CLINICAL STUDY

Decreased soluble leptin receptor levels in women with polycystic ovary syndrome

Susanne Hahn, Uwe Haselhorst², Beate Quadbeck, Susanne Tan, Rainer Kimmig¹, Klaus Mann and Onno E Janssen

Division of Endocrinology, Department of Medicine and ¹Department of Obstetrics and Gynecology, University of Duisburg-Essen, Hufelandstr. 55, 45122 Essen, Germany and ²Biofocus, Recklinghausen, Germany

(Correspondence should be addressed to O E Janssen; Email: onno.janssen@uni-essen.de)

Abstract

Objective: Polycystic ovary syndrome (PCOS) is associated with insulin resistance and a high incidence of obesity. Leptin, the product of the *ob* gene, is involved in the regulation of energy balance and obesity and circulates in both free and bound forms. The soluble leptin receptor (sOB-R) is the most important leptin-binding protein, thus influencing the biologically active free leptin level.

Design: We assessed the correlation of metabolic and endocrine parameters with leptin and sOB-R levels in 122 PCOS women (aged 27 ± 5.7 years) and 81 healthy controls (aged 25 ± 4.0 years).

Methods: Leptin and sOB-R levels were measured using ELISA kits. In addition, anthropometric variables, body fat and endocrine parameters were evaluated and a glucose tolerance test performed to assess indices of insulin resistance and glucose metabolism.

Results: In PCOS patients, no correlation was found between leptin or sOB-R and parameters of hyperandrogenism. However, as expected, body mass index (BMI), body fat, waist circumference and indices of insulin resistance were significantly correlated with leptin in PCOS subjects and controls. In a subgroup analysis of lean, overweight and obese PCOS patients, significant differences were found in leptin (29.7 ± 20.7 vs 45.4 ± 25.0 vs 67.7 ± 28.8 ng/ml, $P < 0.0001$) and sOB-R (8.0 ± 3.4 vs 6.4 ± 2.5 vs 5.7 ± 2.3 ng/ml, $P < 0.05$). Compared with BMI-matched controls, lean PCOS patients had lower sOB-R levels (8.0 ± 3.4 vs 12.7 ± 4.7 ng/ml, $P < 0.0001$) and higher free leptin indices (4.5 ± 3.9 vs 2.8 ± 2.2 , $P = 0.0285$).

Conclusion: Taking into account that low sOB-R levels supposedly compensate diminished leptin action, PCOS *per se* might cause leptin resistance.

European Journal of Endocrinology 154 287–294

Introduction

Polycystic ovary syndrome (PCOS) is among the most common endocrine disorders, affecting more than 5% of women of reproductive age (1, 2). An association of PCOS with peripheral insulin resistance, compensatory hyperinsulinemia and alterations in β -cell function as the cause of its predisposition to develop a metabolic syndrome (type 2 diabetes mellitus, hypertension, lipid disorders, obesity) has been established (3). Most relevant risk factors of cardiovascular disease are elevated in PCOS women (4). However, it is unclear whether this risk results in an increase in cardiovascular morbidity and mortality later in life. On the other hand, there is clear evidence that insulin resistance and hyperinsulinemia aggravate ovarian androgen overproduction and enhance skin manifestations. According to the majority of studies, most PCOS women are insulin resistant and overweight or obese. Adipose tissue has

been established as a major endocrine organ. Through the release of peptides, such as adiponectin, resistin and leptin (5), it is involved in the pathogenesis of several metabolic disorders. Leptin, the gene product of the *ob* gene, is thought to provide the central nervous system with feed-back information about fat storage of the body. Thus, leptin is thought to be a part of the regulation of appetite, food intake and the metabolic rate (6). Obese humans present with hyperleptinemia as an indicator of leptin resistance which, in turn, has been suggested to play a major role in the pathogenesis of obesity (7).

The consensus of published studies did not find a PCOS-specific influence on leptin, as no significant differences were found between body mass index (BMI)-matched PCOS women and healthy controls (8–11). The main determinants of leptin concentration in these studies were fat mass and the degree of obesity or insulin resistance.

In mice, a genetic defect in the *ob* gene results in severe obesity and type 2 diabetes mellitus, as well as in infertility (6). In this mouse model, leptin injections restore fertility. Leptin treatment in normal female mice accelerates puberty (12). In humans, granulosa cells have been shown to secrete leptin (13) and leptin seems to influence adrenal androgen formation (14). However, the role of leptin in ovarian function or on the reproductive system remains unclear. On the one hand, leptin levels did not influence follicular maturation and ovulation in PCOS patients (10) but, on the other hand, Carmina and co-workers (15) demonstrated a negative correlation between leptin and dehydroepiandrosterone sulphate (DHEAS) levels in 21 lean PCOS women, suggesting that androgens may play a role in suppressing serum leptin. Another study showed that leptin concentrations correlated inversely with luteinizing hormone (LH) levels, independent of body weight and insulin resistance (16). In contrast again, Laughlin *et al.* (17) proposed that leptin is neither involved in the hypersecretion of LH nor in the regulation of LH pulsatility. During lactational amenorrhoea Sir-Petermann *et al.* (18) also showed that leptin secretion is not related to LH secretion.

