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The relationship between lipocalin-2 and free testosterone levels in polycystic ovary syndrome

Związek między stężeniami lipokaliny-2 i testosteronu wolnego w zespole policystycznych jajników

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Abstract

Introduction: Lipocalin-2 is an adipokine that is mainly produced from adipocytes and macrophages. Data related to PCOS and other obesity-associated disorders have shown divergent results. Here, we studied lipocalin-2 concentrations in women with PCOS and in healthy women, and investigated the potential contributors underlying lipocalin association with PCOS.

Material and methods: Forty-four women with PCOS and 47 age- and BMI-matched healthy women were enrolled. Fasting serum glucose, insulin, homeostasis model assessment of insulin resistance (HOMA-IR), high-sensitivity C-reactive protein (hs-CRP), and free testosterone levels were measured. The body fat percentage was measured by bioelectrical impedance.

Results: Lipocalin-2 concentrations were significantly higher in the PCOS group than in the control group (55.74 ± 17.54 ng/mL *vs.* 36.46 ± 19.62 ng/mL, p = 0.011). There was a correlation between lipocalin-2 levels and free testosterone. In a multiple regression model, the body fat percentage, HOMA-IR, and hs-CRP were not associated with lipocalin-2. However, only free testosterone was associated with lipocalin-2. A "lipocalin-2 = 11.214 + (1.943 × free-testosterone)" equation was obtained.

Conclusions: Serum lipocalin-2 levels were higher in women with PCOS, and only free testosterone was associated with lipocalin-2. Lipocalin-2 levels and their influencing factors have discrepant results in both PCOS and other obesity- or insulin resistance-related metabolic disorders. Thus, the potential role of lipocalin-2 in PCOS should be clarified. **(Endokrynol Pol 2017; 68 (1): 7–12)**

Key words: *PCOS; lipocalin-2; free testosterone*

Streszczenie

Wstęp: Lipokalina-2 to adipokina produkowana głównie przez adipocyty i makrofagi. Wyniki badań dotyczących zespołu policystycznych jajników (PCOS) i innych zaburzeń związanych z otyłością są rozbieżne. Autorzy ocenili stężenia lipokaliny-2 kobiet z PCOS i u zdrowych kobiet oraz zbadali potencjalne czynniki determinujące związek lipokaliny z PCOS.

Materiał i metody: Do badania włączono 44 kobiety z PCOS i 47 dobranych pod względem wieku i wskaźnika masy ciała zdrowych kobiet. Zmierzono stężenie glukozy i insuliny w surowicy na czczo, wskaźnik insulinooporności w modelu homeostazy HOMA-IR, stężenie białka C-reaktywnego oznaczonego metodą wysokoczułą (hs-CRP) i testosteronu wolnego. Zmierzono również procentową zawartość tkanki tłuszczowej w organizmie, stosując metodę impedancji bioelektrycznej.

Wyniki: Stężenia lipokaliny-2 były istotnie wyższe w grupie PCOS niż w grupie kontrolnej (55,74 ± 17,54 ng/ml *vs.* 36,46 ± 19,62 ng/ml, p = 0.011). Stwierdzono korelację między stężeniami lipokaliny-2 i testosteronu wolnego. W modelu regresji wielokrotnej nie wykazano zależności między procentową zawartością tkanki tłuszczowej w organizmie, wskaźnikiem HOMA-IR i stężeniem hs-CRP a stężeniem lipokaliny-2. Stwierdzono tylko związek między stężeniami testosteronu wolnego i lipokaliny-2. Zależność tę opisuje równanie: stężenie lipokaliny-2 = 11,214 + (1,943 × stężenie testosteronu wolnego).

