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The adiponectin-to-leptin ratio in women with polycystic ovary syndrome: relation to insulin resistance and proinflammatory markers

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Abstract

Central adiposity plays an important role in the insulin resistance of the polycystic ovary syndrome (PCOS) through the dysregulated production of various adipokines. Polycystic ovary syndrome has also been described as a low-grade inflammation state characterized by elevated levels of C-reactive protein (CRP). Furthermore, CRP is a strong independent predictor of the metabolic syndrome and cardiovascular disease. Recently, the adiponectin-to-leptin (A/L) ratio has been proposed as a potential atherogenic index in obese type 2 diabetic patients. The aim of this study was to evaluate the potential role of the A/L ratio in the metabolic and proinflammatory phenotype of PCOS. We studied 74 Greek women with PCOS (38 normal-weight and 36 overweight-obese women). The A/L ratio was negatively correlated with BMI ($r = -0.79$ $P < .001$), homeostasis model assessment ($r = -0.642$, $P < .001$), triglycerides ($r = -0.67$, $P < .001$), and total cholesterol ($r = -0.38$, $P < .01$), and positively correlated with high-density lipoprotein cholesterol ($r = 0.38$, $P < .01$) and sex hormone–binding globulin ($r = 0.39$, $P = .001$). After controlling for BMI, the A/L ratio was independently associated with insulin resistance indexes and triglycerides. Furthermore, the A/L ratio was negatively correlated with CRP ($r = -0.746$, $P < .0001$). Multiple regression analysis revealed that BMI and the A/L ratio were the only independent significant determinants of CRP ($\beta = .436$, $P = .003$ and $\beta = -.398$, $P = .007$, respectively). Studying normal-weight and overweight-obese women separately, we found an independent association between the A/L ratio and CRP in both groups ($\beta = -.460$, $P = .009$ in normal-weight women and $\beta = -.570$, $P = .001$ in overweight-obese women). In conclusion, the A/L ratio may serve as a biomarker of both insulin resistance and low-grade inflammation, providing the link between these cardiovascular risk factors in women with PCOS.

1. Introduction

Polycystic ovary syndrome (PCOS) is characterized by chronic anovulation and hyperandrogenism but also shares components of the metabolic syndrome (MS) manifested by abdominal obesity, insulin resistance, dyslipidemia, and endothelial dysfunction [1,2]. Central adiposity appears to play an important role in the metabolic phenotype through the production of various adipocyte-derived cytokines and proteins known as adipokines [3]. Furthermore, PCOS has been described as a state of chronic low-grade inflammation, mainly characterized by a modest rise in serum C-reactive protein (CRP) compared to that in weight-matched controls [4-6]. Beyond being a marker of inflammation, CRP has also prognostic value in the MS and is considered an independent predictor of cardiovascular disease in both men and women [7,8].

Much attention has been directed at 2 major adipocyte-derived cytokines, leptin and adiponectin, thought to be involved in the regulation of metabolic homeostasis [3]. The way that leptin and adiponectin regulate metabolic homeostasis is different in some ways and complementary in others. Leptin exerts central regulation affecting food intake and energy expenditure, while both leptin and adiponectin have a peripheral regulatory role increasing tissue fat oxidation and thereby insulin sensitivity [9-11]. Serum leptin concentrations are significantly elevated proportionally to the degree of adiposity, whereas adiponectin levels are paradoxically reduced in individuals with obesity and insulin resistance [12,13]. In addition, leptin appears to have proinflammatory properties [14], whereas adiponectin is considered an anti-

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inflammatory cytokine [15]. As a result, the adiponectin-to-leptin (A/L) ratio has been evaluated and proposed as a potential index linking central obesity with its metabolic and inflammatory comorbidities. More precisely, this ratio has been considered as a marker for insulin resistance and atherosclerosis in obese type 2 diabetic patients as well as in subjects without hyperglycemia [16-19]. The ratio has also been suggested to predict insulin sensitivity and potential cardiovascular risk in HIV-infected patients [20].

Although there is some discrepancy in literature, most studies agree that serum leptin concentrations in women with PCOS are similar to those in weight-matched controls and that leptin levels are related to obesity [21-26]. On the other hand, serum adiponectin levels have been reported to be lower in women with PCOS compared to controls [27-32].

The purpose of this study was to assess the possible association between the A/L ratio and markers of insulin resistance as well as inflammation among women with PCOS, and to investigate whether this association is affected by the degree of adiposity or other metabolic variables characterizing PCOS.