Both metabolic and endocrine parameters might correlate better with free leptin rather than with total leptin levels, as the former represents the biologically active fraction that is available for direct interaction with its receptor. The levels of free leptin can either be measured directly or assessed by determination of the soluble leptin receptor (sOB-R), which accounts for the majority of serum leptin-binding activity (19). The sOB-R has been shown to correlate inversely with total serum leptin levels and BMI in women of reproductive age (20). Kado *et al.* (20) hypothesized that obese women are able to conversely increase their leptin activity by minimizing the bound leptin fraction, thus influencing the biological activity of leptin. In line with this, Sinha *et al.* (21) reported that in lean subjects leptin circulates predominantly in the bound form, whereas in obese patients, due to low sOB-R levels, leptin circulates mostly in the free form. Similar results were found for Japanese men and women, showing a negative correlation between sOB-R levels and BMI, parameters of insulin resistance, cholesterol and leptin levels (22). Weight loss, following gastric restrictive surgery, leads to a reduction in circulating leptin levels and to an increase in sOB-R (23). In 114 Greek students, determination of total energy intake and macronutrient composition by food records was undertaken in order to assess an association between sOB-R and dietary variables. The sOB-R was mostly correlated with the total amount of energy intake and not so much with food composition (24). In adolescent girls with anorexia nervosa, higher sOB-R levels and a lower free leptin index (FLI) were found compared with healthy control women (25). In this cohort, sOB-R levels were also correlated

with cortisol levels. Additionally, sOB-R and FLI but not total leptin levels were closely related to parameters of sexual maturation and the onset of puberty (26).

We here present an analysis of sOB-R and its correlation with obesity, leptin resistance, insulin resistance and other endocrine parameters in PCOS women.

Subjects and methods

Study population

PCOS patients ($n = 122$) were recruited from the out-patient clinics of the Division of Endocrinology, Department of Medicine and the Department of Gynecology at the University of Duisburg-Essen. Some patients were also attracted by the clinic's PCOS homepage (www.pco-syndrom.de). Based on the criteria derived from the 1990 National Institutes of Health (NIH) conference, diagnosis of PCOS was established when either oligomenorrhea (cycles lasting longer than 35 days) or amenorrhea (less than two menstrual cycles in the past 6 months) and either clinical signs of hyperandrogenism (hirsutism or obvious acne or alopecia and/or an elevated total testosterone in combination with an elevated free androgen index (FAI) (normal range: testosterone < 2.0 nmol/l, FAI < 3.8) were found, and other pituitary, adrenal or ovarian diseases could be excluded. In addition, in all women an adrenocorticotropin test with measurement of 17-hydroxyprogesterone was performed. When the difference of both values (baseline and after 60 min) was greater than 2 ng/ml or the stimulated value was greater than 10 ng/ml, a genetic analysis (21-hydroxylase deficiency) was added. Hirsutism was routinely graded by two physicians independently using the common modified Ferriman–Gallwey (FG) score. FG scores never differed by more than 2 and when not identical were re-evaluated by a third physician and the median value used. This method to assess hirsutism requires the visual scoring of the extent of terminal hairs in nine body areas, namely (a) upper lip, (b) chin, (c) chest, (d) upper abdomen, (e) lower abdomen, (f) upper back, (g) lower back, (h) thighs and (i) upper arms. The lower arms or lower legs are not included in the hair assessment. Each area is scored from 0 to 4, resulting in a possible maximum score of 36. Hirsutism was diagnosed when a score above 5 was evaluated. All PCOS women also fulfilled the 2003 Rotterdam criteria. All recruited women were required to be otherwise healthy. Healthy controls ($n = 81$) were matched according to BMI (< 25 kg/m²) with a subgroup of lean PCOS women ($n = 44$). Controls were recruited from a screening program for employees instituted at the University of Duisburg-Essen. In control women, all NIH criteria of PCOS were excluded. PCOS as well as control subjects had not taken any medication known to affect carbohydrate metabolism or endocrine parameters for at least

3 months before entering the study. Women taking contraceptive pills were also excluded from the study.

Data collection

In PCOS subjects and control women, clinical parameters were assessed by physical examination, including the degree of hirsutism by evaluating the FG score and anthropometric measurements including body weight in kg (BW) and waist (W) and hip (H) circumference in cm. Body fat (F) was measured using the Body FAT Watcher (NAIS Wellnesslife GmbH, Düsseldorf, Germany) and BMI was calculated as $\text{weight}/(\text{height})^2$ (kg/m^2). Parameters of insulin resistance and β -cell function were evaluated using a 3-h oral glucose tolerance test (OGTT). After an overnight fast of 12 h, patients ingested 75 g glucose and had their glucose and insulin levels determined at baseline and at 30, 60, 90, 120 and 180 min. Insulin resistance and β -cell function were defined by the homeostasis model assessment (HOMA) (27) and hyperinsulinemia by calculating the area under the insulin response curve (AUC-I). In addition, whole-body insulin sensitivity ($\text{ISI}_{\text{Matsuda}}$) (28), which combines hepatic and peripheral insulin sensitivity, was measured by the formula: $10\,000/\text{square root of (fasting glucose} \times \text{fasting insulin)} \times (\text{mean glucose} \times \text{mean insulin during OGTT (times 0, 30, 60, 90, 120 min))}$. Prior to OGTT (between 0800 and 0900 h), blood samples were drawn for serum leptin, sOB-R and all other hormonal and metabolic parameters. The FAI was estimated as $\text{testosterone (nmol/l)/sex hormone-binding globulin (SHBG) (nmol/l)} \times 100$. The ratio of leptin/sOB-R was used as the FLI. Except for amenorrhoeic women, all laboratory determinations were performed in the early follicular phase of the cycle.

