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ScienceDirect
Metabolism Clinical and Experimental 60 (2011) 366–372
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Total and high–molecular weight adiponectin in women with the polycystic ovary syndrome

Edmond P. Wickham III [a,b,*], Kai I. Cheang[c], John N. Clore[a], Jean-Patrice Baillargeon[d], John E. Nestler[a,e]

[a]Department of Internal Medicine, Virginia Commonwealth University, PO Box 980111, Richmond, VA 23298-0111, USA
[b]Department of Pediatrics, Virginia Commonwealth University, PO Box 980111, Richmond, VA 23298-0111, USA
[c]Department of Pharmacy, Virginia Commonwealth University, PO Box 980111, Richmond, VA 23298-0111, USA
[d]Department of Medicine, University of Sherbrooke, Sherbrooke J1H 5N4, Canada.
[e]Department of Obstetrics and Gynecology, Virginia Commonwealth University, PO Box 980111, Richmond, VA 23298-0111, USA

Received 26 May 2009; accepted 23 February 2010

Abstract

Adiponectin, an adipokine with antidiabetic properties, forms multimers; and the high–molecular weight (HMW) form most closely correlates with insulin sensitivity (S_i). Therefore, we hypothesize that HMW adiponectin levels are decreased in women with polycystic ovary syndrome (PCOS), a condition characterized by insulin resistance, compared with healthy controls and that HMW adiponectin correlates with testosterone and S_i. A cross-sectional study involving 13 women with PCOS and 13 age- and body mass index–matched healthy controls was performed. Waist-to-hip ratios (WHRs), glucose, insulin, sex hormone–binding globulin, total testosterone, and total and HMW adiponectin levels were measured after an overnight fast. Free testosterone was calculated from sex hormone–binding globulin and total testosterone, and S_i was determined using a frequently sampled intravenous glucose tolerance test. The study's primary outcomes were differences in total and HMW adiponectin between women with PCOS and healthy control women. Total adiponectin ($P < .01$), HMW adiponectin ($P < .01$), and the ratio of HMW to total adiponectin ($P = .03$) were lower in women with PCOS compared with healthy women. Total and HMW adiponectin levels correlated inversely with WHR ($P < .01$) and free testosterone ($P < .01$) and positively with S_i ($P < .001$). Using forward stepwise multivariate analysis, HMW adiponectin and WHR, but not PCOS status, were independent predictors of S_i. Women with PCOS have lower total and HMW adiponectin levels compared with healthy women. High–molecular weight adiponectin also comprises a smaller proportion of total circulating adiponectin in women with PCOS. Alterations in HMW adiponectin levels in women with PCOS may contribute to the insulin resistance intrinsic to the syndrome.
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1. Introduction

The polycystic ovary syndrome (PCOS) is characterized by chronic anovulation and hyperandrogenism and affects an estimated 6% to 10% of women of reproductive age [1]. Although much remains unknown regarding the pathophysiology of PCOS, insulin resistance appears to play a central role in the syndrome's development. Lean women with PCOS appear to have a form of insulin resistance intrinsic to the syndrome [2]. In addition to this intrinsic insulin resistance, obese women with PCOS also demonstrate the burden of insulin resistance associated with excess adiposity [2]. In women with PCOS, insulin resistance is manifest clinically in increased incidence of glucose intolerance and overt type 2 diabetes mellitus [1].

Adipose tissue is an active endocrine organ, releasing a variety of bioactive peptides and adipokines that modulate the body's metabolism at local and systemic levels [3]. One specific adipokine, adiponectin, has a strong inverse relationship with obesity and insulin resistance and may have unique antidiabetic, anti-inflammatory, and antiatherogenic properties [3,4]. Not only are adiponectin levels decreased in patients with impaired glucose tolerance (IGT)

Approved by the institutional review board at Virginia Commonwealth University.

* Corresponding author. Division of Endocrinology and Metabolism, Virginia Commonwealth University, PO Box 980111, Richmond, VA 23298-0111, USA. Tel.: +1 804 828 9696; fax: +1 804 828 8389.
E-mail address: ewickham@mcvh-vcu.edu (E.P. Wickham).