2. Subjects and methods

2.1. Subjects

The study population consisted of 74 Greek women (aged 16-37 years; mean age, 22.6 ± 5.8 years) with PCOS. Diagnosis of PCOS was based on the criteria proposed by the 1990 National Institutes of Health-National Institute of Child Health and Human Development conference on PCOS. These criteria are ovulatory dysfunction, clinical evidence of hyperandrogenism, and/or hyperandrogenemia, and exclusion of related disorders such as congenital adrenal hyperplasia, hyperprolactinemia, or Cushing syndrome [33]. Hyperandrogenism was defined by the clinical presence of hirsutism (Ferriman-Gallwey score >8), acne, or alopecia, and/or elevated androgen levels. Menstrual dysfunction was defined by the presence of oligomenorrhea or amenorrhea. In those patients who were on medication, treatment was discontinued at least 6 months before their inclusion in the study. None of the patients had clinical evidence of recent or acute infection.

All women with PCOS were studied in the early follicular phase (days 3-5). The BMI of each patient was calculated as the weight in kilograms divided by the square of height in meters. Blood pressure was measured twice in the right arm in a sitting position after 5 minutes resting using a mercury sphygmomanometer, and the average of the 2 measurements was used in the analysis. Blood samples were drawn after overnight fasting for the measurement of adiponectin, leptin and CRP levels, fasting serum glucose and insulin, lipid profile, serum gonadotropins (luteinizing hormone, follicle-stimulating hormone), total testosterone, and sex hormone–binding globulin (SHBG). The free androgen index (FAI) was calculated using the formula: [total testosterone (nmol/L)/SHBG (nmol/L)] × 100. Insulin resistance was assessed by the homeostasis model assessment (HOMA) using a computerized calculator found on www.dtu.ox.ac.uk/homa. The study protocol was approved by the hospital ethics committee, and all subjects studied gave their informed consent.

2.2. Hormonal assays

A morning (7:00-9:00 AM) fasting venous blood sample was collected. Serum specimens were separated by centrifugation and stored at −70°C. Serum adiponectin levels were determined using a sensitive enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). The kit is designed to measure total (low, middle, and high molecular weight) human adiponectin levels. The intra-assay coefficients of variation (CVs) ranged from 2.5% to 4.7%, whereas the interassay CVs ranged from 5.8% to 6.5%. The minimum detectable concentration was 0.25 mg/L. Serum leptin concentrations were measured using a sensitive enzyme-linked immunosorbent assay (Active Human Leptin ELISA, DSL-10-23100, Diagnostic System, Webster, TX). The minimum detectable concentration was 0.05 μg/L. The intra- and interassay CVs were less than 4.8% and 4.3%, respectively. The measurements of CRP were performed by an ultrasensitive latex particle–enhanced immunonephelometric assay on the BN ProSpec nephelometer (Dade Behring, Liederbach, Germany). Both inter- and intra-assay CVs were less than 0.5%. The lower sensitivity of the high-sensitivity CRP (hs-CRP) assay was 0.2 mg/L. Serum glucose was determined by the hexokinase method using a glucose analyzer (Olympus 600, Clinical Chemistry Analyzer, Olympus Diagnostica, O'Callaghans Mills, County Clare, Ireland). Insulin was measured using a microparticle enzyme immunoassay on an AXSYM Immunoanalyzer (Abbott Laboratory, Abbott Park, IL). The CV of this method was 5%. The insulin assay detects human insulin with no cross-reactivity to proinsulin or C-peptide. Total testosterone was determined using a chemiluminescent microparticle immunoassay on an Abbott-ARCHITECT Immunoanalyzer (Abbott Laboratory). The CVs were 4% for total testosterone. Sex hormone–binding globulin was measured by the chemiluminescent immunometric method (IMMULITE 2000 Immunoanalyzer, Diagnostic Products Co, CA) and the CV was 5.5%. Total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglycerides were determined by enzymatic methods (Olympus 600, Clinical Chemistry Analyzer, Olympus Diagnostica). Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation: LDL = total cholesterol (mg/L) − HDL-C (mg/L) − triglycerides (mg/L)/5 [34]. The intra-assay CVs given for the various assays were based on kit package inserts and were corrected according to the laboratory experience where it was necessary.