Biochemical assays

Automated chemiluminescence immunoassay systems were used for the determination of LH, estradiol, testosterone, cortisol, thyrotropin (TSH), cholesterol (CHOL), triglycerides (TG) and blood glucose (G) (ADVIA Centaur; Bayer Vital, Fernwald, Germany), androstenedione, DHEAS, insulin and SHBG (IMMULITE 2000; DPC Biermann, Bad Nauheim, Germany) and insulin-like growth factor (IGF-I) (Nichols Advantage, Nichols Institute Diagnostics, Bad Vilbel, Germany). The glycosylated fraction of hemoglobin-A1 (HbA1c) was determined by an automated HPLC method on an A1C 2.2 glycohemoglobin analyser (TOSOH-Eurogenetics, Cologne, Germany). Leptin and sOB-R were measured using ELISA kits (Diagnostic Systems Laboratories (DSL), Webster, TX, USA). The DSL ACTIVE Human Leptin ELISA is an enzymatically amplified 'two-step' sandwich-type immunoassay. The DSL ACTIVE Leptin Soluble Receptor ELISA is a 'one-step' sandwich-type immunoassay. The theoretical sensi-

tivity or minimum detection limit as calculated by interpolation of the mean plus two standard deviations of 12 replicants of the zero standard was 0.05 ng/ml for leptin and 0.14 ng/ml for sOB-R. Intra-assay variation was <5% and interassay variation was <8% for all measured parameters.

Ethical approval

The study protocol was approved by the Ethics Committee of the University of Duisburg-Essen. All participants gave written informed consent before entering the study.

Statistical analysis

Data are presented as median plus range for non-parametric data. For better comparison, means $\pm$ S.D. is also shown. Correlations between variables were examined by Spearman's correlation coefficient (r_s) because analysed data were not normally distributed.

Differences between the groups were evaluated with the Mann-Whitney U test. *P* values <0.05 were considered significant. The box-plot graph was computed using GraphPad Prism 4 (GraphPad Software, Inc., San Diego, CA, USA).

Results

In PCOS women, leptin levels correlated significantly with BMI (Fig. 1). In addition, leptin was negatively correlated with sOB-R levels both in PCOS patients and in controls, but sOB-R levels were lower in PCOS women than in healthy women (Fig. 2). In PCOS patients, leptin was correlated with metabolic parameters such as insulin resistance, glucose and lipid metabolism (Table 1). A significant correlation was also observed between sOB-R and BMI ($r_s = -0.21$, $P = 0.0285$), body fat, waist and insulin resistance (IR) but not with serum lipids or parameters of glucose metabolism (Table 1). Analysis of endocrine parameters revealed a significant correlation between leptin and FAI ($r_s = 0.29$, $P = 0.0022$) as well as SHBG ($r_s = -0.43$, $P < 0.0001$), but not with total testosterone ($r_s = -0.15$), androstenedione ($r_s = -0.10$), DHEAS ($r_s = -0.04$), estradiol ($r_s = -0.01$), LH ($r_s = 0.09$), cortisol ($r_s = -0.08$) or IGF-I ($r_s = 0.00$) (all $P > 0.05$). No correlation between hirsutism scores and leptin or sOB-R was found. Interestingly, both leptin ($r_s = 0.25$, $P = 0.0060$) and sOB-R ($r_s = -0.22$, $P = 0.0173$) correlated with TSH. No correlations were found between sOB-R and sex steroids, cortisol or lipids.

Lean PCOS patients ($n = 44$) and BMI-matched controls ($n = 81$) did not differ in anthropometric parameters or leptin levels (Table 2). Although ten PCOS patients had a HOMA-IR >2 and five of these had a

Figure 1 Correlation of BMI and leptin levels in PCOS women. Total leptin was determined in 122 PCOS patients as described in Subjects and methods. Spearman's evaluation of correlation gave an $r_s = 0.56$, $P < 0.0001$.

HOMA-IR > 2.6 (the limit for insulin resistance for this age group in our reference population), mean HOMA-IR of the PCOS patients was not significantly different from that of the control group (Table 2). However, sOB-R levels were significantly lower in lean PCOS women compared with these controls (Fig. 3). As expected from the lower sOB-R, PCOS patients had a higher FLI than controls (PCOS 4.5 ± 3.9 vs controls 2.8 ± 2.2 , $P = 0.0285$). Lean PCOS women had significantly lower triglyceride levels (0.82 ± 0.36 vs 1.09 ± 0.48 mmol/l, $P = 0.0021$) and total cholesterol levels (4.7 ± 0.7 vs 5.2 ± 1.1 mmol/l, $P = 0.0164$), not explained by the differences in TSH, as thyroid function was not correlated with lipid metabolism in women with normal body weight.

In the lean PCOS subgroup, correlation of leptin with BMI remained significant ($r_s = 0.5041$, $P = 0.0007$) but not the correlation between leptin and sOB-R ($r_s = -0.0689$, $P = 0.664$). PCOS subjects were found to have higher androgen levels and hirsutism scores (7 ± 4 vs 2 ± 2) than controls but did not

differ in cortisol or IGF-I levels (data not shown). PCOS patients also had significantly higher TSH levels than controls (2.0 ± 1.0 vs 1.5 ± 0.6 mU/l, $P = 0.0038$), in part due to four PCOS patients with elevated TSH levels (3.6–5.0 mU/l). However, excluding these from the evaluation ($n = 40$; TSH 1.8 ± 0.8 mU/l), differences in TSH levels and sOB-R levels (PCOS 7.95 ± 3.4 vs controls 12.6 ± 4.7 ng/ml) still remained significant ($P = 0.0159$ and $P < 0.0001$ respectively). Thus, while TSH correlated with sOB-R in the entire PCOS cohort, no such correlation was found in lean PCOS patients and healthy controls.

