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ORIGINAL INVESTIGATION

Hormonal and adiposity state of women with polycystic ovary syndrome: implication of adiponectin and leptin

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Abstract

Obesity and insulin resistance are frequently seen comorbidities in patients with polycystic ovary syndrome (PCOS), affecting the already disturbed metabolism of these patients. Disturbed secretion of adiponectin and leptin could be one of the contributing factors of obesity and insulin resistance in patients with PCOS. The aim of this study was to determine the levels of adiponectin and leptin in PCOS patients, as well as their association with other components of the syndrome. This cross-sectional study determined clinical, hormonal, and biochemical markers in 61 women with PCOS and 56 controls. There was a statistically significant difference in adiponectin and leptin between the groups ($p>0.001$). There was a significant negative correlation between adiponectin, body mass index (BMI), and waist circumference ($r= -0.478$; -0.452, $p<0.001$) and a negative correlation with testosterone, free androgen index (FAI), insulin, and the homeostasis model assessment for insulin resistance (HOMA-IR). A positive correlation between adiponectin, sex hormone binding globulin (SHGB), and fasting glucose levels was present. Correlation analysis of leptin with other metabolic parameters showed a positive correlation with BMI, waist circumference, insulin, and HOMA-IR. A significant inverse correlation was present between leptin and SHGB. In conclusion, adiponectin and leptin may serve as potential biomarkers of insulin resistance. Determining levels of adiponectin and leptin in the early course of this syndrome may enable earlier diagnosis of insulin resistance, or even early prevention in PCOS patients.

Key words: adiponectin, leptin, polycystic ovary syndrome, obesity, insulin resistance

1. Introduction

Presently, obesity is a major problem around the globe primarily because of its associated conditions like cardiovascular disease, metabolic disturbances, type 2 diabetes, and certain types of cancer. In 2008, global reports stated that one in every 3 adults was overweight and 1 in every 9 was obese. Beyond this global average, at least 1 in 5 women were obese in 117 countries and at least 1 in 5 men were obese in 73 countries. Notably, the prevalence of obesity has accelerated in the last decade compared to the 1980s and 1990s [1]. Obesity is a multifactorial disease caused by chronic energy imbalance. When the energy input surpasses the output, there is an increase in adipose tissue. Storing energy is one of the biological imperatives of life of almost all animal species. Lipids are the primary source of stored energy in mammals, and the main storage location is the white adipose tissue. When circulating and stored carbohydrates cannot meet the body's energy needs, fatty acids in the white adipose tissue are mobilised through the process of lipolysis, a breakdown of triglycerides to glycerol and free fatty acids [2]. White adipose tissue is composed of adipocytes, i.e. stroma which is composed of preadipocytes, macrophages, endothelial cells, leukocytes and fibroblasts. In the last decade it has become clear that adipose tissue is an organ with an active function in the metabolic processes that maintain energy homeostasis. The production of the adipokines is influenced by food intake and the degree of alimentation. Some hormones that are products of adipose tissue like leptin, visfatin, resistin, apelin and adiponectin, have a major role in the regulation of weight. The central effects of these adipokines are expressed through the regulation of appetite and energy output. Also, they affect the insulin sensitivity of tissues, utilizing the stored lipids and oxidative capacity [3]. Obesity impairs this balance through several direct and indirect mechanisms. It has been proven that adipose tissue impairs the secretion and the bio avidity of sex hormones. Indirectly, its effects are demonstrated through insulin and the adipokines leptin, adiponectin, resistin, and omentin [4]. In 2002, Staiger et al. explained the relationship between adipokines and adipose tissue with the modulation of insulin sensitivity. He explained that the production of adiponectin is regulated by intra-abdominal visceral fat tissue and that it is inversely correlated with serum insulin [5]. One of the reproductive dysfunctions, which are most often related to obesity, is the polycystic ovarian syndrome (PCOS). PCOS is a heterogeneous endocrine disorder whose pathogenesis has not been fully explained yet. It is globally present in 7-10% of women of reproductive age. Obesity is a health problem with a growing prevalence and women with PCOS are subject to a higher risk of weight gain and obesity compared to the rest of the population [6]. Approximately 50-70% of women with PCOS have an altered level of insulin resistance and hyperinsulinemia [7], and they have a higher risk of developing dyslipidemia, cardiovascular disorders, as well as type 2 diabetes [8]. Complex hormonal mechanisms work to support the reproductive system in order to maintain control over the menstrual period, ovulation, and the development of the endometrium. Insulin resistance (IR) affects the metabolic changes but the causes of insulin resistance have not been explained yet [9]. Insulin resistance along with hyperinsulinemia plays a major role in the initiation of hyperandrogenism by increasing the biosynthesis of androgenic hormones in the ovaries. In the long run, insulin resistance with hyperinsulinemia increases the risk of metabolic disorders such as impaired glucose tolerance, type 2 diabetes, and cardiovascular disorders [10,11]. Two studies by Bulent et al. and Panidis et al. have illustrated that in PCOS, the hyperinsulinemia, dyslipidemia, and/or hypertension are significantly correlated with obesity [9,12]. Some of the adipokines produced by adipose tissue also demonstrate a close correlation with the presence of insulin resistance in obese patients. In 2012, Spanos et al. demonstrated that overweight patients have impaired adipokine production, which he related with insulin resistance, hyperandrogenism and PCOS [13].