2.3. Statistical analysis

Normal distribution of continuous parameters was tested by Kolmogorov-Smirnov test. Variables not normally

Table 1
Anthropometric and blood chemistry characteristics of women with PCOS subdivided in 2 groups: normal-weight (BMI <25 kg/m^2) and overweight-obese (BMI ≥25 kg/m^2) women with PCOS

	Total PCOS	Normal-weight women with PCOS	Overweight-obese women with PCOS	*P* value
No. of women	74	38	36	
Age (y)	22.6 ± 5.8	21 ± 4.0	24.1 ± 6.9	NS
BMI (kg/m^2)	26.6 ± 6.9	21.2 ± 1.9	31.9 ± 5.7	<.001
Systolic blood pressure (mm Hg)	116 ± 10	112 ± 8	117 ± 8	.05
Diastolic blood pressure (mm Hg)	70 ± 6	69 ± 7	71 ± 5	NS
LH/FSH	1.4 ± 1.0	1.4 ± 0.9	1.4 ± 1.1	NS
SHBG (nmol/L)	37.9 ± 26.4	46.7 ± 29.4	28.8 ± 19.4	<.001
FAI	13.7 ± 10.4	8.8 ± 7.1	18.7 ± 16.8	<.001
Total testosterone (μg/dL)	1.0 ± 0.6	0.9 ± 0.5	1,1 ± 0,6	NS
HOMA index	1.8 ± 1.3	1.1 ± 0.5	2.4 ± 1.5	<.001
Total cholesterol (mg/L)	183.3 ± 35.0	175.8 ± 30.9	189.8 ± 37.5	NS
HDL-C (mg/L)	48.8 ± 16.7	50.6 ± 10.6	47.0 ± 20.9	NS
LDL-C (mg/L)	166.2 ± 30.8	163.3 ± 29.5	168.7 ± 32.1	NS
Triglycerides (mg/L)	85.4 ± 50.0	62.3 ± 22.1	105.6 ± 58.5	<.001
CRP (mg/L)	2.1 ± 2.0	0.9 ± 0.8	3.4 ± 2.6	<.001
Adiponectin (mg/L)	11.4 ± 5.3	12.8 ± 4.7	9.9 ± 5.5	.002
Leptin (μg/L)	30.1 ± 19.3	16.9 ± 10.0	43.7 ± 17.1	<.001
Adiponectin/leptin × 10^3	994 ± 765	1253 ± 1201	263 ± 196	<.001

NS indicates nonsignificant; LH, luteinizing hormone; FSH, follicle-stimulating hormone.

distributed were logarithmically transformed before analysis and the values presented were back-transformed. Differences between 2 continuous parameters were estimated using *t* test. Associations between adipokines, CRP, and metabolic parameters were examined by Pearson correlation coefficient, and partial correlations were also performed between these parameters after adjustment for BMI and age. Stepwise multiple regression analysis was performed to identify independent predictors of CRP levels. Continuous data are expressed as the mean ± SD. *P* value of less than .05 was set as statistically significant. All analyses used the SPSS (version 12.0, SPSS, Chicago, IL).

3. Results

3.1. Characteristics of women with PCOS

The study population was subdivided into 2 groups: group 1 consisted of 38 patients with normal weight (BMI <25 kg/m^2) and group 2 of 36 overweight-obese patients (BMI ≥ 25 kg/m^2). The characteristics of women with PCOS and the comparisons between the 2 groups are presented in Table 1. Overweight-obese women with PCOS exhibited higher mean levels in parameters associated with insulin resistance and subclinical inflammation and lower mean A/L ratio compared with normal-weight women with PCOS.

3.2. Correlations between adipokines and metabolic variables in women with PCOS

Table 2 shows the correlation coefficients of the relationship between the adipokines adiponectin and leptin with metabolic variables in women with PCOS. Serum adiponectin levels were negatively correlated with BMI, HOMA index, and triglycerides, and positively correlated with SHBG and HDL-C ($P < .05$), while there was a negative correlation with FAI of borderline significance ($P = .07$). After controlling for BMI and age, adiponectin was only significantly correlated in a negative way with HOMA index ($r = -0.279$, $P = .03$) and triglycerides ($r = -0.324$, $P = .01$) and in a positive way with HDL-C levels ($r = 0.402$, $P = .002$), whereas no association was observed with other parameters. These correlations between adiponectin and HOMA index, triglycerides, and HDL-C levels were observed even when overweight-obese women with PCOS were studied separately, whereas in normal-weight patients, adiponectin was only correlated with triglycerides ($r = -0.449$, $P = .001$) and HDL-C levels ($r = 0.592$, $P = .02$).