To evaluate the impact of BMI on leptin and sOB-R levels, PCOS patients were stratified into three groups according to BMI: < 25 kg/m², PCOS lean, $n = 44$; 25 – 29.9 kg/m², the overweight group, $n = 20$ and ≥ 30 kg/m², PCOS obese, $n = 58$. As expected, metabolic variables correlated with BMI in PCOS patients (Table 2). Leptin increased and sOB-R decreased with higher BMI values. SHBG levels were

Figure 2 Correlation of leptin with sOB-R levels in PCOS patients and healthy controls. Leptin and sOB-R levels were determined in 122 PCOS patients (●) and 81 healthy controls (□) by ELISA as described in Subjects and methods. In both PCOS and controls, leptin was significantly correlated with sOB-R ($r_s = -0.38$, $P < 0.0001$ and $r_s = -0.27$, $P = 0.0142$ respectively). However, the sOB-R levels of the PCOS patients were shifted to lower values by about 4 ng/ml.

Table 1 Correlation of leptin and sOB-R levels with metabolic parameters in PCOS women.

Variable	Leptin (ng/ml)		sOB-R (ng/ml)	
	r_s	P	r_s	P
BW	0.48	<0.0001	-0.19	NS
F	0.66	<0.0001	-0.30	0.0029
W	0.54	<0.0001	-0.30	0.0068
ISI ^{Matsuda}	-0.43	0.0002	0.20	NS
HOMA-IR	0.47	<0.0001	-0.22	0.0191
I _{fasting}	0.48	<0.0001	-0.23	0.0121
AUC-I	0.46	<0.0001	-0.21	0.0307
TG	0.35	0.0002	-0.08	NS
G _{fasting}	0.33	0.0027	-0.04	NS
HbA1c	0.24	0.0112	-0.06	NS

I, insulin. NS, not significant.

also different between the groups, but all other endocrine parameters were similar (Table 2).

The PCOS cohort was also divided into a hyperandrogenemic group (HA) ($n = 103$) and a group with normal testosterone levels (N) ($n = 19$) to analyse the impact of androgens on leptin and sOB-R levels. Neither group differed in hirsutism scores (HA 10.4 ± 7.3 vs N 10.1 ± 6.0 , $P = 0.8796$). Leptin and sOB-R values were similar in the HA and the N subgroup (leptin $P = 0.75$, sOB-R $P = 0.645$). Furthermore, oligomenorrhoeic ($n = 78$) and amenorrhoeic ($n = 44$) PCOS women had similar leptin and sOB-R levels (data not shown).

Discussion

Leptin levels are increased in obesity and may play a role in the development of insulin resistance and type 2 diabetes mellitus (7). In our study, leptin levels correlated significantly with BMI and body weight in both PCOS women and controls. The difference in mean leptin levels in lean, overweight and obese patients was highly significant. In agreement with previous studies (8), leptin correlated with other metabolic parameters including lipid metabolism and insulin resistance in this German PCOS cohort. However, in conformity with other studies (9), our lean PCOS women and healthy controls, with similar anthropometric variables and parameters of insulin resistance, did not differ in total leptin levels. Similar results were found in PCOS patients from Australia (29), Brazil (16), Canada (10), Finland (30), Italy (15), Sweden (8), Turkey (11) and the USA (17, 31).

In the entire PCOS cohort, as well as in the lean subgroup, no correlation was found between leptin concentrations and androgens or gonadotropins. The correlation of leptin with FAI resulted from its influence on SHBG. The inverse correlation between leptin and SHBG has previously been described by Laughlin *et al.* (17). Another study on leptin also reported a

Table 2 Characteristics of controls ($n = 81$), lean ($n = 44$), overweight (ow; $n = 20$) and obese ($n = 58$) PCOS patients. Values are means $\pm$ S.D. (median and range).