Leptin is a cytokine that is produced by the white adipose tissue. It is a protein composed of 167 amino acids and its molecular mass is 16kDa. It is a key hormone that participates in maintaining energy homeostasis and weight through the limitation of food intake and an increase in energy output. This protein is coded by the ob gene and is produced in mature and differentiated adipocytes. Leptin regulates food intake and energy output by binding to the long form of the leptin receptor (Ob-Rb) in the hypothalamus, providing information about the quantity of stored energy [14]. Several studies indicate that this hormone is involved in several different physiological processes, such as metabolic control, control of growth, reproduction, puberty, hematopoiesis, angiogenesis, and blood pressure regulation. The study by Carmina E. et al. 2005 and Chakrabarti J. 2013, demonstrates that in PCOS patients there is a positive

correlation between leptin and body weight, expressed through BMI, as well as with insulin sensitivity, and that the correlation between leptin and insulin resistance directly depends on body weight [15,16]. Adiponectin is a protein composed of 244 amino acids, with a molecular mass of 30 kDa. Adiponectin is synthesized and secreted only by the adipose tissue. Its concentration in the blood ranges from 5 to 30 mg/ml and about 0.01% of the total plasma proteins belong to it. Adiponectin is a hydrophilic protein composed of a carboxy-terminal globular domain, variable region and collagen domain, and amino-terminal domain. A decrease in adiponectin concentration is associated with dyslipidemia and atherosclerosis. The most potent form, with the largest part in metabolic processes, is the adiponectin high molecular weight complex (HMW) [17]. There is a strong correlation between decreased total adiponectin concentrations and type 2 diabetes, and what is significant here is that there is a decrease in only HMW while the concentration of the other multimeric forms remains unchanged. The latest studies have shown that in PCOS patients, reduced levels of adiponectin correlate with the degree of obesity [12,18]. On the contrary, Spranger J et al. 2004 illustrates that PCOS is not associated with reduced levels of adiponectin, but that adiponectin has an independent association with obesity and insulin resistance both in healthy control groups and in women with PCOS [19.] The exact mechanism through which adiponectin improves insulin sensitivity should be further examined. Adiponectin levels have been shown to have a negative correlation with the individual features of PCOS, including luteinizing hormone (LH) concentrations, testosterone levels, and a positive correlation with sex hormone binding globulin (SHGB) [20,21]. Since adiponectin and leptin levels may influence metabolic disturbances, the aim of this study was to determine the levels of these adipokines in women with and without PCOS and to evaluate the possible relationship between these adipokines and anthropometric indices, BMI, fasting insulin, and homeostatic insulin resistance index (HOMA–IR), as well as other components of the syndrome.