0026-0495/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2010.02.019

and type 2 diabetes mellitus, but hypoadiponectinemia also independently predicts the future development of type 2 diabetes mellitus in healthy individuals [4].

In regard to alterations in adiponectin levels among women with PCOS, studies have reported conflicting results, with some groups documenting decreased levels of adiponectin in PCOS women compared with weight- and body mass index (BMI)–matched controls [5-10], but other studies showing no difference after controlling for obesity [11-13]. Even among studies demonstrating lower adiponectin levels in PCOS women, the relationships among adiponectin and central obesity, insulin resistance, and testosterone remain unclear [5,7,9,10,14].

Adiponectin forms multimers through disulfide bonds and exists in several oligomeric forms in serum: low–molecular weight complexes composed of trimers, hexamers, and high–molecular weight (HMW) multimers consisting of 12 to 18 subunits [3]. Increasing evidence suggests that the various isoforms of adiponectin have different biologic activities, with the ratio of HMW adiponectin to total adiponectin, not the absolute serum adiponectin level, most closely correlating with measures of insulin sensitivity (S_i) [3,15]. Furthermore, in high-risk adults, decreased HMW adiponectin at baseline is a stronger risk factor for progression to type 2 diabetes mellitus than total adiponectin level [16].

Differences in adiponectin multimers, as opposed to total adiponectin levels, may explain the apparent contradictions between adiponectin levels and anthropometric and biochemical characteristics reported in previous studies of women with PCOS. Testosterone selectively inhibits the in vitro secretion of HMW adiponectin by adipocytes [17], supporting the hypothesis that HMW adiponectin may be decreased in women with PCOS, a syndrome characterized by hyperandrogenism.

We hypothesized that women with PCOS would demonstrate lower HMW adiponectin levels than healthy women after controlling for obesity and that HMW adiponectin levels would correlate inversely with insulin resistance and serum testosterone levels. To test this hypothesis, we compared HMW adiponectin levels in women with PCOS with those of age- and BMI-matched healthy women. In addition, we investigated the relationship between adiponectin multimers and insulin resistance in women with and without PCOS.

2. Materials and methods

Twenty-six women (13 PCOS women and 13 healthy women) enrolled in a cross-sectional study previously performed at Virginia Commonwealth University [18] were matched for BMI and age using multivariate minimum distance matching with the nearest neighbor approach. All women were between 18 and 40 years of age and had BMIs not exceeding 40 kg/m^2. None of the women had diabetes mellitus or received oral contraceptives or other medications known to affect S_i for at least 2 months before study participation. *Polycystic ovary syndrome* was defined according to the 1990 National Institute of Child Health and Human Development conference criteria: oligomenorrhea (≤8 menstrual periods in the previous year); hyperandrogenism (elevated serum total or free testosterone concentration); and exclusion of secondary causes of ovulatory dysfunction or hyperandrogenism [19]. Specifically, serum prolactin, thyroid function tests, and 17α-hydroxyprogesterone levels were obtained to exclude hyperprolactinemia, thyroid dysfunction, and nonclassic adrenal hyperplasia, respectively. Women in the PCOS group with IGT were not excluded because IGT is a common comorbidity in PCOS. Healthy women did not have clinical evidence of hyperandrogenism; had regular menstrual cycles, normal androgen levels, and normal glucose tolerance; and did not have a history of gestational diabetes or a first-degree relative with diabetes. The study was approved by the institutional review board at Virginia Commonwealth University, and each subject gave written informed consent.

