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The effects of obesity and polycystic ovary syndrome on serum lipocalin-2 levels: a cross-sectional study

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Abstract

Background: Lipocalin-2 is a novel adipokine that appears to play a role in the development of insulin resistance. Serum lipocalin-2 levels are elevated in obese patients. Obesity and insulin resistance are cardinal characteristics of the polycystic ovary syndrome (PCOS). However, there are limited data on serum lipocalin-2 levels in patients with PCOS. The aim of the present study was to assess serum lipocalin-2 levels in PCOS.

Methods: We studied 200 patients with PCOS and 50 healthy female volunteers.

Results: Serum lipocalin-2 levels were slightly higher in women with PCOS compared with controls (65.4 +/- 34.3 vs. 60.3 +/- 26.0 ng/ml, respectively) but this difference did not reach statistical significance. In contrast, lipocalin-2 levels were higher in overweight/obese women with PCOS than in normal weight women with the syndrome (76.2 +/- 37.3 vs. 54.5 +/- 27.2 ng/ml, respectively; $p < 0.001$). Serum lipocalin-2 levels were also higher in overweight/obese controls compared with normal weight controls (70.1 +/- 24.9 vs. 50.5 +/- 23.7 ng/ml, respectively; $p = 0.004$). In the total study population (patients with PCOS and controls), lipocalin-2 levels were independently correlated with the body mass index ($p < 0.001$). In women with PCOS, lipocalin-2 levels were independently correlated with the waist ($p < 0.001$).

Conclusions: Obesity is associated with elevated serum lipocalin-2 levels. In contrast, PCOS does not appear to affect lipocalin-2 levels.

Background

Polycystic ovary syndrome (PCOS) is characterized by hyperandrogenism (biochemical hyperandrogenemia and/or clinical manifestations of hyperandrogenemia), chronic oligo- or anovulation and polycystic ovaries on ultrasonography [1,2]. Obesity, usually of the central type, is included in the cardinal characteristics of the syndrome, as it is present in varying degrees (30-70%) and is directly linked to increased peripheral insulin resistance (IR) [3-5].

Insulin resistance, via the resulting hyperinsulinemia, significantly contributes to the endocrine and metabolic disturbances observed in PCOS [6,7]. Insulin has been

shown to stimulate theca cell androgen synthesis and suppress sex hormone-binding globulin (SHBG) in the liver, further increasing the free portion of circulating androgens [8,9]. In addition, adiposity contributes to the conversion of Δ_4 -androstendione (Δ_4 -A) to the most potent androgen, testosterone (T), because adipocytes have been shown to express significant amounts of the enzyme 17 β -hydroxysteroid dehydrogenase-ketosteroid reductase [10,11].

Lipocalin-2 belongs to the superfamily of lipocalins and was first isolated in human neutrophils. Lipocalin-2 is a 25 kDa glucoprotein that consists of 178 aminoacid residues and is covalently linked to metalloproteinases [12,13]. The gene that encodes its synthesis is located on chromosome 9 (9q34.11) and was characterized in 1997 [14]. Lipocalin-2 mRNA has been isolated in the bone marrow, as well as in tissues exposed to microorganisms

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(respiratory and alimentary tract, genitourinary system). In addition, lipocalin-2 is expressed in several types of cells, including adipocytes, endothelial cells, macrophages, vascular smooth muscle cells, hepatocytes, endometrial cells and splenic cells [15-22].

Most investigators reported increased serum lipocalin-2 levels in obese patients [23,24]. In addition, males have higher serum lipocalin-2 levels and this gender difference is present in both normal weight and obese subjects [23]. Moreover, lipocalin levels are elevated in patients with cardiovascular diseases and might represent an independent cardiovascular risk factor [24].

Since a considerable proportion of patients with PCOS has obesity (particularly abdominal), IR, glucose intolerance, type 2 diabetes mellitus (T2DM) and low-grade inflammation, i.e. disorders where lipocalin-2 secretion is affected, the present study was designed to assess a) serum lipocalin-2 levels in normal weight and overweight/obese patients with PCOS, and, b) the association between serum lipocalin-2 levels and anthropometric, metabolic, hormonal and ultrasonographic features of PCOS.