Materials and methods

This cross-sectional study was conducted at the department of Clinical biochemistry at the University Clinic of Gynecology and Obstetrics in Skopje, Republic of Macedonia, in the period between October 2013 and September 2015. This study included 61 premenopausal women, ages 18 to 40 years with PCOS and a control group of 56 normo-ovulatory women with regular menstrual cycles, without clinical or biochemical signs of hyperandrogenism and no prior known endocrine diseases. The diagnosis of PCOS was made based on the 2003 Rotterdam criteria, when at least 2 of the 3 characteristics were present: irregular menstrual cycles –oligo (six or fewer menstrual periods per year), or amenorrhea (the cessation of menses for longer than 6 months), clinical and/or biochemical confirmation of hyperandrogenism, and/or polycystic ovaries on ultrasound (defined as the presence of 10 or more follicles 2-9 mm and/or increased ovarian volume >10mm) [22]. All participants answered a questionnaire that included age, marital status, lifestyle habits, and use of vitamin supplements. Patients with abnormal levels of prolactin, thyroid hormones, renal or hepatic dysfunction, type 1 or type 2 diabetes mellitus, congenital adrenal hyperplasia, or a history of cardiovascular disease were excluded. All patients were informed about the study protocol and gave written informed consent.

Clinical assessment and anthropometric parameters including body weight, height, and waist and hip circumference were recorded for all patients. A transvaginal ultrasound scan of the ovaries was performed using a 6 MHz 3-D transducer in order to determine the number of antral follicles. Following a 12-hour overnight fast, blood samples were taken from subjects in the follicular phase of the menstrual cycles or at any given day in patients with absent menstrual cycles in the previous two or more months. Direct in- vivo methods for measuring insulin sensitivity are rather complex. Static models of fasting glucose and insulin measurements correlate highly with dynamic measurements by glucose loading [23]. The presence of IR was investigated using basal fasting insulin concentrations, fasting glucose concentrations and the homeostasis model assessment (HOMA-IR). The homeostasis model assessment for insulin resistance (HOMA-IR) was calculated using the equation: fasting plasma insulin μU/ml × glucose (mmol/L/22.5) [24]. Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), estradiol (E2), insulin (INS) total testosterone (TTES), dehydroepiandrosterone sulfate (DHEA-S), androstenedione (AND), thyroid-stimulating hormone (TSH), and sex hormone binding globulin (SHGB) were measured by chemiluminescent immunometric assay on Siemens, Immulite 2000 HP automated immunoassay analyzer. Fasting levels of plasma glucose were measured immediately after venipuncture using the

enzymatic glucose oxidase method on an automated analyzer, Roche, Cobas Integra 400 plus. Total leptin levels were determined by IBL Leptin enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle, using commercially available kits. Total adiponectin levels were measured by an ELISA competitive enzyme immunoassay for quantitative measurement of human adiponectin, using commercially available kits (BV51001 Human Adiponectin, IBL International, Hamburg, Germany). The free androgen index was calculated according to the standard equation: testosterone (nmol/l) x 100/SHBG (nmol/l).

All statistical procedures were performed using the Statistical Package for the Social Sciences (SPSS version 17.0 for Windows, SPSS Inc.). Results were expressed as mean ± S.D and range. Normality of distribution was evaluated with one- sample Kolmogorov–Smirnoff test. To determine the relationship between analyzed variables, Spearman's correlation test was used. The clinical and laboratory characteristics in the groups were compared by analysis of variance (ANOVA test). Means between every group of every parameter were compared with post hoc ANOVA. Bivariate correlation analysis (calculation of the Pearson coefficient) was used to explore the possible correlation of adiponectin to each parameter. Statistical significance was set at P-value <0.05.