Subjects were admitted to the General Clinical Research Center after a 12-hour overnight fast. The PCOS women were studied during the equivalent of the follicular phase of the menstrual cycle as documented by a serum progesterone not exceeding 6 nmol/L. Healthy women were studied during the midfollicular phase of menstrual cycles (days 5-9), which most closely approximates the hormonal milieu of anovulatory women with PCOS. Height and weight were measured to the nearest 0.1 cm or 0.1 kg using a precision stadiometer or digital scale, respectively. Waist circumference (WC) and hip circumference (HC) were measured to the nearest 0.1 cm at a level midway between the lowest rib margin and the iliac crest and at the widest level over the greater trochanters, respectively. Waist-to-hip ratios (WHRs) were determined from WC and HC values. Blood pressure was measured with each subject supine using an appropriately sized cuff and an automated device (Dinamap Pro 100; General Electric Healthcare, Chalfont St Giles, United Kingdom).

On the first day, fasting baseline laboratory tests and a 2-h oral glucose tolerance test (OGTT) with 75 g dextrose were performed. Fasting blood samples were obtained for determination of plasma insulin, glucose, sex hormone–binding globulin (SHBG), and total and HMW adiponectin. During the OGTT, blood samples were collected every 15 minutes for determination of serum glucose and insulin concentrations. Impaired fasting glucose (IFG) and IGT were defined using current American Diabetes Association (ADA) recommendations [20].

On the second day, after a 12-h overnight fast, S_i was determined by a frequently sampled intravenous glucose tolerance test [21]. At time zero, 300 mg/kg dextrose was administered intravenously; and 0.03 U/kg insulin was administered intravenously 20 minutes later. Twenty-seven blood samples were collected for determination of insulin and glucose during the 3-hour duration of the protocol. Data

were analyzed with the Minimal Model Identification Software (MINMOD Millennium, version 6.02, 2001) [22], which yields quantitative determination of tissue S_i.

2.1. Laboratory assays

Blood samples were centrifuged immediately, and sera were stored at −70°C until assayed. Plasma glucose levels were measured using glucose oxidase methodology (YSI 2300 Stat Plus Glucose Analyzer; Yellow Springs Instruments, Yellow Springs, OH), and plasma insulin levels were measured using an enzyme-linked immunosorbent assay (ELISA) for human insulin (ALPCO Diagnostics, Windham, NH). Serum total testosterone levels were was determined by radioimmunoassay (Diagnostic Products, Los Angeles, CA). Sex hormone–binding globulin concentrations were determined as previously described [23,24]; and serum free testosterone was calculated by the method of Sodergard et al [25], assuming a serum albumin concentration of 40 g/L. To avoid interassay variation, all samples were analyzed in duplicate in a single assay for each hormone. The intraassay coefficients of variation (CVs) for insulin and steroid hormone assays were 5.5% and less than 10%, respectively.

Quantification of total and HMW adiponectin species was performed using a double monoclonal sandwich ELISA method (Daiichi Pure Chemical, Tokyo, Japan; distributed by ALPCO Diagnostics). This method uses pretreatment with proteases for selective measurement of human multimeric adiponectin. This assay demonstrates a sensitivity of 0.04 ng/mL, an interassay CV less than 15%, and an intraassay CV of 5.3% and 3.3% for total and HMW adiponectin, respectively [26]. Adiponectin levels were described as the following: total adiponectin, HMW adiponectin (absolute serum level determined by ELISA), and the ratio of HMW to total adiponectin (S_A).

2.2. Statistical analysis

Results not normally distributed were log-transformed for all statistical analyses and reported back-transformed in their original units. All results were reported as means, or geometric means for log-transformed variables, with 95% confidence intervals. P values < .05 were considered significant. All statistical analyses were performed using JMP 7.0 software (SAS Institute, Cary, NC).

Variable comparisons between PCOS and healthy women were made with the Student unpaired 2-tailed t test. Although paired t tests could be performed in light of the matched pairs of PCOS and healthy women, analyses were performed with t tests for independent samples to provide more conservative estimates. Univariate correlation analysis was performed using Pearson correlation test. A forward stepwise multiple linear regression analysis was performed to determine the best model to predict S_i considering biologically plausible predictors of S_i, that is, PCOS status, BMI, WHR, total testosterone, free testosterone, total adiponectin, HMW adiponectin, and S_A. These independent variables were selected based on literature and/or because their correlations with S_i were significant in the univariate analyses. Biologically plausible interactions among these variables (PCOS status × BMI, PCOS status × WHR, and free testosterone × HMW adiponectin) were also entered into the model.