Methods

Patients

We studied 200 women with PCOS [age 24.5 ± 5.3 years, body mass index (BMI) 27.0 ± 6.4 kg/m²] (Group I). We also studied 50 healthy women (age 32.6 ± 4.7 years, mean BMI 25.1 ± 4.0 kg/m²) with normal ovulating cycles (28 ± 2 days, blood progesterone levels >10 ng/ml in two consecutive cycles), no signs of hyperandrogenism and normal sonographic appearance of the ovaries (control group, Group II)(Table 1). All women with PCOS were outpatients at the Gynecological Endocrinology Infirmary of the Second Department of Obstetrics and Gynecology, Aristotle University of Thessaloniki, Greece, who had presented with at least one of the following signs: oligomenorrhea, fertility problems, hirsutism, acne or male-pattern alopecia. Women of the control group were healthy volunteers.

Diagnosis of PCOS was based on the revised criteria of Rotterdam (see study protocol)[1,2]. None of the women studied had galactorrhea or any endocrine or systemic disease that could possibly affect reproductive physiology. No woman reported use during the last semester of any medication that could interfere with the normal function of the hypothalamic-pituitary-gonadal axis. When basic 17α -hydroxyprogesterone (17α -OHP) levels were >1.5 ng/ml, the Synacthen test (0.25 mg/1 ml; Novartis Pharma S.A., Rueil-Malmaison, France) was performed to rule out congenital adrenal hyperplasia. Other causes of hyperandrogenemia, including prolactinoma, Cushing's syndrome and androgen secreting tumors were also excluded. Informed consent was

obtained from all women, and the study was approved by the institutional review board. The study met the requirements of the 1975 Helsinki guidelines.

Study protocol

In all women, body weight, height and waist circumference (W) were measured. Body weight was measured with analog scales and in light clothing; height was measured barefoot with a stadiometer. The BMI was calculated by dividing weight (in kg) by height squared (in m) to assess obesity. The W was obtained as the smallest circumference at the level of the umbilicus.

Baseline blood samples were collected between days 3 and 7 of the menstrual cycle in the control group and between 3 to 7 days after a spontaneous bleeding episode in patients with PCOS, after an overnight fast. The circulating levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), T, Δ_4 -A, dehydroepiandrosterone sulfate (DHEA-S), 17α -OHP, SHBG, glucose, insulin, thyroid stimulating hormone (TSH) and free thyroxin (FT4) were measured. Immediately after the baseline blood sampling an oral glucose tolerance test (OGTT) was performed; 75 g of glucose were administered orally and serum glucose levels were determined after 30, 60, 90 and 120 min. At the same day transvaginal ultrasonography was performed and the volume of each ovary was determined, as well as the number of follicles in each ovary.

Patients with PCOS were divided according to BMI in Subgroups I α [BMI <25 kg/m²; n = 100, age 23.4 ± 4.5 years, BMI 22.1 ± 1.8 kg/m²] and I β (BMI >27 kg/m²; n = 100, age 25.7 ± 5.8 years, BMI 31.9 ± 5.6 kg/m²). Controls were also divided according to BMI in subgroups II α [BMI <25 kg/m²; n = 25, age 31.3 ± 4.5 years, BMI 21.9 ± 1.6 kg/m²] and II β (BMI >27 kg/m²; n = 25, age 33.9 ± 4.6 years, BMI 28.3 ± 3.0 kg/m²).

Methods

Plasma glucose, insulin, FSH, LH, PRL, androgens, 17α -OHP, TSH and FT4 concentrations were measured as previously described [25]. Serum lipocalin-2 levels were determined with an enzyme-linked immunosorbent assay (human lipocalin-2/NGAL Elisa, BioVendor Laboratorni medicina a.s., Modrice, Czech Republic). Lower levels of detection was 0.02 ng/ml, the intra-assay coefficients of variation for low and high levels were 8.38 and 7.03%, respectively, and the inter-assay coefficients of variation for low and high lipocalin-2 levels were 9.73 and 9.77%, respectively. Free androgen index (FAI) was determined as follows: FAI = T (nmol/l) \times 100/SHBG (nmol/l) [26]. The homeostasis model assessment of IR (HOMA-IR) index was calculated as follows: HOMA-IR = fasting insulin (mIU/l) \times glucose (mg/dl)/405 [27]. The quantitative insulin sensitivity check index