Table 1. Clinical and anthropometric characteristics of PCOS patients and controls

	PCOS (n = 61)	Control (n = 56)	P
Age (years)	24.15±3.8	25.9±4.98	ns
BMI (kg/m2)	27.8±6.3	24.73±5,86	0.004
Waist (cm)	96.11±15.4	87.56±16,86	0.001
Hip (cm)	109.5±1.6	106.86±16,58	ns
WHR	0.84±0.17	0.81±0.06	ns
FSH (mIU/l)	5.57±1.2	7.15±1.0	0.010
LH (mIU/l)	11.81±4.6	4.63±1.3	0.010
LH/FSH	2.16±0.8	0.74±0.74	0.010
Prolactin (ng/ml)	11.43±3.8	13.11±4.1	ns
Estradiol (pmol/l)	57.31±13.9	45.70±12.2	ns
TSH (mIU/ml)	2.56±2.41	2.21±0.79	ns
DHEAS (µg/ml)	3.52±2.66	2.12±0.76	0.010
Testosterone (nmol/l)	2.23±0.8	0.89±0.3	0.010
Androstendione (ng/ml)	4.83±1.38	2.25±0.74	0.010
Free androgen index	5.15±2.7	1.70±0.9	0.010
SHBG (nmol/l)	36.5 ±24.5	52.15±20.7	0.019
Fasting glucose (mmol/l)	5.2±0.45	5.07±0.33	0.010
Fasting insulin (mIU/l)	16.97±16.7	6.99±4.39	0.001
HOMA-IR	3.38±3.08	1.6±1.09	0.001
Adiponectin (ng/ml)	11.75±4.96	16.02±7.1	0.001
Leptin (ng/ml)	9.16±7.15	3.66±6.80	0.001

FSH - Follicle-stimulating hormone, LH - luteinizing hormone, TSH - thyroid-stimulating hormone, FAI - free androgen index, SHGB - sex hormone binding globulin, HOMA-IR - homeostasis model assessment for insulin resistance, ns - not significant

Results

From a total of 117 individuals who participated in this study, 61 were diagnosed with PCOS according to the Rotterdam diagnostic criteria and 56 served as controls with normal hormonal status and ovulation. Both cases and controls were similar in age (24.2 ± 3.8 versus 25.1 ± 4.5 years). There was no significant difference between single and married woman. The majority of participants were non-smokers (n= 84, 71.8%, p=0.001), and the majority didn't have any sports activity (n=96, 82.1%, p< 0.001). Characteristics of the studied population are presented in Table 1. As expected, statistically significant differences among cases and controls were observed for levels of FSH, LH, and the LH/FSH ratio (p<0.01). Biochemical markers of hyperandrogenemia were also markedly higher as follow: DHEAS, testosterone, and androstenedione (p < 0.01). SHGB was statistically lower and the calculated free androgen index (FAI) was higher in women with PCOS (p<0.01). All women with PCOS had a higher BMI compared with the control group (27.8 ± 6.3 versus 24.7 ± 5.9, p<0.01). Waist circumference in women with PCOS was significantly higher. There was no statistically significant difference in hip circumference and waist to hip ratio (WHR) between the two groups even though PCOS patients had a higher waist circumference than the control group. Fasting glucose levels were significantly elevated in women with PCOS (p<0.01). Fasting insulin, and HOMA–IR were also statistically significantly higher in PCOS patients (p<0.001). Adiponectin levels were statistically significantly decreased in woman with PCOS compared to controls (p<0.001) (Figure 1). A statistically significant difference was found between the group of women with PCOS and the control group in terms of elevated leptin levels (p<0.001) (Table 1, Figure 1).

The Pearson correlation coefficient test was used to determine correlations between serum leptin and adiponectin levels and anthropometric characteristics, as well as biochemical variables in the PCOS group. A statistically significant negative correlation was found between adiponectin and BMI as well as with waist circumference (r= -0.478, p<0.001; -0.452, p<0.001). A statistically significant positive correlation was found among adiponectin and LH as well as the LH/ FSH ratio. Adiponectin levels inversely correlated with testosterone, FAI, insulin, and HOMA–IR. A positive correlation was found among adiponectin and SHGB and fasting glucose levels (Table 2).