3. Results

Table 1 contains the clinical and biochemical characteristics of women with PCOS and age- and BMI-matched healthy control women. Women with PCOS had significantly higher WHR compared with healthy women ($P = .02$). Total and free testosterone values were also significantly higher in women with PCOS compared with controls ($P < .001$). Mean area under the curve $(AUC)_{glucose}$ and $AUC_{insulin}$ during the OGTT were both significantly higher in PCOS women compared with healthy women ($P < .01$ and $< .001$, respectively). Mean S_i was substantially lower among women with PCOS compared with healthy controls ($P < .01$). Four of the women with PCOS had IGT; 1 woman with IGT also had IFG. None of the healthy women had IFG or IGT.

3.1. Total and HMW adiponectin levels

Both total and HMW adiponectin were decreased in women with PCOS compared with healthy women ($P < .01$). Furthermore, among women with PCOS, HMW multimers comprised a smaller proportion of total circulating adiponectin levels (S_A) than in control women ($P = .03$). The observed differences in total adiponectin ($P < .01$), HMW adiponectin ($P = .01$), and S_A ($P = .02$) between PCOS and healthy women remained significant even after excluding the group pairs containing women with IGT. Furthermore, given the difference in WHR between women with PCOS and controls, multivariate regression analysis was performed to determine if group differences in adiponectin remained significant after controlling for WHR. After adjusting for observed differences in WHR, total adiponectin (partial $P = .02$) and HMW adiponectin levels (partial $P = .04$) remained different between groups. Similarly, after controlling for group differences in S_i by separate multivariate regression analyses, differences in total adiponectin according to PCOS status remained significant (partial $P = .04$). However, differences in HMW adiponectin levels between PCOS and healthy women were no longer significant after controlling for S_i.

3.2. Correlation between adiponectin levels and clinical/biochemical characteristics

Given the association between hypoadiponectinemia and increased adiposity, correlations between adiponectin and anthropometric characteristics were performed (Table 2). Considering all women (N = 26), both total and HMW adiponectin levels had strong inverse linear relationships ($r = -0.58$ for both) with WHR. A significant negative linear

Table 1
Clinical and biochemical characteristics of women with PCOS and healthy control women

Variable	PCOS subjects	Healthy subjects	*P* value
n	13	13	
Age (y)	29.9 (25.7-34.1)	30.7 (26.5-34.9)	.77
BMI (kg/m^2)	30.7 (26.5-34.9)	30.0 (25.8-34.2)	.80
WC (cm)	90.8 (82.0-99.6)	88.7 (79.9-97.5)	.73
HC (cm)	109.0 (101.2-116.9)	115.1 (107.3-123.0)	.27
WHR	0.83 (0.79-0.86)	0.77 (0.73-0.80)	.02
Systolic blood pressure (mm Hg)	116 (108.1-123.3)	113 (105.7-120.9)	.65
Diastolic blood pressure (mm Hg)	70 (64.7-74.6)	67 (61.6-71.5)	.37
Insulin (ρmol/L)[a]	42.4 (27.1-66.0)	22.2 (13.9-35.4)	.05
Glucose (mmol/L)	4.51 (4.20-4.80)	4.42 (4.12-4.72)	.69
SHBG (nmol/L)[a]	87.9 (54.8-141.2)	139.8 (87.1-224.6)	.17
Total testosterone (nmol/L)[a]	3.23 (2.45-4.25)	1.32 (1.00-1.74)	<.001
Free testosterone calculation (ρmol/L)[a]	30.9 (19.8-48.9)	9.4 (5.9-15.0)	<.001
$AUC_{glucose}$ (mmol·min^{-1}·L^{-1})[b]	838.7 (772.0-905.4)	673.6 (607.0-740.3)	<.01
$AUC_{insulin}$ (nmol·min^{-1}·L^{-1})[a,b]	48.43 (34.00-68.97)	17.63 (12.38-25.11)	<.001
S_i[c]	4.3 (-0.14-8.73)	13.5 (9.04-17.91)	<.01
Total adiponectin (μg/mL)	3.5 (2.47-4.45)	6.0 (5.00-6.99)	.001
HMW adiponectin (μg/mL)	1.3 (0.68-1.93)	2.7 (2.11-3.35)	<.01
HMW to total adiponectin	0.35 (0.30-0.41)	0.44 (0.39-0.50)	.03