Table 1 Anthropometric, hormonal, metabolic and ultrasonographic characteristics of all patients with polycystic ovary syndrome (PCOS) and all controls

	Group I (patients with PCOS) (n = 200)	Group II (controls) (n = 50)	p (adjusted for age and BMI)
Age (years)	24.5 ± 5.3	32.6 ± 4.7	NA
BMI (kg/m ²)	27.0 ± 6.4	25.1 ± 4.0	NA
Waist (cm)	83.5 ± 14.9	80.8 ± 10.1	NS
FSH (mIU/ml)	6.4 ± 1.9	7.9 ± 2.8	0.007
LH (mIU/ml)	7.6 ± 5.0	5.9 ± 2.8	NS
Prolactin (ng/ml)	14.3 ± 6.9	12.2 ± 4.3	NS
Testosterone (ng/dl)	75.1 ± 30.1	32.9 ± 14.4	<0.001
Δ ₄ -A (ng/ml)	2.9 ± 1.0	1.7 ± 0.5	<0.001
DHEA-S (ng/ml)	3106.0 ± 1300.8	1944.6 ± 811.8	<0.001
FAI	7.64 ± 6.1	1.98 ± 1.16	0.002
17α-OHP (ng/ml)	1.1 ± 0.5	0.7 ± 0.3	0.010
SHBG (nmol/l)	47.1 ± 28.1	69.2 ± 33.7	NS
Glucose (mg/dl)	98.5 ± 21.5	97.0 ± 9.8	NS
Insulin (μIU/ml)	12.4 ± 9.1	9.2 ± 6.8	NS
Glucose/insulin	11.52 ± 6.7	14.86 ± 9.03	NS
HOMA-IR	3.22 ± 3.90	2.24 ± 1.71	NS
QUICKI	0.34 ± 0.03	0.35 ± 0.03	NS
Area under the OGTT curve	15143.9 ± 3134.1	14565.6 ± 3352.5	NS
Mean ovarian volume (cm ³)	9.8 ± 4.9	5.3 ± 1.8	< 0.001
Mean number of ovarian follicles	10.8 ± 4.7	6.2 ± 1.9	< 0.001
Lipocalin (ng/ml)	65.4 ± 34.3	60.3 ± 26.0	NS

NA, not applicable; NS, not significant; BMI, body mass index; FSH, follicle stimulating hormone; LH, luteinizing hormone; Δ₄-A, Δ₄-androstenedione; DHEA-S, dehydroepiandrosterone sulfate; FAI, free androgen index; 17α-OHP, 17α-hydroxyprogesterone; SHBG, sex hormone-binding globulin; OGTT, oral glucose tolerance test; HOMA-IR, homeostasis model assessment of insulin resistance; QUICKI, quantitative insulin sensitivity check index

(QUICKI) was calculated according to the following formula: QUICKI = 1/[log Insulin (mIU/l) + log Glucose (mg/dl)][28].

Transvaginal ultrasonography

Transvaginal ultrasonography was performed by an experienced operator in all women. Ovarian volume was calculated as follows: Ovarian volume = (π/6) × ovarian length × ovarian height × ovarian width. Polycystic ovaries were diagnosed when ≥ 12 follicles with a diameter of 2-9 mm were present in one or both ovaries, or when the ovarian volume was > 10 cm³.

Statistical analysis

Data analysis was performed with the statistical package SPSS (version 17.0; SPSS Inc., 233 South Wacker Drive, 11th Floor, Chicago, IL). All tested parameters followed normal distribution as assessed with the Kolmogorov-Smirnov test and are reported as mean ± SD. Because women with PCOS were younger and had greater BMI than controls (p < 0.001 and p = 0.009, respectively), comparisons between patients and controls were performed with analysis of covariance (ANCOVA) adjusting for age and BMI. Because normal weight women with

PCOS were younger than obese/overweight women with PCOS (p = 0.002), comparisons between these groups were performed with ANCOVA adjusting for age. Because normal weight controls were younger than obese/overweight controls (p = 0.046), comparisons between these groups were performed with ANCOVA adjusting for age. Changes between baseline and end-of-treatment were assessed with the paired samples t-test. Independent correlations between lipocalin-2 levels and other parameters were assessed with stepwise linear regression analysis including parameters that were significantly correlated with lipocalin-2 levels in univariate analysis. In all cases, a p value < 0.05 was considered significant.