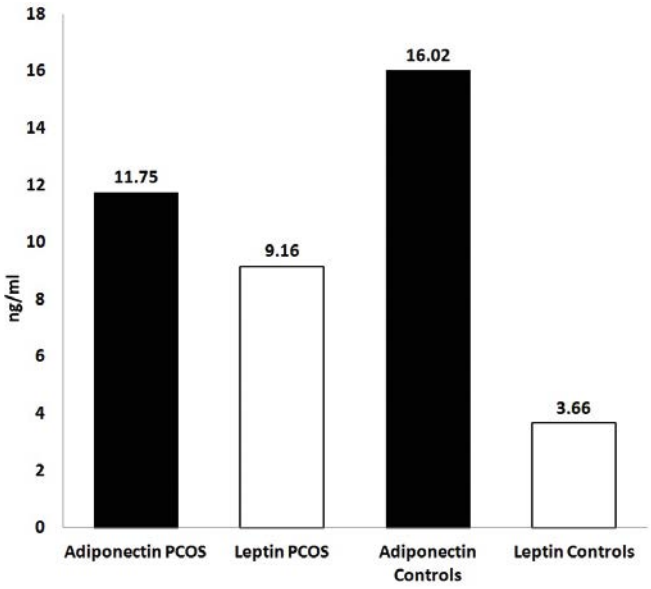

Figure 1. Adiponectin and leptin levels in PCOS patients and controls

Correlation analysis of leptin with other metabolic parameters showed a strong positive correlation with BMI, waist circumference, insulin, and HOMA-IR. There was no statistically significant positive correlation between leptin and fasting glucose levels. A statistically significant inverse correlation was present between leptin and SHGB, but not with other hormonal variables (Table 2).

Discussion

Adipose tissue disorders have raised a lot of scientific interest primarily because of the increasing global obesity epidemic [1]. In this manner, some studies have considered insulin resistance a key of adipose tissue disorders. PCOS is a multifactorial disease, with a complex pathophysiological mechanism involving several metabolic abnormalities. Multiple metabolic disturbances like hyperinsulinemia and insulin resistance characterize PCOS, which can lead to an increased risk of diabetes mellitus, dyslipidemia, and atherosclerosis [7,8]. PCOS is the most common cause of anovulation and impaired carbohydrate metabolism in woman of reproductive age. According to the WHO, the prevalence of impaired glucose tolerance in women with PCOS is 20-40%, compared to 5% in the control population, and approximately 50% of PCOS cases present with insulin resistance [25]. In addition to insulin resistance and

Table 2. Correlation coefficients between serum adiponectin, leptin and other clinical parameters

Correlation coefficients	Adiponectin		Leptin	
	r	P	r	P
Age (years)	- 0.185	0.150	- 0.185	0.150
BMI (kg/m2)	- 0.478	<0.001	0.439	<0.001
Waist	-0.452	<0.001	0.446	<0.001
FSH (mIU/l)	-0.038	0.930	-0.008	0.93
LH (mIU/l)	0.351	0.001	-0.092	0.246
LH/FSH ratio	0.34	0.001	-0.080	0.275
DHEA-S (µg/ml)	- 0.047	0.670	0.195	0.079
Testosterone (nmol/l)	- 0.226	0.040	-0.097	0.228
FAI	- 0.479	<0.001	0.137	0.152
SHBG (nmol/l)	0.362	0.005	-0.224	0.046
Fasting glucose (mmol/l)	0.289	0.024	0.136	0.149
Fasting insulin (mIU/l)	- 0.231	0.037	0.331	0.005
HOMA-IR	-0.229	0.038	0.421	0.001

FSH - Follicle-stimulating hormone, LH - luteinizing hormone, FAI - free androgen index, SHGB - sex hormone binding globulin, HOMA-IR - homeostasis model assessment for insulin resistance

central obesity, which are among the most intriguing characteristic of PCOS, secreted hormones from adipose tissue- adipokines have attracted attention. Adiponectin is possibly the most interesting adipocytokine primarily released from adipose tissue. Adiponectin has well-established anti-atherogenic, anti-inflammatory and insulin sensitizing features. Adiponectin levels are markedly decreased in obese patients when compared with normal weight patients. Furthermore, adiponectin levels are inversely correlated with the degree of insulin resistance [14,19,20]. Taking into account obesity and insulin resistance, the role of adipokines in the pathogenesis of PCOS has been assessed in our study. Patients that were included in this study were divided into two groups according to the Rotterdam diagnostic criteria for PCOS [22]. The two groups were evaluated for specific features that could have an impact on the laboratory results. There were no differences in age, life habits and level of physical activity between the groups. There were differences in BMI and waist circumference among the studied group of women.