Wnioski: Stężenia lipokaliny-2 w surowicy były wyższe u kobiet z PCOS. Jedynym parametrem związanym ze stężeniem lipokaliny-2 było stężenie testosteronu wolnego. Dane dotyczące stężenia lipokaliny-2 i wpływających na nie czynników w PCOS i innych zaburzeniach metabolicznych związanych z otyłością lub insulinoopornością są rozbieżne. Potencjalna rola lipokaliny-2 w PCOS wymaga wyjaśnienia. **(Endokrynol Pol 2017; 68 (1): 7–12)**

Słowa kluczowe: *PCOS; lipokalina-2; wolny testosteron*

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Abbreviations

BMI — body mass index
HOMA-IR — homeostasis model assessment of insulin resistance
hs-CRP — high sensitivity C-reactive protein
IL-6 — interleukin 6
PCOS — polycystic ovary syndrome
TNF-α — tumour necrosis factor alpha

Introduction

Polycystic ovary syndrome (PCOS) is the one of the most common hormonal disorders in women of reproductive age [1–3]. It has been proposed that insulin resistance may have a pivotal role in the development of PCOS; nonetheless, the pathophysiology of this disease has not been wholly elucidated [4]. This syndrome is associated with a higher incidence of sub-clinical chronic inflammation, obesity, and related disorders including insulin resistance, metabolic syndrome, diabetes mellitus, hyperlipidaemia, and cardiovascular risk [2, 5–6]. Hyperinsulinaemia and decreasing insulin sensitivity usually contribute to metabolic disturbances in PCOS [2]. Insulin induces androgen production in the cells and suppresses the sex hormone binding globulin in liver; thus, the concentrations of more potent forms of plasma-free androgens increase [6]. Moreover, excess adipose tissue due to hyperinsulinaemia may be due to a more potent testosterone from androstenedione based on the enhanced activity of 17 β hydroxy steroid dehydrogenase activity in adipose tissue [6].

In obesity, inflammatory cytokines are abundantly expressed by enlarged adipocytes and activated macrophages in adipose tissue. Some of these inflammatory cytokines, including interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF-α), can directly decrease insulin sensitivity by inhibiting insulin's peripheral actions. Nevertheless, several adipocytokines such as adiponectin and visfatin produced from adipose tissue have shown insulin-sensitising activity [7]. Lipocalin-2 is an adipocytokine. This protein is expressed in tissues exposed to microorganisms (respiratory and alimentary tract, genitourinary tract) and endothelial cells, vascular smooth muscle cells, hepatocytes, endometrial cells, and splenic cells [7, 8]. Lipocalin-2 binds to bacterial iron-laden siderophores to inhibit bacterial growth [9]. Several studies have been published reporting either increased, decreased, or unchanged lipocalin levels in both PCOS and other insulin resistance-related disorders, but the potential mechanism underlying these inconsistent results has not been wholly elucidated [3, 6–8, 10–14].

In recent studies it has been suggested that androgens and sexual dimorphism may contribute to lipocalin-2 levels [15–18]. Here, we studied lipocalin-2 levels in women with both PCOS and BMI-matched controls and measured potential confounders associated with lipocalin levels in PCOS.

Material and methods

Study design

The current study was performed according to the terms of the World Medical Association Declaration of Helsinki. This study was conducted in the outpatient gynaecology clinic of Manisa Sarigol Hospital, Manisa, Turkey and was performed with approval of the Ethics Committee. A concise and clear summary of the study protocol was given to each volunteer; all participants provided written informed consent. Women with PCOS and healthy women in whom age and body mass index (BMI) were matched were consecutively included in the study. All recruited women were Caucasian.

PCOS group

In the absence of other hyperandrogenic conditions, a diagnosis of PCOS was based on at least two of the following criteria [19]: 1) oligo- or anovulatory menstrual disturbance as represented by infrequent bleeding at intervals > 35 days [19]; 2) Clinical and/or biochemical signs of hyperandrogenism. Clinical hyperandrogenism was defined by a Ferriman-Gallwey score of at least 8, while biochemical hyperandrogenism was defined as elevated free T levels (> 0.034 nmol/L) [19–21]; 3) The existence of ultrasonographic findings of polycystic ovarian morphology (with one ovary being sufficient for diagnosis) as defined as the presence of 12 or more follicles measuring 2–9 mm in diameter and/or an ovarian volume of greater than 10 mL (without a cyst or dominant follicle) in each ovary [19]. Women with PCOS did not receive any medication.

Control group

Women admitted to the hospital for routine examinations or symptoms of dysmenorrhoea, urinary incontinence, and premenstrual syndrome were enrolled in the control group. The menstrual cycle periods were between 26 and 32 days, and there were no clinical or biochemical findings of hyperandrogenism.