Results

The anthropometric, hormonal, metabolic and ultrasonographic features of women with PCOS and controls are shown in Table 1. Women with PCOS had lower plasma FSH levels and higher plasma T, Δ₄-A, DHEA-S, FAI and 17α-OHP levels than controls. In addition, women with PCOS had greater mean ovarian volume and a higher mean number of ovarian follicles than controls. There were no differences in plasma glucose or

insulin levels, glucose/insulin ratio, the area under the OGTT curve and the indices HOMA-IR and QUICKI between women with PCOS and controls. Serum lipocalin-2 levels were slightly higher in women with PCOS compared with controls (65.4 ± 34.3 vs. 60.3 ± 26.0 ng/ml, respectively) but this difference did not reach statistical significance.

The anthropometric, hormonal, metabolic and ultrasonographic features of normal weight and overweight/obese women with PCOS are shown in Table 2. Overweight/obese women with PCOS had greater BMI and W than normal weight women with PCOS. Plasma SHBG levels were lower and the FAI was higher in the former. Moreover, plasma insulin levels, the area under the OGTT curve and the HOMA-IR index were higher, whereas the glucose/insulin ratio and the QUICKI were lower in overweight/obese women with PCOS than in normal weight women with PCOS. Serum lipocalin-2 levels were also higher in overweight/obese women with PCOS (76.2 ± 37.3 vs. 54.5 ± 27.2 ng/ml in normal weight women with PCOS; $p < 0.001$).

The anthropometric, hormonal, metabolic and ultrasonographic features of normal weight and overweight/obese controls are shown in Table 3. Overweight/obese controls had greater BMI and W than normal weight controls. Serum lipocalin-2 levels were also higher in

overweight/obese controls (70.1 ± 24.9 vs. 50.5 ± 23.7 ng/ml in normal weight controls; $p = 0.004$). In contrast, there were no differences in hormone levels between the two groups except plasma LH levels that were higher in normal weight controls ($p = 0.011$). In addition, there were no differences in plasma glucose and insulin levels, the glucose/insulin ratio, the area under the OGTT curve and the indices HOMA-IR and QUICKI between normal weight controls and overweight/obese controls.

In the total sample of patients ($n = 250$), serum lipocalin-2 levels were negatively correlated with the QUICKI ($r = -0.221$, $p < 0.001$), the glucose/insulin ratio ($r = -0.183$, $p = 0.004$) and plasma SHBG levels ($r = -0.131$, $p = 0.039$) and positively correlated with the waist/hip ratio ($r = 0.317$, $p < 0.001$), W ($r = 0.313$, $p < 0.001$), BMI ($r = 0.304$, $p < 0.001$), HOMA-IR ($r = 0.221$, $p < 0.001$) and plasma insulin ($r = 0.200$, $p = 0.002$) and glucose levels ($r = 0.191$, $p = 0.002$). In step-wise linear regression analysis, serum lipocalin-2 levels were independently correlated with BMI ($p < 0.001$; Figure 1).

In women with PCOS ($n = 200$), serum lipocalin-2 levels were negatively correlated with the QUICKI ($r = -0.265$, $p < 0.001$), the glucose/insulin ratio ($r = -0.245$, $p < 0.001$) and plasma SHBG levels ($r = -0.152$, $p = 0.031$) and positively correlated with the waist/hip ratio

Table 2 Anthropometric, hormonal, metabolic and ultrasonographic characteristics of normal weight and overweight/obese patients with polycystic ovary syndrome (PCOS).