Variable	Controls	PCOS lean	PCOS ow	PCOS obese
Age (years)	25.0 $\pm$ 4.0 (24, 20–26) ^b	27.0 $\pm$ 5.6 (27, 16–37)	26.0 $\pm$ 5.6 (27, 17–36)	29.0 $\pm$ 6.1 (28, 16–40)
BMI (kg/m ²)	21.5 $\pm$ 2.0 (21, 18–24)	21.0 $\pm$ 1.9 (22, 18–24)	27.0 $\pm$ 1.6 (27, 25–29) ^a	37.0 $\pm$ 5.1 (37, 30–48) ^a
W (cm)	76.4 $\pm$ 6.6 (75, 64–94)	74.4 $\pm$ 7.1 (75, 59–92)	87.7 $\pm$ 10.5 (86, 73–115) ^a	106.4 $\pm$ 13.2 (104, 88–155) ^a
F (%)	26.7 $\pm$ 2.8 (26.5, 22.4–33)	28.0 $\pm$ 2.9 (28.6, 23–34)	33.4 $\pm$ 2.7 (32.4, 27.4–38.7) ^a	44.7 $\pm$ 6.0 (43.6, 33.5–56) ^a
I _{fasting} (pmol/l)	44.9 $\pm$ 19.7 (43, 14.4–100.8)	41.0 $\pm$ 20.1 (38, 12.6–90)	63.3 $\pm$ 31.1 (58, 23.4–119) ^b	153.0 $\pm$ 98.1 (126, 28–486) ^a
HOMA- β (%)	1.7 $\pm$ 1.0 (1.5, 0.5–3.1)	1.5 $\pm$ 0.7 (1.4, 0.4–3.4)	2.2 $\pm$ 1.1 (1.9, 0.8–4.5) ^b	6.1 $\pm$ 4.0 (5.1, 0.8–17.3) ^a
ISI ^{Matsuda}	136.3 $\pm$ 62.8 (137, 39.3–321.6)	123.0 $\pm$ 62.9 (100, 50–339)	194.0 $\pm$ 110.8 (171, 57–416) ^b	281.0 $\pm$ 143.8 (242, 87–840) ^a
AUC-I	8.7 $\pm$ 3.5 (8.6, 2.9–18.3)	9.5 $\pm$ 4.3 (9, 3.4–18.8)	4.9 $\pm$ 2.8 (4, 2.2–13) ^a	2.3 $\pm$ 1.9 (2, 0.5–4.7) ^a
G _{fasting} (mg/dl)	149.0 $\pm$ 78.6 (121.5, 50–347)	143.1 $\pm$ 89.5 (103, 53–424)	199.0 $\pm$ 100.6 (161, 76–442) ^b	461.0 $\pm$ 389.3 (413, 109–2700) ^a
CHOL (mmol/l)	83.4 $\pm$ 6.7 (83.5, 63–97)	84.0 $\pm$ 7.3 (84, 71–99)	88.0 $\pm$ 9.2 (88, 74–107)	95.0 $\pm$ 11.7 (90, 69–127) ^a
Leptin (ng/ml)	5.2 $\pm$ 1.1 (5.1, 3.1–9.6) ^b	4.7 $\pm$ 0.7 (4.7, 3.5–6.3)	4.9 $\pm$ 1.1 (4.9, 3.2–6.6)	5.1 $\pm$ 1.2 (4.9, 2.2–10.9) ^b
sOB-R (ng/ml)	12.7 $\pm$ 4.7 (11.9, 5.7–28.5) ^a	29.7 $\pm$ 20.7 (22.6, 6.8–91.9)	45.4 $\pm$ 25.0 (36.6, 7.2–91.9) ^b	67.7 $\pm$ 28.8 (67.1, 13.5–148) ^a
Testosterone (nmol/l)	1.3 $\pm$ 0.4 (1.4, 0.6–2.0) ^a	8.0 $\pm$ 3.4 (7.5, 3.2–19.7)	6.4 $\pm$ 2.5 (6.2, 1.2–10.8) ^b	5.7 $\pm$ 2.3 (5.2, 1.8–11.8) ^a
Androstenedione (nmol/l)	1.9 $\pm$ 0.6 (1.9, 0.7–3.2) ^a	2.6 $\pm$ 0.9 (2.4, 0.5–5.4)	2.7 $\pm$ 0.7 (2.7, 1.7–4.4)	2.6 $\pm$ 0.7 (2.5, 1–4.5)
TSH (mU/l)	1.5 $\pm$ 0.6 (1.4, 0.5–3.5) ^b	4.1 $\pm$ 1.6 (3.9, 1–7.5)	4.3 $\pm$ 1.9 (4.1, 0.8–8.8)	4.0 $\pm$ 1.7 (3.8, 1.3–7.9)
SHBG (nmol/l)	69.9 $\pm$ 40.7 (67.7, 38.4–137)	2.0 $\pm$ 1.0 (1.8, 0.4–5)	34.5 $\pm$ 17.8 (30.3, 11–66) ^b	22.2 $\pm$ 1.2 (2.0, 0.3–6.5)

^a $P < 0.0001$, ^b $P < 0.05$ vs lean PCOS women.

Figure 3 sOB-R levels in lean PCOS patients and healthy controls. sOB-R was measured in 44 lean PCOS women and 81 healthy controls (BMI ranged in both groups from 18 to 24 kg/m²) as described in Subjects and methods. The difference in sOB-R levels was significant by Mann–Whitney U test, $P < 0.0001$. Data are shown by box and whiskers graph. The box extends from the 25th to the 75th percentile, with a line at the median indicating the 50th percentile. The whiskers represent the ranges extending from the lowest to the highest value.

correlation of leptin with FAI (8). Our results are also in agreement with data from Pirwany and colleagues (10) who did not find any correlation between leptin and testosterone as well as LH but a significant correlation between leptin and FAI. Furthermore, they did not find any correlation of leptin levels and follicular development or ovulation. We also did not find differences in leptin levels in either oligomenorrhoeic or amenorrhoeic women.

Glucocorticoids are potent regulators of leptin expression. In rats, the administration of glucocorticoids leads to an up-regulation of leptin expression (32). In contrast, Cushing's syndrome does not elevate leptin levels in humans (33). On the other hand, leptin reduced cortisol secretion by 52% in adrenocortical cells isolated from obese patients (34). We did not find a correlation between leptin and cortisol levels or IGF-I, consistent with other studies (35, 36). A positive correlation of leptin with estradiol levels was detected in 28 PCOS women published by Mendonca *et al.* in 2004 (37). No such correlation was found in a study from Sweden (8) and in our study population.