Data are means (95% confidence intervals) unless otherwise noted. To convert values for insulin to micro–international units per milliliter, divide by 6.945; to convert values for glucose to milligrams per deciliter, divide by 0.0555; to convert values for total testosterone to nanograms per deciliter, divide by 0.0347; to convert values of free testosterone to nanograms per deciliter, divide by 34.7.

[a] Geometric means.

[b] Determined during 2-hour OGTT.

[c] Determined by frequently sampled intravenous glucose tolerance test.

relationship between S_A and WHR ($r = -0.52$) was also observed. However, significant relationships among other anthropometric measures (WC, HC, or BMI) and adiponectin (including total adiponectin, HMW adiponectin, or S_A) were not identified.

Next, the relationships between adiponectin and androgens were considered. Although total and HMW adiponectin correlated with total testosterone, stronger inverse relationships were observed between free testosterone and both total and HMW adiponectin ($r = -0.57$ and -0.55, respectively). Moreover, the inverse relationships between free testosterone and both total and HMW adiponectin remained significant after controlling for BMI by multivariate regression analysis (data not shown).

Finally, the relationships between parameters of glucose homeostasis and adiponectin were explored. There were no significant linear relationships between adiponectin and fasting glucose or insulin levels. However, total adiponectin, HMW adiponectin, and S_A all positively correlated with estimates of S_i ($r = 0.63$, 0.64, and 0.54, respectively). Further supporting the strong correlation between adiponectin and S_i, total adiponectin and HMW adiponectin were also found to inversely correlate with both $AUC_{glucose}$ and $AUC_{insulin}$ determined during an OGTT (Table 2).

Table 2
Univariate analysis of anthropometric/biochemical characteristics and total adiponectin, HMW adiponectin, and the ratio of HMW to total adiponectin when women with PCOS and healthy women were analyzed together (N = 26)

Variable	Total adiponectin		HMW adiponectin		HMW to total adiponectin	
	Correlation	*P* value	Correlation	*P* value	Correlation	*P* value
BMI	−0.18	.38	−0.21	.30	−0.33	.10
WHR	−0.58	<.01	−0.58	<.01	−0.52	<.01
Fasting glucose	−0.15	.47	−0.11	.59	−0.04	.85
Fasting insulin[a]	−0.32	.11	−0.33	.10	−0.39	.045
Total testosterone[a]	−0.41	.04	−0.41	.04	−0.32	.11
SHBG[a]	0.46	.02	0.43	.03	0.24	.23
Free testosterone calculation[a]	−0.57	<.01	−0.55	<.01	−0.36	.07
$AUC_{glucose}$[b]	−0.53	<.01	−0.43	.03	−0.24	.25
$AUC_{insulin}$[a,b]	−0.42	.03	−0.42	.03	−0.45	.02
S_i[c]	0.63	<.001	0.64	<.001	0.54	<.01

[a] Log-transformed before analysis.

[b] Determined during a standard 2-hour OGTT.

[c] Determined by frequently sampled intravenous glucose tolerance test.

Table 3
Univariate analysis of anthropometric/biochemical characteristics and S_i in all women (N = 26)

Variable	S_i[a]	
	Correlation	P value
BMI	−0.31	.12
WC	−0.38	.06
WHR	−0.63	<.001
Total testosterone[b]	−0.38	.06
Free testosterone calculation[b]	−0.44	.02
Total adiponectin	0.63	<.001
HMW adiponectin	0.64	<.001
HMW to total adiponectin	0.54	<.01

[a] Determined by frequently sampled intravenous glucose tolerance test.
[b] Log-transformed before analysis.