	Group I α (normal weight patients with PCOS) (n = 100)	Group I β (overweight/obese patients with PCOS) (n = 100)	p (adjusted for age)
Age (years)	23.4 \pm 4.5	25.7 \pm 5.8	NA
BMI (kg/m ²)	22.1 \pm 1.8	31.9 \pm 5.6	<0.001
Waist (cm)	72.8 \pm 5.3	94.2 \pm 13.6	<0.001
FSH (mIU/ml)	6.8 \pm 1.9	6.0 \pm 1.7	0.001
LH (mIU/ml)	8.5 \pm 5.5	6.7 \pm 4.4	0.025
Prolactin (ng/ml)	14.7 \pm 6.6	13.9 \pm 7.3	NS
Testosterone (ng/dl)	74.7 \pm 27.3	75.4 \pm 32.7	NS
Δ_4 -A (ng/ml)	2.9 \pm 1.1	2.9 \pm 0.9	NS
DHEA-S (ng/ml)	3148.6 \pm 1218.2	3063.1 \pm 1384.1	NS
FAI	5.80 \pm 4.19	9.51 \pm 7.19	<0.001
17 α -OHP (ng/ml)	1.1 \pm 0.5	1.1 \pm 0.5	NS
SHBG (nmol/l)	59.0 \pm 30.5	35.2 \pm 19.4	<0.001
Glucose (mg/dl)	94.9 \pm 10.0	102.1 \pm 28.3	NS
Insulin (μ IU/ml)	8.6 \pm 5.0	16.1 \pm 10.6	<0.001
Glucose/insulin	13.68 \pm 6.02	9.36 \pm 6.61	<0.001
HOMA-IR	2.04 \pm 1.34	4.38 \pm 5.08	<0.001
QUICKI	0.35 \pm 0.03	0.32 \pm 0.03	<0.001
Area under the OGTT curve	14496.9 \pm 2725.9	15797.4 \pm 3388.2	0.006
Mean ovarian volume (cm ³)	7.7 \pm 3.7	11.9 \pm 5.0	<0.001
Mean number of ovarian follicles	10.9 \pm 5.2	10.8 \pm 4.1	NS
Lipocalin (ng/ml)	54.5 \pm 27.2	76.2 \pm 37.3	<0.001

Abbreviations are defined in Table 1.

Table 3 Anthropometric, hormonal, metabolic and ultrasonographic characteristics of normal weight and overweight/obese controls

	Group II α (normal weight controls) (n = 25)	Group II β (overweight/obese controls) (n = 25)	p (adjusted for age)
Age (years)	31.3 \pm 4.5	33.9 \pm 4.6	NA
BMI (kg/m ²)	21.9 \pm 1.6	28.3 \pm 3.0	<0.001
Waist (cm)	73.7 \pm 5.6	87.9 \pm 8.5	<0.001
FSH (mIU/ml)	8.1 \pm 2.5	7.8 \pm 3.2	NS
LH (mIU/ml)	7.1 \pm 3.3	4.8 \pm 1.5	0.011
Prolactin (ng/ml)	12.1 \pm 3.8	12.4 \pm 4.7	NS
Testosterone (ng/dl)	36.4 \pm 14.5	29.4 \pm 13.8	NS
Δ_4 -A (ng/ml)	1.8 \pm 0.4	1.7 \pm 0.6	NS
DHEA-S (ng/ml)	2138.9 \pm 788.6	1750.4 \pm 803.1	NS
FAI	1.89 \pm 0.99	2.07 \pm 1.31	NS
17 α -OHP (ng/ml)	0.7 \pm 0.3	0.7 \pm 0.3	NS
SHBG (nmol/l)	76.4 \pm 30.8	61.9 \pm 35.5	NS
Glucose (mg/dl)	95.1 \pm 7.8	98.9 \pm 11.2	NS
Insulin (μ U/ml)	8.2 \pm 7.0	10.3 \pm 6.6	NS
Glucose/insulin	15.48 \pm 7.27	14.23 \pm 10.62	NS
HOMA-IR	1.96 \pm 1.76	2.53 \pm 1.63	NS
QUICKI	0.36 \pm 0.03	0.35 \pm 0.04	NS
Area under the OGTT curve	13009.8 \pm 1680.8	16121.4 \pm 3883.3	0.002
Mean ovarian volume (cm ³)	5.3 \pm 1.9	5.3 \pm 1.8	NS
Mean number of ovarian follicles	6.0 \pm 1.9	6.4 \pm 1.9	NS
Lipocalin (ng/ml)	50.5 \pm 23.7	70.1 \pm 24.9	0.004

Abbreviations are defined in Table 1.

($r = 0.348$, $p < 0.001$), W ($r = 0.343$, $p < 0.001$), BMI ($r = 0.314$, $p < 0.001$), HOMA-IR ($r = 0.265$, $p < 0.001$) and plasma insulin ($r = 0.254$, $p < 0.001$) and glucose levels ($r = 0.162$, $p = 0.002$). In stepwise linear regression analysis, serum lipocalin-2 levels were independently correlated with the W ($p < 0.001$; Figure 2).