We excluded: pregnant women, women with hyperprolactinaemia, breastfeeding women, Cushing syndrome, congenital adrenal hyperplasia and other diseases of the adrenal gland, thyroid disorders, impaired glucose tolerance, type 1 or type 2 diabetes mellitus, hepatic disease, as well as women receiving hormonal contraceptive methods, insulin sensitisers, oral antidiabetics, or insulin.

Anthropometric evaluation

Patient height (centimetres) and weight (kilogram) were measured using standard measures with bare feet and daily light clothes. The body mass index (BMI) was calculated using the formula: weight (kg)/square of height (m^2). The body fat percentage was measured in all women using the bioelectrical impedance analysis method (Tanita Body Fat Analyzer -TBF-401, Tanita Co, Tokyo, Japan).

Laboratory studies

All blood samples were obtained after an overnight fast and during the early follicular phase (between the third and fifth days) of a spontaneous menstrual cycle. Serum fasting glucose concentrations were measured by a glucose oxidase method (BT products, Izmir, Turkey), and the intra-assay coefficient of variation (CV) was 1.1%; inter-assay CV was 2.5%. Serum fasting insulin concentrations were measured with an electrochemiluminescence immunoassay (Roche Diagnostics GmbH, Mannhein, Germany) with an intra-assay CV of 1.9% and inter-assay CV of 2%. The serum-free testosterone concentrations were measured by enzyme immunoassay method (IBL International, Hamburg, Germany), and the intra-assay CV was 3.3% with an inter-assay CV of 5.46%. The serum high-sensitivity C-reactive protein (hs-CRP) concentrations were measured with an ELISA technique using an appropriate kit (Circulex, USA), and the intra-assay CV was 3.8% with an inter-assay CV of 5.2%. Serum lipocalin-2 concentrations were measured by enzyme-linked immunosorbent assay (ELISA) kit (RayBiotech Human Lipocalin-2 ELISA kit, RayBiotech Inc, Norcross,GA, USA) based on sandwich enzyme immunoassay technique according to the manufacturer's recommendations. The intra-assay CV was < 10%, while the inter-assay CV was < 12%. The homoeostasis model assessment of insulin resistance (HOMA-IR) index was calculated as follows: HOMA-IR = fasting insulin (μU/mL) × fasting glucose (mg/dL)/405 [22].

Statistical analysis

The normality of the data was tested using the Kolmogorov-Smirnov test, and all continuous variables showed normal distribution ($p > 0.05$). The power analysis was performed using G* Power software 3.0.10 for Windows (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany). Continuous variables were presented as mean ± standard deviation. Demographic and laboratory characteristics of the subjects with and without PCOS were compared using an independent sample t-test (two-tailed). Pearson's correlation analyses of the relationship between the variables were examined with each other. A multiple linear regression model was established with independent variables that could affect the level of lipocalin-2. Statistical analyses were performed with Statistical Package for Social Sciences (SPSS) version 13.0. (SPSS Inc., Chicago, IL, USA). Statistical significance was defined as $p < 0.05$.

Results

Power analysis calculations based on a pilot study of circulating lipocalin-2 levels showed that the required size of the study population was 32 subjects per group ($\alpha = 0.05$ and the study power = 0.90).

Forty-four women with PCOS and forty-seven age- and BMI-matched healthy controls were recruited. The clinical and laboratory characteristics of the women included in the study are shown in Table I. Among women with PCOS and healthy women, there was no significant difference between age ($p = 0.708$), weight ($p = 0.541$), height ($p = 0.318$), and BMI ($p = 0.823$). However, the body fat percentage (36.861 ± 8.215% *vs.* 31.448 ± 5.791%, $p = 0.02$) and Ferriman-Galleway Score (11.07 ± 3.59 *vs.* 5.71 ± 2.86, $p = 0.01$) in the PCOS group were significantly higher than the control group. There was no significant difference in serum fasting

Table I. ***Comparison of demographic and laboratory characteristics of the women with PCOS and the control group without PCOS***

Tabela I. ***Porównanie danych demograficznych i parametrów laboratoryjnych w grupie kobiet z PCOS i w grupie kontrolnej***