In PCOS women, the present study confirms that circulating leptin levels correlate with parameters of insulin resistance and the metabolic syndrome but has no detectable effect on the reproductive features of PCOS. Thus, leptin appears to impair ovulation and reproduction only in extreme leptin deficiency, such as in the *ob/ob* mouse. No such extreme leptin deficiency has been described in PCOS patients.

Some studies have focused on the relationship between leptin and thyroid function. Thyroid hormones regulate the expression of leptin mRNA and secretion of leptin by adipocytes *in vitro* (38). In PCOS women, free thyroxine (fT4) was found to be lower than in controls (39) in some studies but not in all (40). However, even in the former study, only a weak and non-significant correlation between fT4 and leptin was found. We have recently described a higher incidence of elevated TSH levels and autoimmune thyroiditis in PCOS women (40), which was confirmed in this study. Pinkney *et al.* (41) found no differences in TSH levels of lean or obese euthyroid patients, with a significant correlation of leptin and TSH only in the obese subgroup. In agreement with these data, TSH correlated with leptin only in overweight and obese, but not in lean PCOS women in our German sample. In another study from Italy, subclinical hypothyroidism did not influence leptin levels in obese patients (42). Thus, the impact of thyroid function on leptin appears to be quite limited.

In obesity, high leptin levels are found to be indicative of leptin resistance (7). Potential mechanisms that may mediate leptin resistance include impairment of brain leptin transport or leptin receptor internalization, receptor mutations and post-receptor signaling defects. Furthermore, the active hormone may be reduced by binding proteins or soluble receptors (43). As only free leptin is found in cerebrospinal fluid, its free fraction rather than total leptin appears to be biologically active (44). sOB-R represents the main leptin-binding compound in plasma, thus regulating its free fraction in the circulation. Several studies have shown that sOB-R levels are inversely correlated with BMI (45), confirmed in our study by comparison of PCOS women divided into different BMI subgroups. sOB-R levels also decreased with higher body fat content and insulin resistance. Lower sOB-R levels increase the free leptin index, a mechanism likely to compensate, at least in part, for leptin resistance. All PCOS women, regardless of their BMI, presented with lower sOB-R levels and higher free leptin indices than healthy controls. The reduction of sOB-R could not be explained by differences in body fat content or other metabolic parameters.

The interplay of sOB-R and the reproductive system is quite unclear. While some studies have found an association of sOB-R with estradiol and free testosterone (46), sex hormones did not affect sOB-R levels in a Japanese population (45). In analogy to leptin, sOB-R did not correlate with androgens, estradiol or gonadotropins in our German PCOS cohort. The inverse relationship of sOB-R with IGF-I described in adolescents (47) could not be confirmed in our study, most likely because of the prevalence of adult women in our study population.

Yannakoulia and co-workers (24) demonstrated that sOB-R is positively correlated with total energy intake and inversely correlated with energy intake from

dietary fat. They speculated that the macronutrient composition of the diet influences serum concentrations of free leptin. Another recent study found a positive correlation between free leptin and dietary carbohydrate content but not with lipid or protein content (48). The authors hypothesized that the reduction of sOB-R levels is tantamount to or represents a compensatory mechanism to overcome leptin resistance. Except for slightly lower triglycerides and cholesterol levels in lean PCOS subjects compared with their BMI-matched controls, we cannot exclude differences in dietary composition in our PCOS and control groups, as no food records from our study population were available.

As neither androgens nor estrogens seem to explain the higher leptin (49) and lower sOB-R levels in women (46), body composition has been speculated to account for these differences. Consistent with this hypothesis, reduction of trunk fat in anorectic women results in higher sOB-R levels and, consequently, lower FLI (50). Waist-to-hip ratio, as an approximate measure of visceral obesity, did not differ in PCOS women and controls in our study (data not shown) but an exact evaluation of trunk, subcutaneous and extremity fat was not performed.

A recently published twin study of pubertal females studying the genetic and environmental influences on the variations of leptin and sOB-R levels showed that leptin is mostly influenced by body composition but sOB-R by genetic background (26). The lower sOB-R levels in PCOS women, unexplained by body weight, body fat content or hormonal parameters, may indicate that genetic determinants are more important than environmental factors in its contribution to free leptin regulation.

In conclusion, we are the first to demonstrate low sOB-R levels in PCOS women as a possible mechanism to compensate for leptin resistance. Neither leptin nor sOB-R levels were influenced by hyperandrogenism or the severity of menstrual disturbances. Rather than environmental factors, food or body composition, genetic differences might account for lower sOB-R levels in PCOS women.