Discussion

Lipocalins are bioactive peptides that belong to adipokines. The lipocalin superfamily includes more than 20 small

extracellular peptides that exert multiple functions mostly after binding to other molecules [29]. They were named lipocalins by Pervaiz and Brew from the Greek words “lipos” (i.e. fat) and “kalyx” (i.e. cup), because of their cup-like molecule [30,31]. Lipocalin-2 has a similar tertiary structure with other lipocalins and its pivotal characteristic is the presence of a hydrophobic calyx that binds to small lipophilic molecules. The main binding part of lipocalin-2 is its small iron-binding molecules [29]. Accordingly, lipocalin-2 both binds and transfers iron, an essential

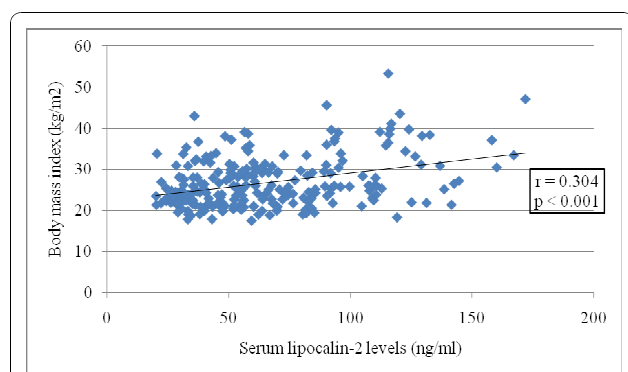


Figure 1 Correlation of serum lipocalin-2 levels with the body mass index in the total study population (Groups 1 and 2, n = 250).

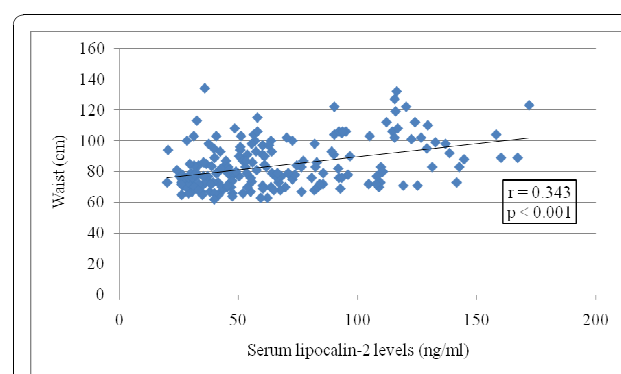


Figure 2 Correlation of serum lipocalin-2 levels with the waist in women with PCOS (Group 1, n = 200).

component for the growth of almost all bacteria. Therefore, lipocalin-2 exerts bacteriostatic actions and appears to play an important role in innate immunity and immune response to bacterial infections [32-35].

In the present study, serum lipocalin-2 levels were marginally higher in women with PCOS compared with controls (65.4 ± 34.3 vs. 60.3 ± 26.0 ng/ml, respectively) but this difference did not reach significance (Table 1). Plasma glucose or insulin levels, the glucose/insulin ratio, the area under the OGTT curve and the indices HOMA-IR and QUICKI also did not differ between women with PCOS and controls. The lack of difference in insulin resistance between patients with PCOS and controls might be partly due to the inclusion of patients with the ovulatory phenotype of PCOS, which is known to have a milder form of the metabolic disturbances [36]; among the 200 patients with PCOS, 50 (25%) had this phenotype. In addition, we used relatively insensitive markers of insulin resistance (i.e. the HOMA-IR and QUICKI indices) instead of the gold standard euglycemic hyperinsulinemic clamp and this might have also precluded the detection of a difference in insulin resistance between patients with PCOS and controls [37,38]. The slightly higher serum lipocalin-2 levels in women with PCOS (Group I) compared with controls (Group II) might be due to the greater BMI in the former (Table 1), since serum lipocalin-2 levels are elevated in obese patients [23,24]. However, the present study suggests that PCOS does not affect serum lipocalin-2 levels.