	PCOS	Control	p[a]
	(n = 44)	(n = 47)	
Age (years)	24.41 ± 4.8	24.83 ± 4.47	NS
Height [cm]	159.76 ± 5.51	158.49 ± 5.03	NS
Weight [kg]	65.09 ± 9.307	64.37 ± 7.479	NS
BMI [kg/m^2]	25.76 ± 6.41	24.97 ± 2.55	NS
Body Fat Percentage	36.861 ± 8.215	31.448 ± 5.791	0.02
Ferriman-Gallwey Score	11.07 ± 3.59	5.71 ± 2.86	0.01
Serum fasting glucose [mg/L]	880.2 ± 75.5	871.5 ± 46.6	NS
Serum fasting insulin [pmol/L]	99.732 ± 55.49	60.491 ± 38.892	0.02
HOMA -IR	3.283 ± 2.05	1.885 ± 1.04	0.014
Free Testosterone [nmol/L]	0.285 ± 0.122	0.133 ± 0.029	0.002
hs-CRP [mg/L]	3.26 ± 1.35	1.36 ± 0.59	0.03
Lipocalin-2 [ng/mL]	55.74 ± 17.54	36.46 ± 19.62	0.011

Results shown as mean ± SD. BMI — body mass index; HOMA-IR — homoeostasis model assessment of insulin resistance; hs-CRP — high sensitivity C-reactive protein; PCOS — polycystic ovary syndrome; [a]Independent samples t-test was used. $P < 0.05$ (significant difference)

Table II. *Results of Pearson's correlation analysis*
Tabela II. *Wyniki analizy korelacji Pearsona*

		Lipocalin-2	hs-CRP	Free Testosterone	BMI	Body Fat percentage
Glucose	r	–0.178	0.019	0.039	0.413	0.338
	p	0.143	0.877	0.752	< 0.001	0.04
Insulin	r	0.122	–0.002	0.125	0.374	0.336
	p	0.317	0.99	0.304	0.002	0.005
HOMA-IR	r	0.088	0.007	0.151	0.434	0.374
	p	0.472	0.957	0.217	< 0.001	0.002
hs-CRP	r	0.179		0.1	0.252	0.368
	p	0.141	Not applicable	0.416	0.037	0.002
Lipocalin-2	r		0.179	0.338	0.116	0.166
	p	Not applicable	0.141	0.004	0.343	0.124
Free Testosterone	r	0.338	0.1		0.099	0.022
	p	0.004	0.416	Not applicable	0.419	0.859
BMI	r	0.116	0.252	0.099		0.908
	p	0.343	0.037	0.419	Not applicable	<0.001
Body Fat Percentage	r	0.166	0.368	0.022	0.908	
	p	0.124	0.002	0.859	< 0.001	Not applicable

Pearson's correlation analysis was used. r: Pearson correlation coefficient. P < 0.05 (significant difference)

serum glucose between the two groups (p > 0.05). Serum fasting insulin (99.732 ± 55.49 pmol/L *vs.* 60.491 ± ± 38.892 pmol/L, p = 0.02), free testosterone (0.285 ± ± 0.122 nmol/L *vs.* 0.133 ± 0.029 pmol/L, p = 0.002), hs-CRP (3.26 ± 1.35 mg/L *vs.* 1.36 ± 0.59 mg/L, p = 0.03), and lipocalin-2 (55.74 ± 17.54 ng/mL *vs.* 36.46 ± 19.62 ng/mL, p = 0.011) concentrations and HOMA-IR (3.283 ± 2.05 *vs.* 1.885 ± 1.04, p = 0.014) in women with PCOS were significantly higher than in healthy women.

The results of Pearson's correlation analysis are shown in Table II. Lipocalin-2 showed a significant positive correlation with the free testosterone (r = 0.338, p = 0.004). There was no significant correlation between lipocalin-2 and body fat percentage (p = 0.124), hs-CRP (p = 0.141), and HOMA-IR (p = 0.472).

A linear regression model was established using independent variables that can influence the levels of lipocalin-2 such as hs-CRP, free testosterone, HOMA-IR, and body fat percentage. According to this model, only free testosterone was associated with lipocalin-2 (β = 0.660, p = 0.007). The "lipocalin-2 = 11.214 + (1.943 × free-testosterone)" equation was determined (Table III).