In the first part of the study we evaluated levels of adiponectin and leptin. Recent literature regarding adiponectin levels in PCOS women has been controversial. It has been suggested that obesity, hyperinsulinemia, insulin resistance, and hyperandrogenism may explain hypoadiponectinemia in women with PCOS [13]. Adiponectin improves beta cell dysfunction and fatty acid beta-oxidation, thereby maintaining insulin sensitivity, and lower levels of adiponectin are associated

with the development of type 2 diabetes mellitus [14]. PCOS patients with higher insulin resistance, calculated with the HOMA-IR index, have altered adiponectin and leptin secretion. Panidis et al. observed lower adiponectin levels among insulin resistant PCOS patients [12]. Pangaribuan et al. studied differences in adiponectin levels among normal weight and obese/overweight PCOS women, demonstrating that adiponectin levels are even lower in obese/overweight PCOS women than in normal weight PCOS women [20]. Our findings support this study and we found that adiponectin levels were lower among women with PCOS compared to controls. In our study, we did not evaluate differences in adiponectin levels in PCOS patients according to their BMI, but we found a strong statistical correlation between adiponectin and anthropometric characteristics among all subjects with PCOS.

Leptin is another cytokine primarily synthesized by adipocytes. Because of the pleotropic nature of leptin and its close connection to the physiological control of metabolism and reproduction, even indirect dysregulation of leptin secretion may have pathophysiological significance in PCOS. Chakrabarti at al. demonstrated that irrespective of BMI or insulin resistance, PCOS patients have higher leptin levels [16]. In our study, we observed similar results finding statistically higher leptin levels in PCOS women. In essence, this study demonstrated that women with PCOS have lower levels of adiponectin, while leptin levels and insulin resistance are increased.

The second part of this study evaluated the correlation between adiponectin, leptin, insulin resistance (HOMA-IR), BMI, and hormone levels. We found a significant statistical correlation between the two studied adipokines and insulin resistance. Adiponectin had a statistically significant negative, and leptin a statistically significant positive correlation with fasting insulin, insulin resistance and BMI. Adipocytes are considered to be endocrine cells that synthesize and release molecules that play an endocrine/paracrine role in reproduction influencing the hypothalamic–pituitary axis [26,27]. Serum leptin is related to estrogens, progesterone, androgens, and insulin. Their role in the regulation of circulating leptin levels and its relationship to reproductive hormones is still unclear. Olszanecka-Glinianowicz et al. found a positive correlation between circulating adiponectin levels and plasma FSH and LH concentrations, postulating the hypothesis that adipokines in PCOS women may participate in the dysregulation of the pituitary–ovarian axis [21]. We only found a statistically strong positive correlation between adiponectin levels, LH and the LH/FSH ratio. As previously described, leptin acts at a central level, stimulating the gonadotrophic axis [26]. Previous studies reported negative correlation between leptin levels and LH in PCOS women even after adjustment for BMI [28]. We did not establish such a correlation, which may be due to a smaller sample. Biochemical hyperandrogenism is one of the main metabolic disturbances in PCOS. Hyperinsulinemia is associated with androgen excess and a depressed level of SHBG and insulin resistance may have an important role in the etiology of PCOS and hyperandrogenism observed in these patients. Different results have been published regarding the relationship between hyperandrogenemia and adipokines [13,16,20,21]. Our study supports the findings that have shown a correlation between adiponectin and leptin with markers of hyperandrogenemia. We demonstrated a significant inverse correlation between adiponectin and testosterone, FAI, and a positive correlation with SHBG. In our study, leptin levels had an inverse correlation with SHGB levels.