References

- Asuncion M, Calvo RM, San Millan JL, Sancho J, Avila S & Escobar-Morreale HF. A prospective study of the prevalence of the polycystic ovary syndrome in unselected Caucasian women from Spain. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 2434–2438.
- Azziz R, Woods KS, Reyna R, Key TJ, Knochenhauer ES & Yildiz BO. The prevalence and features of the polycystic ovary syndrome in an unselected population. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 2745–2749.
- Dunaif A, Segal KR, Futterweit W & Dobrjansky A. Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. *Diabetes* 1989 **38** 1165–1174.
- Loverro G, Lorusso F, Mei L, Depalo R, Cormio G & Selvaggi L. The plasma homocysteine levels are increased in polycystic ovary syndrome. *Gynecologic and Obstetric Investigation* 2002 **53** 157–162.
- Zhang Y, Proenca R, Maffei M, Barone M, Leopold L & Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994 **372** 425–432.
- Janeckova R. The role of leptin in human physiology and pathophysiology. *Physiological Research* 2001 **50** 443–459.
- Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL *et al.* Serum immunoreactive leptin concentrations in normal-weight and obese humans. *New England Journal of Medicine* 1996 **334** 292–295.
- Gennarelli G, Holte J, Wide L, Berne C & Lithell H. Is there a role for leptin in the endocrine and metabolic aberrations of polycystic ovary syndrome? *Human Reproduction* 1998 **13** 535–541.
- Maliqueo M, Perez-Bravo F, Calvillan M, Piwonka V, Castillo T & Sir-Petermann T. Relationship between leptin and insulin sensitivity in patients with polycystic ovary syndrome (In Spanish). *Medicina Clinica* 1999 **113** 526–530.
- Pirwany IR, Fleming R, Sattar N, Greer IA & Wallace AM. Circulating leptin concentrations and ovarian function in polycystic ovary syndrome. *European Journal of Endocrinology* 2001 **145** 289–294.
- Telli MH, Yildirim M & Noyan V. Serum leptin levels in patients with polycystic ovary syndrome. *Fertility and Sterility* 2002 **77** 932–935.
- Ahima RS, Dushay J, Flier SN, Prabakaran D & Flier JS. Leptin accelerates the onset of puberty in normal female mice. *Journal of Clinical Investigation* 1997 **99** 391–395.
- Agarwal SK, Vogel K, Weitsman SR & Magoffin DA. Leptin antagonizes the insulin-like growth factor-I augmentation of steroidogenesis in granulosa and theca cells of the human ovary. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 1072–1076.
- Biason-Lauber A, Zachmann M & Schoenle EJ. Effect of leptin on CYP17 enzymatic activities in human adrenal cells: new insight in the onset of adrenarche. *Endocrinology* 2000 **141** 1446–1454.
- Carmina E, Ferin M, Gonzalez F & Lobo RA. Evidence that insulin and androgens may participate in the regulation of serum leptin levels in women. *Fertility and Sterility* 1999 **72** 926–931.
- Spritzer PM, Poy M, Wiltgen D, Mylius LS & Capp E. Leptin concentrations in hirsute women with polycystic ovary syndrome or idiopathic hirsutism: influence on LH and relationship with hormonal, metabolic, and anthropometric measurements. *Human Reproduction* 2001 **16** 1340–1346.
- Laughlin GA, Morales AJ & Yen SS. Serum leptin levels in women with polycystic ovary syndrome: the role of insulin resistance/hyperinsulinemia. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 1692–1696.
- Sir-Petermann T, Recabarren SE, Lobos A, Maliqueo M & Wildt L. Secretory pattern of leptin and LH during lactational amenorrhoea in breastfeeding normal and polycystic ovarian syndrome women. *Human Reproduction* 2001 **16** 244–249.
- Lammert A, Kiess W, Bottner A, Glasow A & Kratzsch J. Soluble leptin receptor represents the main leptin binding activity in human blood. *Biochemical and Biophysical Research Communications* 2001 **283** 982–988.
- Kado N, Kitawaki J, Koshiba H, Ishihara H, Kitaoka Y, Teramoto M & Honjo H. Relationships between the serum levels of soluble leptin receptor and free and bound leptin in non-pregnant women of reproductive age and women undergoing controlled ovarian hyperstimulation. *Human Reproduction* 2003 **18** 715–720.
- Sinha MK, Opentanova I, Ohannesian JP, Kolaczynski JW, Heiman ML, Hale J, Becker GW, Bowsher RR, Stephens TW & Caro JF. Evidence of free and bound leptin in human circulation. Studies in lean and obese subjects and during short-term fasting. *Journal of Clinical Investigation* 1996 **98** 1277–1282.
- Ogawa T, Hirose H, Yamamoto Y, Nishikai K, Miyashita K, Nakamura H, Saito I & Saruta T. Relationships between serum soluble leptin receptor level and serum leptin and adiponectin levels, insulin resistance index, lipid profile, and leptin receptor gene polymorphisms in the Japanese population. *Metabolism* 2004 **53** 879–885.