Discussion

While serum lipocalin concentrations were elevated in women with PCOS, lipocalin 2 was not correlated with HOMA-IR, hs-CRP, and body fat percentage. Only free testosterone was associated with lipocalin-2.

It is still unclear whether circulating lipocalin-2 levels show differences according to insulin resistance or obesity-related disorders [8]. It has been reported that lipocalin-2 levels are elevated in patients with type 2 diabetes and obese animals. In addition, the expression of lipocalin-2 was augmented by some agents that decrease insulin sensitivity, including TNF-α and corticosteroids [8]. Another study demonstrated that circulating lipocalin-2 was associated with waist circumference, body fat percentage, chronic inflammatory markers, fasting glucose, and insulin [7, 8]. After adjusting the BMI, serum lipocalin-2 correlated with glucose, HOMA-IR, and hs-CRP. It has been suggested that lipocalin-2 is an independent risk factor in insulin resistance and diabetes [23]. Conversely, Zhang et al. reported that increased lipocalin-2 levels may have exhibited anti-inflammatory effects. Hence, lipocalin-2 has a protective mechanism against inflammation in obesity and insulin resistance [14].

In a recent study, De la Chesnaye et al. reported that plasma lipocalin-2 concentrations were significantly reduced in Mexican patients with long-term type 2 diabetes mellitus versus healthy individuals [24]. Furthermore, Wallenius et al. reported that lipocalin-2 was correlated with IL-6, but not associated with insulin sensitivity as determined via a euglycaemic hyperinsulinaemic clamp in healthy men. This suggests that lipocalin-2 reflects inflammation, but not insulin sensitivity [25]. The features of insulin resistance increased in lipocalin-2 knock-out mice [26].

Table III. *Evaluation of the effects of lipocalin-2 on hs-CRP, free testosterone, HOMA-IR, and body fat percentage by using multiple linear regression analysis method*

Tabela III. *Ocena wpływu stężenia lipokaliny-2 na stężenia hs-CRP i testosteronu wolnego oraz wartość wskaźnika HOMA-IR i procentowej zawartości tkanki tłuszczowej w organizmie przy użyciu analitycznego modelu regresji liniowej wielokrotnej*

	β	95% CI		P
		Lower	Upper	
Variables				
(Constant)	11.214	–2.465	24.893	0.321
hs-CRP	0.743	–0.351	1.837	0.541
Body fat percentage	1.265	–0.214	2.744	0.326
HOMA-IR	0.873	–0.126	1.872	0.537
Free-testosterone	1.943	0.125	3.761	0.006*

Multiple linear regression analysis was used. β — Unstandardised regression coefficient; CI — Confidence interval; A P value of < 0.05 was considered significant (*). According to this model, the "lipocalin-2 = 11.214 + (1.943 × free-testosterone)" equation was determined

Subclinical inflammation is a key mediator of obesity-associated metabolic disorders. Choi et al. found that lipocalin-2 concentrations did not differ between obese and normal weight women. Exercise training and weight loss did not affect lipocalin-2 or the well-established inflammatory marker hs-CRP [27]. Due to its autocrine or paracrine effects, lipocalin-2 antagonised the inflammatory effects of TNF-α in macrophages [8, 14]. Moghadasi et al. reported that there was no significant relationship between hs-CRP and plasma lipocalin-2 in men [28]. According to a linear regression model in our study, we showed no association between lipocalin-2 and hs-CRP.

Likewise, studies of other obesity or insulin resistance-related metabolic disorders show divergent results between lipocalin-2 and PCOS. Gencer et al. found that lipocalin-2 concentrations were significantly lower in women with PCOS. Moreover, lipocalin-2 was not associated with cardiovascular risk indicators as defined by echocardiographic examination [10]. Diamanti-Kandarakis et al. demonstrated that circulating lipocalin-2 levels were significantly lower in women with PCOS and were not associated with obesity, insulin resistance, and estradiol concentrations [3]. Although women with PCOS have numerous risk factors that can cause cardiovascular disease, it was reported that the incidence of cardiovascular events was not elevated in PCOS [29]. Therefore, the results of Diamanti-Kandarakis highlighted that lipocalin-2 is related to cardiovascular events rather than atherosclerotic heart disease; lipocalin-2 may have a protective role in PCOS [3, 10].