Table 2
Correlations between adiponectin, leptin, and the A/L ratio with CRP and metabolic parameters in women with PCOS

	Adiponectin (*r*)	Leptin (*r*)	A/L ratio (*r*)
Age	−0.135	0.227	−0.237
BMI	−0.525***	0.729***	−0.799***
LH/FSH	0.119	−0.119	0.145
SHBG	0.349**	−0.308*	0.391**
FAI	−0.199	0.319*	−0.337**
Total testosterone	−0.097	0.249*	−0.241*
HOMA	−0.515***	0.517***	−0.642***
Total cholesterol	−0.135	0.416**	−0.387**
Triglycerides	−0.469***	0.493***	−0.668***
HDL-C	0.495***	−0.218	0.382**
LDL-C	−0.023	0.312*	−0.257
CRP	−0.585***	0.629***	−0.746***
Adiponectin		−0.290*	0.651***
Leptin	−0.290*		−0.915***

Correlations remaining significant after adjustment for age and BMI are underlined.
* $P < .05$.
** $P < .01$.
*** $P < .001$.

Leptin was positively correlated with BMI, HOMA index, triglycerides, total cholesterol, LDL-C, total testosterone, and FAI, and negatively correlated with SHBG, whereas there was no correlation with HDL-C. All these associations were body weight–dependent and were lost after controlling for BMI.

3.3. Associations of the A/L ratio with metabolic parameters in women with PCOS

There was an inverse correlation between serum leptin and adiponectin levels ($r = -0.290$, $P = .03$). This correlation remained significant after controlling for age, BMI, and insulin resistance indexes ($P = .02$). The A/L ratio was correlated negatively with BMI, HOMA index, triglycerides, total cholesterol, total testosterone, and FAI. On the other hand, there was a positive correlation with SHBG and HDL-C, whereas the association between the A/L ratio and LDL-C was of borderline significance ($P = .06$) (Table 2). No association was found between A/L ratio and blood pressure. Even after controlling for age and BMI, the A/L ratio was significantly correlated with HOMA index ($P = .04$), triglycerides ($P = .002$), and total cholesterol ($P = .03$). These associations were also found when normal-weight and overweight-obese women with PCOS were studied separately.

3.4. Association of the A/L ratio with proinflammatory markers in women with PCOS

Both leptin and adiponectin were significantly correlated with CRP levels in an opposite manner ($P < .001$ for all correlations). After controlling for BMI and age, the only remaining significant association of CRP was with the A/L ratio ($P = .008$) and adiponectin levels ($P = .02$). Stepwise multiple regression analysis revealed that BMI and the A/L ratio were the only independent significant determinants of CRP levels ($\beta = .436$, $P = .003$ and $\beta = -.398$, $P = .007$, respectively). Performing the stepwise multiple regression analysis without including the A/L ratio, we found adiponectin to be a strong predictor of CRP levels but with a less strong β than the A/L ratio ($\beta = -.331$).

Multiple regression analysis was performed separately in normal-weight women with PCOS and overweight-obese patients to further define the relationship between adipokines and CRP. In both groups the A/L ratio was the only independent parameter contributing significantly to CRP ($\beta = -.460$, $P = .009$ for normal-weight patients and $\beta = -.570$, $P = .001$ for overweight-obese patients).

4. Discussion

In the present study we assessed the association of the A/L ratio with metabolic and insulin resistance variables in Greek women with PCOS. In addition, we explored the relation between this ratio and CRP, a marker of systemic low-grade inflammation, and investigated whether this association is affected by metabolic variables characterizing the syndrome. Two main points have emerged from this study.

First, there was an inverse relationship between serum adiponectin and leptin concentrations, which was independent of BMI and insulin resistance indexes in our women with PCOS. This suggests that the secretion of these adipokines from adipose tissue is reciprocally regulated by some common factor or that adiponectin and leptin regulate each other in PCOS, as has been reported for obese type 2 diabetic patients [16-18].