- 23 van Dielen FM, van't Veer C, Buurman WA & Greve JW. Leptin and soluble leptin receptor levels in obese and weight-losing individuals. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 1708–1716.
- 24 Yannakoulia M, Yiannakouris N, Bluher S, Matalas AL, Klimis-Zacas D & Mantzoros CS. Body fat mass and macronutrient intake in relation to circulating soluble leptin receptor, free leptin index, adiponectin, and resistin concentrations in healthy humans. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 1730–1736.
- 25 Misra M, Miller KK, Almazan C, Ramaswamy K, Aggarwal A, Herzog DB, Neubauer G, Breu J & Klibanski A. Hormonal and body composition predictors of soluble leptin receptor, leptin, and free leptin index in adolescent girls with anorexia nervosa and controls and relation to insulin sensitivity. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 3486–3495.
- 26 Li HJ, Ji CY, Wang W & Hu YH. A twin study for serum leptin, soluble leptin receptor and free insulin-like growth factor-I in pubertal females. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 3659–3664.
- 27 Matthews D, Hosker J, Rudenski A, Naylor B, Treacher D & Turner R. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985 **28** 412–419.
- 28 Matsuda M & DeFronzo R. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* 1999 **22** 1462–1470.
- 29 Chapman IM, Wittert GA & Norman RJ. Circulating leptin concentrations in polycystic ovary syndrome: relation to anthropometric and metabolic parameters. *Clinical Endocrinology* 1997 **46** 175–181.
- 30 Rouru J, Anttila L, Koskinen P, Penttilä TA, Irjala K, Huupponen R & Koulou M. Serum leptin concentrations in women with polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 1697–1700.
- 31 Mantzoros CS, Dunaif A & Flier JS. Leptin concentrations in the polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 1687–1691.
- 32 De Vos P, Saladin R, Auwerx J & Staels B. Induction of ob gene expression by corticosteroids is accompanied by body weight loss and reduced food intake. *Journal of Biological Chemistry* 1995 **270** 15958–15961.
- 33 Koistinen H. Serum leptin concentration is normal in Cushing syndrome and in acromegaly. *Diabetologia* 1996 **39** A59.
- 34 Bornstein SR, Uhlmann K, Haidan A, Ehrhart-Bornstein M & Scherbaum WA. Evidence for a novel peripheral action of leptin as a metabolic signal to the adrenal gland: leptin inhibits cortisol release directly. *Diabetes* 1997 **46** 1235–1238.
- 35 Gomez JM, Maravall FJ, Gomez N, Navarro MA, Casamitjana R & Soler J. Interactions between serum leptin, the insulin-like growth factor-I system, and sex, age, anthropometric and body composition variables in a healthy population randomly selected. *Clinical Endocrinology* 2003 **58** 213–219.
- 36 Kowalska I, Kinalski M, Strackowski M, Wolczyński S & Kinalska I. Insulin, leptin, IGF-I and insulin-dependent protein concentrations after insulin-sensitizing therapy in obese women with polycystic ovary syndrome. *European Journal of Endocrinology* 2001 **144** 509–515.
- 37 Mendonca HC, Montenegro RM Jr, Foss MC, Silva de Sa MF & Ferriani RA. Positive correlation of serum leptin with estradiol levels in patients with polycystic ovary syndrome. *Brazilian Journal of Medical and Biological Research* 2004 **37** 729–736.
- 38 Yoshida T, Monkawa T, Hayashi M & Saruta T. Regulation of expression of leptin mRNA and secretion of leptin by thyroid hormone in 3T3-L1 adipocytes. *Biochemical and Biophysical Research Communications* 1997 **232** 822–826.
- 39 Erel CT, Senturk LM, Kaleli S, Gezer A, Baysal B & Tasan E. Is serum leptin level regulated by thyroid functions, lipid metabolism and insulin resistance in polycystic ovary syndrome? *Gynecological Endocrinology* 2003 **17** 223–229.
- 40 Janssen OE, Mehlmauer N, Hahn S, Offner AH & Gartner R. High prevalence of autoimmune thyroiditis in patients with polycystic ovary syndrome. *European Journal of Endocrinology* 2004 **150** 363–369.
- 41 Pinkney JH, Goodrick SJ, Katz J, Johnson AB, Lightman SL, Coppack SW & Mohamed-Ali V. Leptin and the pituitary-thyroid axis: a comparative study in lean, obese, hypothyroid and hyperthyroid subjects. *Clinical Endocrinology* 1998 **49** 583–588.
- 42 Tagliaferri M, Berselli ME, Calo G, Minocci A, Savia G, Petroni ML, Viberti GC & Liuzzi A. Subclinical hypothyroidism in obese patients: relation to resting energy expenditure, serum leptin, body composition, and lipid profile. *Obesity Research* 2001 **9** 196–201.
- 43 Houseknecht KL, Baile CA, Matteri RL & Spurlock ME. The biology of leptin: a review. *Journal of Animal Science* 1998 **76** 1405–1420.
- 44 Landt M, Parvin CA & Wong M. Leptin in cerebrospinal fluid from children: correlation with plasma leptin, sexual dimorphism, and lack of protein binding. *Clinical Chemistry* 2000 **46** 854–858.
- 45 Shimizu H, Shimomura K, Negishi M, Masunaga M, Uehara Y, Sato N, Shimomura Y, Kasai K & Mori M. Circulating concentrations of soluble leptin receptor: influence of menstrual cycle and diet therapy. *Nutrition* 2002 **18** 309–312.
- 46 Chan JL, Bluher S, Yiannakouris N, Suchard MA, Kratzsch J & Mantzoros CS. Regulation of circulating soluble leptin receptor levels by gender, adiposity, sex steroids, and leptin: observational and interventional studies in humans. *Diabetes* 2002 **51** 2105–2112.
- 47 Kratzsch J, Lammert A, Bottner A, Seidel B, Mueller G, Thiery J, Hebebrand J & Kiess W. Circulating soluble leptin receptor and free leptin index during childhood, puberty, and adolescence. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 4587–4594.
- 48 Magni P, Liuzzi A, Ruscica M, Dozio E, Ferrario S, Bussi I, Minocci A, Castagna A, Motta M & Savia G. Free and bound plasma leptin in normal weight and obese men and women: relationship with body composition, resting energy expenditure, insulin-sensitivity, lipid profile and macronutrient preference. *Clinical Endocrinology* 2005 **62** 189–196.
- 49 Caro JF, Sinha MK, Kolaczynski JW, Zhang PL & Considine RV. Leptin: the tale of an obesity gene. *Diabetes* 1996 **45** 1455–1462.
- 50 Misra M, Soyka LA, Miller KK, Grinspoon S, Levitsky LL & Klibanski A. Regional body composition in adolescents with anorexia nervosa and changes with weight recovery. *American Journal of Clinical Nutrition* 2003 **77** 1361–1367.

Received 27 June 2005

Accepted 24 October 2005