On the other hand, several studies showed no significant differences in lipocalin-2 levels between women complicated with PCOS and healthy controls [6,11]. However, Cakal et al.reported that serum lipocalin-2 concentrations significantly increased in women with PCOS versus age- and BMI-matched controls. Furthermore, lipocalin-2 was correlated with HOMA-IR, testosterone, and $DHEASO_4$. It has been suggested that lipocalin-2 can reflect insulin resistance in PCOS [12]. We found high lipocalin-2 levels in women with PCOS and did not find any significant association between lipocalin-2 and cardiovascular marker hs-CRP or HOMA-IR. There were no significant differences in circulating lipocalin-2 levels in overweight women versus lean women in both PCOS and healthy controls [10]. In our study, lipocalin-2 levels and body fat percentages were higher in PCOS than in BMI-matched controls — still lipocalin-2 was not associated with body fat percentage.

Several studies have suggested that there are potential interconnections between lipocalin-2 and androgens [15–18, 30–31]. The presence of the putative androgen response elements in the promoter regions of lipocalin-encoding genes was described in rat studies as well as the regulatory effects of androgens on lipocalin-2 expression [18, 30]. Furthermore, lipocalin-2 influences the aromatase enzyme activity, which has been defined as a key enzyme of conversion of androgens to oestrogen in adipose tissue and granulosa cells [15, 16, 18]. In women with PCOS versus BMI matched controls, Martinez-Garcia et al. demonstrated that lipocalin-2 expression increased in adipose tissue but not in skeletal muscle. Men were included in this study, and lipocalin-2 production in women with PCOS showed masculine patterns. Therefore, the authors concluded that differences in hormonal function of adipose tissue between men and women may depend on androgen concentrations because the production of several adipocytokines in women with complicated PCOS shows masculine patterns [18]. Lipocalin-2 is an adipokine that is mostly produced by adipose tissue [8, 12]. In our study, only free testosterone was associated with lipocalin-2, although the body fat percentage increased in PCOS. We obtained the "lipocalin-2 = 11.214+(1.943 × free-testosterone)" equation.

There were some limitations to our study. First, although the power analysis suggested that 32 subjects offered 90% power, this number is relatively small. Second, if serum levels of other adipocytokines (adiponectin, vaspin, and omentin) and androgens ($DHEAS0_4$ and androstenedione) were measured, we could glean more information about the relationship between PCOS and lipocalin-2. Finally, insulin resistance should have been evaluated with more complicated clamp techniques.

Conclusions

In conclusion, serum lipocalin-2 concentrations were higher in women with PCOS. Only free testosterone was associated with lipocalin-2. Lipocalin-2 levels and its influencing factors exhibited discrepant results in both PCOS and other obesity- or insulin-resistance-related metabolic disorders; therefore, the potential role of lipocalin-2 in PCOS needs to be clarified.