In conclusion, we demonstrated that in PCOS patients, adiponectin levels are decreased and leptin levels are increased, independent of BMI. We found a negative correlation between adiponectin and insulin resistance, and a positive correlation between leptin and insulin resistance in PCOS women. We suggest that impaired adipokine secretion is associated with PCOS pathogenesis, and can influence the development of insulin resistance. We support the importance of adiponectin and leptin as biomarkers of the degree of insulin resistance. Maintaining an equilibrium of adipokine secretion may represent a key strategy for the prevention of metabolic comorbidities of PCOS. It is suggested that the early regulation of serum adiponectin levels in obese subjects with PCOS by treating of obesity, especially in the young, can lower the risk of many comorbidities associated with PCOS.

Authors' contributions

AAB gave the idea of this study and for the article, wrote the paper, participated in drafting the article and gave her final approval. SJM and DPI participated in data acquisition and gave their final approval. BZI performed statistical analyses and critically revised the manuscript, gave suggestions regarding data analysis and gave her final approval.

References

1. Stevens GA, Singh GM, Lu Y, Danaei G, Lin JK, Finucane MM, et al. National, regional, and global trends in adult overweight and obesity prevalences. Popul Health Metr 2012;10:22.
https://doi.org/10.1186/1478-7954-10-22

2. Bowers RR, Festuccia WTL, Song CK, Shi H, Migliorini RH, Bartness TJ. Sympathetic innervation of white adipose tissue and its regulation of fat cell number. Am J Physiol - Regul Integr Comp Physiol 2004;286:R1167–75.
https://doi.org/10.1152/ajpregu.00558.2003

3. Leal Vde O, Mafra D. Adipokines in obesity. Clinica Chimica Acta 2013;419:87-94.
https://doi.org/10.1016/j.cca.2013.02.003

4. Brewer CJ, Balen AH. The adverse effects of obesity on conception and implantation. Reproduction 2010;140:347–64.
https://doi.org/10.1530/REP-09-0568

5. Staiger H, Tschritter O, Machann J, Thamer C, Fritsche A, Maerker E, et al. Relationship of serum adiponectin and leptin concentrations with body fat distribution in humans. Obesity Research 2003;11:368–72.
https://doi.org/10.1038/oby.2003.48

6. 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. J Clin Endocrinol Metab 2004;89:2745–9.
https://doi.org/10.1210/jc.2003-032046

7. Chen X, Jia X, Qiao J, Guan Y, Kang J. Adipokines in reproductive function - a link between obesity and polycystic ovary syndrome. J Mol Endocrinol 2013;50:R21-37.
https://doi.org/10.1530/JME-12-0247

8. Ovalle F, Ricardo Azziz. Insulin resistance, polycystic ovary syndrome, and type 2 diabetes mellitus. Fertility and Sterility 2002;77:1095–105.
https://doi.org/10.1016/S0015-0282(02)03111-4

9. Yildiz BO, Knochenhauer ES, Azziz R. Impact of Obesity on the Risk for Polycystic Ovary Syndrome. J Clin Endocrinol Metab. 2008;93:162–8.
https://doi.org/10.1210/jc.2007-1834

10. Teede H, Deeks A, Moran L. Polycystic ovary syndrome: a complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan. BMC Med. 2010;8:41.
https://doi.org/10.1186/1741-7015-8-41

11. Wild AR, Carmina E, Diamanti-kandarakis E, Dokas A, Ecobar-Morreale HF, Futterweit W, et al. Assessment of cardiovascular risk and prevention of cardiovascular disease in women with the polycystic ovary syndrome: A consensus statement by the androgen excess and polycystic ovary syndrome (AE-PCOS) society. J Clin Endocrinol Metab 2010;95:2038–49.
https://doi.org/10.1210/jc.2009-2724

12. Panidis D, Kourtis A, Farmakiotis D, Mouslech T, Rousso D, Koliakos G. Serum adiponectin levels in women with polycystic ovary syndrome. Hum Reprod 2003;18: 1790–96.
https://doi.org/10.1093/humrep/deg353

13. Spanos N, Tziomalos K, Macut D, Koiou E, Kandaraki EA, Delkos D, et al. Adipokines, insulin resistance and hyperandrogenemia in obese patients with polycystic ovary syndrome: cross-sectional correlations and the effects of weight loss. Obes Facts 2012;5:495–504.
https://doi.org/10.1159/000341579

14. Bastard JP, Maachi M, Lagathu C, Kim MJ, Caron M, Vidal H, et al. Recent advances in the relationship between obesity, inflammation, and insulin resistance. Eur Cytokine Netw 2006;17:4–12.