Overweight/obese women with PCOS (subgroup I β) had higher serum lipocalin-2 levels than normal weight women with PCOS (subgroup I α) ($p < 0.001$; Table 2). Similarly, overweight/obese controls (subgroup II β) had higher serum lipocalin-2 levels than normal weight controls (subgroup II α) ($p = 0.004$; Table 3). In the total study population ($n = 250$), in stepwise linear regression analysis, serum lipocalin-2 levels were independently correlated with BMI ($p < 0.001$; Figure 1). Moreover, in women with PCOS ($n = 200$), in stepwise linear regression analysis, serum lipocalin-2 levels were independently correlated with W ($p < 0.001$; Figure 2). A significant increase in serum lipocalin-2 levels has been previously reported in obese patients [23,24]. In addition, the elevated serum lipocalin-2 levels in obese patients correlate with anthropometric, hormonal and metabolic parameters [23]. Moreover, the strong correlation between serum lipocalin-2 levels and both the HOMA-IR index and plasma glucose levels, which is not affected after adjusting for the BMI, suggests that lipocalin-2 might represent an independent risk factor for development of IR and hyperglycemia.

There are only two studies that assessed serum lipocalin-2 levels in patients with PCOS [39,40]. However, these two studies yielded conflicting results. In the first

study, serum lipocalin-2 levels were determined in 40 patients with PCOS and 40 controls, aged 25.4 ± 4.5 and 27.4 ± 4.4 years, respectively, and with BMI of 25.3 ± 3.8 and 23.4 ± 2.4 kg/m², respectively [39]. The matrix metalloproteinase-9 (MMP-9)/neutrophil gelatinase-associated lipocalin (NGAL) complex was also measured. Serum lipocalin-2 and MMP-9/NGAL complex levels were lower in patients with PCOS than in controls ($p < 0.001$ for both comparisons)[39]. The investigators suggested that NGAL and MMP-9/NGAL complex levels should be further evaluated in patients with PCOS, because the decreased levels of these atherogenic molecules might protect patients with PCOS against cardiovascular disease (CVD). In the second study, serum lipocalin-2 levels were measured in 30 patients with PCOS and 30 controls [40]. Receiver operating characteristic curves were plotted to determine the serum levels of lipocalin-2 that indicate the presence of IR. This study showed that lipocalin-2 levels are elevated in patients with PCOS compared with controls ($p < 0.001$) and that lipocalin-2 may prove to be a useful marker of IR in patients with PCOS [40]. In the present study we evaluated a substantially larger number of patients with PCOS ($n = 200$) and we did not observe significant differences in serum lipocalin-2 levels between patients with PCOS and controls (Table 1). However, overweight/obese patients with PCOS and overweight/obese controls had significantly higher lipocalin-2 levels than normal weight patients with PCOS and normal weight controls, respectively ($p < 0.001$ and $p = 0.004$, respectively; Table 2 and 3).

It has been reported that serum lipocalin-2 levels are elevated in patients with CVD and might represent an independent cardiovascular risk factor [24]. It has also been reported that gelatinase B (also known as MMP-9), an endopeptidase capable of degrading the molecular components of the extracellular matrix, is associated with increased risk for abdominal aortic aneurysm, atherosclerosis and plaque rupture [41,42]. Therefore, MMP-9 is considered to be an important mediator of vascular remodeling and plaque instability [43]. Physical disruption of the atherosclerotic plaque triggers thrombus formation, which might lead to myocardial infarction (MI). MMP-9 action is enhanced by NGAL, also known as lipocalin-2 [44]. The formation of the MMP-9/lipocalin-2 complex is crucial for atherotic plaque erosion and thrombus formation [19,45,46]. Hemdahl et al have shown increased expression of lipocalin-2 and co-localization with MMP-9 in atherosclerotic plaques and MI lesions [47].

Conclusions

Our findings suggest that PCOS is not associated with significant changes in serum lipocalin-2 levels. On the other hand, obese patients have elevated serum

lipocalin-2 levels, regardless of the presence of PCOS. The increased serum lipocalin-2 levels in overweight and obese patients with PCOS potentially represent a useful marker of IR.

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Authors' contributions

DP conceived of the study, and participated in its design and coordination and drafted the manuscript. KT performed the statistical analysis and helped to draft the manuscript. All authors helped to draft the manuscript, and read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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