Previous experimental and clinical studies have indicated that adiponectin is down-regulated in hypertrophic adipose tissue and that hypoadiponectinemia is a reflection of visceral adiposity [10,35] Furthermore, the degree of hypoadiponectinemia in obesity is related to insulin resistance rather than to body fat content [13]. This is consistent with adiponectin's biological effects including stimulation of fatty acid oxidation, suppression of hepatic gluconeogenesis, and inhibition of inflammatory response [10,15]. These beneficial effects are associated with insulin receptor phosphorylation, activation of intracellular adenosine monophosphate kinase, and modulation of nuclear factor–κB [36-38]. On the other hand, serum leptin levels rise in proportion to body adiposity [39,40]. Importantly, obese individuals appear to be resistant to the central hypothalamic effects of leptin [41]. Therefore, the catabolic pathways involved in the reduction of appetite and increase in energy expenditure are not activated and the excess body weight is maintained. However, there is no documented evidence for peripheral leptin resistance regarding the proinflammatory and other effects of leptin in obesity and related disorders. Although we did not directly assess body fat distribution in our women with PCOS, the analysis of the association of the 2 adipokines with insulin resistance and metabolic parameters supports previous data. Indeed, serum adiponectin levels in our patients were negatively associated with insulin resistance and proatherogenic lipid parameters independently of BMI, whereas the positive association between leptin with these parameters was lost after controlling for BMI.

The second and more important point that emerged from our analysis was the strong independent association of the A/L ratio not only with adiposity but also with insulin resistance and atherogenic lipoprotein profile in women with PCOS. This association was observed in both normal-weight and overweight-obese women with PCOS. This is in accordance with previous studies showing that this ratio is a sensitive and reliable marker of insulin resistance, as well as a potential atherogenic index in patients with type 2 diabetes mellitus [16-18]. The A/L ratio has also been suggested to predict insulin sensitivity and cardiovascular risk in HIV-infected patients [20].

Furthermore, the A/L ratio was found to be highly predictive of hs-CRP levels in women with PCOS, and this relation was superior to the association between leptin or adiponectin alone with this inflammatory marker. Although BMI was also a strong predictor of CRP in our patients, the

fact that the association of the A/L ratio with CRP was confirmed even when normal-weight women with PCOS were studied separately shows that the link between the A/L ratio and CRP is independent of body weight. Previous studies have pointed out the independent association between leptin or adiponectin alone with CRP in healthy humans and patients with type 2 diabetes mellitus [42-49]. The novel finding of the present study is an even stronger association between the A/L ratio with hs-CRP levels in women with PCOS leading to the conclusion that this ratio could also serve as a marker of inflammation.

The independent association between the A/L ratio and CRP suggests that both adipokines may directly influence CRP production and inflammatory processes in PCOS. It has been shown that these adipokines may influence CRP by influencing the production and action of the proinflammatory cytokines tumor necrosis factor α and interleukin 6 in opposite ways [50,51]. In addition, leptin receptors have been found to have signaling capabilities of IL-6–type cytokine receptors, and thus, leptin, acting through its receptors in the liver, may directly induce CRP production [52]. In this context, hyperleptinemia has been associated with the development of steatohepatitis in patients with MS [52]. On the other hand, a recent report revealed that CRP messenger RNA is also expressed in human adipose tissue and its levels are inversely correlated with adiponectin messenger RNA levels, leading to the conclusion that the expression of adiponectin and CRP is reciprocally regulated in adipose tissue [44].

It is also known that both adipokines may play a role in the regulation of inflammatory processes in the vascular endothelium. Adiponectin suppresses the expression of the adhesion molecules intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and P selectin, inhibiting monocyte adherence to endothelial cells, and also protects against oxidative stress [53,54]. Leptin, on the other hand, promotes vascular proliferation and calcification and increases oxidative stress [53]. The independent association of the A/L ratio with CRP could provide an additional mechanism in this proatherogenic process because CRP is not merely a marker of inflammation but may be directly involved in vascular atherogenesis. Indeed, CRP has been shown to promote the secretion of inflammatory mediators in the vascular endothelium, and also to increase cell adhesion molecule expression, and decrease endothelial nitric oxide synthase expression and activity [55-57]. The present study does not support that this association may be specific to PCOS, but it may be more prominent in this condition because women with PCOS present characteristics of the MS.

As a conclusion, this study supports the importance of the A/L ratio as a biomarker of insulin resistance and systemic low-grade inflammation in women with PCOS. Taking into consideration that both of these conditions are implicated in the development of atherosclerosis and cardiovascular disease, the A/L ratio could also serve as a risk marker for coronary artery disease. Therefore, modulating the balance of these adipokines may represent a novel therapeutic strategy aiming in the prevention of the metabolic comorbidities of PCOS.

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