15. Carmina EN, Orio F, Palomba S, Cascella T, Longo RA, Colao AM, et al. Evidence for altered adipocyte function in polycystic ovary syndrome. European Journal of Endocrinology 2005;152:389–94.
https://doi.org/10.1530/eje.1.01868

16. Chakrabarti J. Serum leptin level in women with polycystic ovary syndrome: correlation with adiposity, insulin, and circulating testosterone. Ann Med Health Sci Res 2013;3:191–6.
https://doi.org/10.4103/2141-9248.113660

17. Schraw T, Wang ZV, Halberg N, Hawkins M, Scherer PE. Plasma adiponectin complexes have distinct biochemical characteristics. Endocrinology 2008;149:2270–82.
https://doi.org/10.1210/en.2007-1561

18. Orio F, Palomba S, Cascella T, Milan G, Mioni R, Pagano C, et al. Adiponectin levels in women with polycystic ovary syndrome. J Clin Endocrinol Metab 2003;88:2619–23.
https://doi.org/10.1210/jc.2002-022033

19. Spranger J, Kroke A, Möhlig M, Bergmann MM, Ristow M, Boeing H, et al. Adiponectin and protection against type 2 diabetes mellitus. The Lancet 2003;361:226–8.
https://doi.org/10.1016/S0140-6736(03)12255-6

20. Pangaribuan B, Yusuf I, Mansyur M, Wijaya A. Serum adiponectin and resistin in relation to insulin resistance and markers of hyperandrogenism in lean and obese women with polycystic ovary syndrome. Ther Adv Endocrinol Metab 2011;2:235–45.
https://doi.org/10.1177/2042018811423770

21. Olszanecka-Glinianowicz M, Kuglin D, Dąbkowska-Huć A, Skałba P. Serum adiponectin and resistin in relation to insulin resistance and markers of hyperandrogenism in lean and obese women with polycystic ovary syndrome. Eur J Obstet Gynecol Reprod Biol 2011;154:51–6.
https://doi.org/10.1016/j.ejogrb.2010.08.022

22. The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2004;19:41-47.
https://doi.org/10.1093/humrep/deh098

23. Patarrão RS, Lautt WW, Macedo MP, Patarrão RS, Lautt WW, Macedo MP. Assessment of methods and indexes of insulin sensitivity. Rev Port Cardiol 2014;09:65–73.
https://doi.org/10.1016/j.rpedm.2013.10.004

24. Katsuki A, Sumida Y, Gabazza EC, Murashima S, Furuta M, Araki-Sasaki R, et al. Homeostasis model assessment is a reliable indicator of insulin resistance during follow-up of patients with type 2 diabetes. Diabetes Care 2001;24:362–5.
https://doi.org/10.2337/diacare.24.2.362

25. Baldani DP, Skrgatic L, Ougouag R. Polycystic Ovary Syndrome: Important Underrecognised Cardiometabolic Risk Factor in Reproductive-Age Women. Int J Endocrinol 2015;2015:786362.
https://doi.org/10.1155/2015/786362

26. Vázquez MJ, Romero-Ruiz A, Tena-Sempere M. Roles of leptin in reproduction, pregnancy and polycystic ovary syndrome: consensus knowledge and recent developments. Metabolism 2015;64:79–91.
https://doi.org/10.1016/j.metabol.2014.10.013

27. Chen CI, Hsu MI, Lin SH, Chang YCI, Hsu CS, Tzeng CR. Adiponectin and leptin in overweight/obese and lean women with polycystic ovary syndrome. Gynecol Endocrinol 2015;31:264–8.
https://doi.org/10.3109/09513590.2014.984676

28. 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. Hum Reprod 2001;16:1340–6.
https://doi.org/10.1093/humrep/16.7.1340