in a cohort of 39 females a statistically significant association between estradiol and leptin plasma levels ($r=0.53$) and an inverse link ($r=-0.43$) between testosterone and leptin in males (19). Krzyżanowska et al. observed a direct estradiol/leptin relation ($r=0.67$; $p=0.03$) in 11 obese women and that link became the inverse one in 16 women with normal BMI range ($r=-0.67$; $p=0.03$) (11). A similar type of estradiol/leptin association was found in 16 normally menstruating women by Śmiarowska et al. and Leenen et al. observed the relation between abdominal obesity and total testosterone level in women (12,20).

Vettor et al. and Perry et al. found a link between plasma leptin and testosterone levels in 65 and 94 women. Correlation coefficient varied from 0.38 to 0.50(131,132). Urbański and Pau showed even a higher correlation coefficient in animal model (r=0.67)(36). In our study testosterone together with insulin accounted for 46% of plama leptin varince in women with BMI ranging from 25 to30.

Insulin also plays a role in regulation of leptin secretion and its serum level. Short lasting (2-4 hours) hyperinsulinemia seems not to exert any influence on leptin level, whereas long lasting one (72 hours) exerts a stimulatory effect on leptin secretion (4,7). Leptin receptors were found in pancreatic β-cells and insulin may also exert its action on carbohydrate metabolism by activation of adrenergic α-receptors in β-cells. Leptin infusion markedly decreased both plasma insulin and glucose levels not only by the mentioned mechanism but also by resulting partly of them increase in insulin sensitivity (20). Insulin and tumor necrosis factor-α (TNF-α) release leptin of its pool in adipose tissue (16). The persistence of high insulin and TNF-α levels are common finding in postmenopausal women (22). Ob gene expression is modulated by insulin as well as by glucocorticoids and catecholamines (20). Rosenbaum showed that in males but not in pre- and postmenopausal women fasting insulin levels correlated significantly with leptin levels. Singha and Caro observed in a group of 275 patients a similar leptin/insulin relation (r=0.57) in a group of 275 subjects (20). Baumgartner et al. revealed by mean of stepwise multiple regression, in a group of 56 postmenopausal women, the independent of body fat mass, insulin role in regulation of leptin plasma concentration (32). Van den Saffele et al. applied multiple linear regression and found that BMI, age and insulin are significant independent correlates of serum leptin in men (44).The leptin infusion leads to the decrease of glucose and insulin levels (20). In our study we observed a direct link between insulin and leptin in obese women (r=0.48; $p<0.05$) and insulin alone accounted for 39% of plasma leptin variance in women with overweight.

Van Rossum et al. applied stepwise regression analysis and found that plasma SHBG was one of the independent predictors of the relative change in leptin concentration and together with body mass and intravisceral fat volume accounted for 48% of plasma leptin variance (45). Relative changes in leptin with weight loss in 54 obese women (mean age 60±6 years old) correlated with SHBG (r=0.38; $p<0.01$). Obese postmenopusal women with lower initial SHBG level and more visceral obesity had a greater decrease in leptin with weight loss.

Our results showed that in certain groups of women age, insulin, SHBG, and sex steroids may influence the plasma leptin level. This influence seems to be secondary to fat stores impact on the leptin level.

## Conclusion

The results presented in this paper confirmed the strong influence of body fat mass on serum leptin concentration. However insulin, SHBG, sex steroids as well as age may also exert secondary influence on plasma leptin level.

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