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References

1. Asunción M, Calvo RM, San Millán JL, et al. A prospective study of the prevalence of the polycystic ovary syndrome in unselected Caucasian women from Spain. J Clin Endocrinol Metab. 2000; 85(7): 2434–2438, doi: 10.1210/jcem.85.7.6682, indexed in Pubmed: 10902790.
2. Kozakowski J, Jeske W, Zgliczyński W, et al. Body composition, glucose metabolism markers and serum androgens - association in women with polycystic ovary syndrome. Endokrynol Pol. 2013; 64(2): 94–100, indexed in Pubmed: 23653271.
3. Diamanti-Kandarakis E, Livadas S, Kandarakis SA, et al. Serum concentrations of atherogenic proteins neutrophil gelatinase-associated lipocalin and its complex with matrix metalloproteinase-9 are significantly lower in women with polycystic ovary syndrome: hint of a protective mechanism? Eur J Endocrinol. 2008; 158(4): 525–531, doi: 10.1530/EJE-07-0822, indexed in Pubmed: 18362300.
4. Apridonidze T, Essah PA, Iuorno MJ, et al. Prevalence and characteristics of the metabolic syndrome in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2005; 90(4): 1929–1935, doi: 10.1210/jc.2004-1045, indexed in Pubmed: 15623819.
5. Kruszyńska A, Słowińska-Srzednicka J, Jeske W, et al. Proinsulin, adiponectin and hsCRP in reproductive age women with polycystic ovary syndrome (PCOS)--the effect of metformin treatment. Endokrynol Pol. 2014; 65(1): 2–10, doi: 10.5603/EP.2014.0001, indexed in Pubmed: 24549596.
6. Panidis D, Tziomalos K, Koiou E, et al. The effects of obesity and polycystic ovary syndrome on serum lipocalin-2 levels: a cross-sectional study. Reprod Biol Endocrinol. 2010; 8: 151, doi: 10.1186/1477-7827-8-151, indexed in Pubmed: 21143924.
7. Wang Yu, Lam KSL, Kraegen EW, et al. Lipocalin-2 is an inflammatory marker closely associated with obesity, insulin resistance, and hyperglycemia in humans. Clin Chem. 2007; 53(1): 34–41, doi: 10.1373/clinchem.2006.075614, indexed in Pubmed: 17040956.
8. Esteve E, Ricart W, Fernández-Real JM. Adipocytokines and insulin resistance: the possible role of lipocalin-2, retinol binding protein-4, and adiponectin. Diabetes Care. 2009; 32 Suppl 2: S362–S367, doi: 10.2337/dc09-S340, indexed in Pubmed: 19875582.
9. van Dam RM, Hu FB. Lipocalins and insulin resistance: etiological role of retinol-binding protein 4 and lipocalin-2? Clin Chem. 2007; 53(1): 5–7, doi: 10.1373/clinchem.2006.080432, indexed in Pubmed: 17202496.
10. Gencer M, Gazi E, Hacıvelioğlu S, et al. The relationship between subclinical cardiovascular disease and lipocalin-2 levels in women with PCOS. Eur J Obstet Gynecol Reprod Biol. 2014; 181: 99–103, doi: 10.1016/j.ejogrb.2014.07.032, indexed in Pubmed: 25145761.
11. Koiou E, Tziomalos K, Katsikis I, et al. Weight loss significantly reduces serum lipocalin-2 levels in overweight and obese women with polycystic ovary syndrome. Gynecol Endocrinol. 2012; 28(1): 20–24, doi: 10.3109/09513590.2011.588745, indexed in Pubmed: 21756081.
12. Cakal E, Ozkaya M, Engin-Ustun Y, et al. Serum lipocalin-2 as an insulin resistance marker in patients with polycystic ovary syndrome. J Endocrinol Invest. 2011; 34(2): 97–100, doi: 10.3275/7077, indexed in Pubmed: 20511727.
13. Stejskal D, Karpísek M, Humenanska V, et al. Lipocalin-2: development, analytical characterization, and clinical testing of a new ELISA. Horm Metab Res. 2008; 40(6): 381–385, doi: 10.1055/s-2008-1062746, indexed in Pubmed: 18393169.
14. Zhang J, Wu Y, Zhang Y, et al. The role of lipocalin 2 in the regulation of inflammation in adipocytes and macrophages. Mol Endocrinol. 2008; 22(6): 1416–1426, doi: 10.1210/me.2007-0420, indexed in Pubmed: 18292240.
15. Guo H, Zhang Y, Brockman DA, et al. Lipocalin 2 deficiency alters estradiol production and estrogen receptor signaling in female mice. Endocrinology. 2012; 153(3): 1183–1193, doi: 10.1210/en.2011-1642, indexed in Pubmed: 22234464.
16. Fried SK, Greenberg AS. Lipocalin 2: a "sexy" adipokine that regulates 17 -estradiol and obesity. Endocrinology. 2012; 153(4): 1582–1584, doi: 10.1210/en.2012-1012, indexed in Pubmed: 22408176.
17. Luque-Ramírez M, Martínez-García MÁ, Montes-Nieto R, et al. Sexual dimorphism in adipose tissue function as evidenced by circulating adipokine concentrations in the fasting state and after an oral glucose challenge. Hum Reprod. 2013; 28(7): 1908–1918, doi: 10.1093/humrep/det097, indexed in Pubmed: 23559188.
18. Martínez-García MÁ, Montes-Nieto R, Fernández-Durán E, et al. Evidence for masculinization of adipokine gene expression in visceral and subcutaneous adipose tissue of obese women with polycystic ovary syndrome (PCOS). J Clin Endocrinol Metab. 2013; 98(2): E388–E396, doi: 10.1210/jc.2012-3414, indexed in Pubmed: 23337724.
19. Legro RS, Arslanian SA, Ehrmann DA, et al. Endocrine Society. Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2013; 98(12): 4565–4592, doi: 10.1210/jc.2013-2350, indexed in Pubmed: 24151290.
20. Lauritsen MP, Bentzen JG, Pinborg A, et al. The prevalence of polycystic ovary syndrome in a normal population according to the Rotterdam criteria versus revised criteria including anti-Mullerian hormone. Hum Reprod. 2014; 29(4): 791–801, doi: 10.1093/humrep/det469, indexed in Pubmed: 24435776.
21. Ferriman D, Gallwey JD. Clinical assessment of body hair growth in women. J Clin Endocrinol Metab. 1961; 21: 1440–1447, doi: 10.1210/jcem-21-11-1440, indexed in Pubmed: 13892577.
22. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and ?-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985; 28(7): 412–419, doi: 10.1007/bf00280883.
23. Jang Y, Lee JHo, Wang Yu, et al. Emerging clinical and experimental evidence for the role of lipocalin-2 in metabolic syndrome. Clin Exp Pharmacol Physiol. 2012; 39(2): 194–199, doi: 10.1111/j.1440-1681.2011.05557.x, indexed in Pubmed: 21689137.
24. De la Chesnaye E, Manuel-Apolinar L, Zarate A, et al. Lipocalin-2 plasmatic levels are reduced in patients with long-term type 2 diabetes mellitus. Int J Clin Exp Med. 2015; 8(2): 2853–2859, indexed in Pubmed: 25932245.
25. Wallenius V, Elias E, Bergstrom GML, et al. The lipocalins retinol-binding protein-4, lipocalin-2 and lipocalin-type prostaglandin D2-synthase correlate with markers of inflammatory activity, alcohol intake and blood lipids, but not with insulin sensitivity in metabolically healthy 58-year-old Swedish men. Exp Clin Endocrinol Diabetes. 2011; 119(2): 75–80, doi: 10.1055/s-0030-1265212, indexed in Pubmed: 21104585.
26. Guo H, Jin D, Zhang Y, et al. Lipocalin-2 deficiency impairs thermogenesis and potentiates diet-induced insulin resistance in mice. Diabetes. 2010; 59(6): 1376–1385, doi: 10.2337/db09-1735, indexed in Pubmed: 20332347.
27. Choi KM, Kim TN, Yoo HJ, et al. Effect of exercise training on A-FABP, lipocalin-2 and RBP4 levels in obese women. Clin Endocrinol (Oxf). 2009; 70(4): 569–574, doi: 10.1111/j.1365-2265.2008.03374.x, indexed in Pubmed: 18710473.
28. Moghadasi M, Mohammadi Domieh A. Effects of Resistance versus Endurance Training on Plasma Lipocalin-2 in Young Men. Asian J Sports Med. 2014; 5(2): 108–114, indexed in Pubmed: 25834704.
29. Wild S, Pierpoint T, McKeigue P, et al. Cardiovascular disease in women with polycystic ovary syndrome at long-term follow-up: a retrospective cohort study. Clinical Endocrinology. 2000; 52(5): 595–600, doi: 10.1046/j.1365-2265.2000.01000.x.
30. Nantermet PV, Xu J, Yu Y, et al. Identification of genetic pathways activated by the androgen receptor during the induction of proliferation in the ventral prostate gland. J Biol Chem. 2004; 279(2): 1310–1322, doi: 10.1074/jbc.M310206200, indexed in Pubmed: 14576152.
31. Asirvatham AJ, Schmidt M, Gao B, et al. Androgens regulate the immune/inflammatory response and cell survival pathways in rat ventral prostate epithelial cells. Endocrinology. 2006; 147(1): 257–271, doi: 10.1210/en.2005-0942, indexed in Pubmed: 16195407.