3.3. Determinants of S_i

Univariate correlations between S_i and anthropometric or biochemical characteristics potentially affecting S_i are outlined in Table 3. A strong inverse linear relationship was observed between S_i and WHR ($r = -0.63$, $P < .001$). Although the relationship between total testosterone and S_i did not attain statistical significance, S_i did correlate negatively with free testosterone ($r = -0.44$, $P = .02$). As outlined previously, significant relationships were observed between S_i and total adiponectin, HMW adiponectin, and S_A.

The best model to predict S_i using stepwise multivariate analysis considering PCOS status, all the variables shown in Table 3, and biologically plausible interactions was $S_i = 43.9 + 2.83 \cdot \text{HMW adiponectin} - 60.8 \cdot \text{WHR}$ (adjusted $R^2 = 0.46$, $P < .001$). Therefore, both HMW adiponectin (positive association, partial $P = .03$) and WHR (negative association, partial $P = .04$) significantly and independently predicted S_i.

4. Discussion

Our study tested the hypotheses that women with PCOS have decreased levels of HMW adiponectin compared with healthy women after controlling for obesity, and that HMW adiponectin would correlate inversely with serum testosterone and insulin resistance. In our population, both HMW and total adiponectin were lower in women with PCOS compared with healthy control women. In addition, in women with PCOS, HMW adiponectin comprised a smaller fraction of total adiponectin than in healthy women.

In our study, women with PCOS had higher WHR than BMI-matched healthy women. Furthermore, total adiponectin, HMW adiponectin, and S_A were all found to correlate negatively with WHR. However, alterations in visceral adiposity alone could not account for the observed differences in adiponectin between groups, as both total and HMW adiponectin levels remained significantly lower in women with PCOS after adjusting for WHR.

In our population, we also observed significant inverse relationships between free testosterone and both total and HMW adiponectin; and these findings are consistent with the results of other studies [17,27,28]. Both total and HMW adiponectin levels are lower in adult men compared with women [3,16,17]. This sexual dimorphism in adiponectin first appears in puberty and correlates with the pubertal increase in testosterone in men [28]. In vitro, testosterone appears to selectively inhibit the secretion of HMW adiponectin by adipocytes [17]. Lastly, total adiponectin (multimers were not assessed) increased in women with hyperandrogenism randomized to receive flutamide, an androgen antagonist [27].

Consistent with the hypothesis that insulin resistance is central to PCOS, we demonstrated that nondiabetic women with PCOS were less insulin sensitive than age- and BMI-matched healthy women. It is possible that hypoadiponectinemia plays a role in the development of insulin resistance in PCOS. However, the literature on the relationship between total adiponectin and S_i in PCOS women is conflicting. Most studies support a positive correlation between total adiponectin and S_i [10,12-14]; however, other studies have not confirmed this relationship [5,7,29,30].

These discrepancies may be influenced by differences in adiponectin multimerization. In our study, total adiponectin, HMW adiponectin, and S_A all exhibited strong positive relationships with S_i. However, WHR and HMW adiponectin, but not total adiponectin or S_A, were the most important predictors of S_i in stepwise multivariate analysis. These findings are consistent with data suggesting that the HMW complex is the biologically active form of adiponectin [3]. It is also important to highlight that, in our study, PCOS group status was not a predictor of S_i independent of differences in HMW adiponectin. This finding supports the hypothesis that the increased burden of insulin resistance observed in women with PCOS may result, in part, from alterations in HMW adiponectin.

The few previous studies investigating the relationship between alterations in adiponectin multimerization among women with PCOS have demonstrated conflicting results [6,31,32]. Similar to our findings, Aroda and colleagues [32] demonstrated decreased total and HMW adiponectin levels among 26 women with PCOS compared with 6 healthy women. Although the group of Aroda et al did report lower ratios of HMW to total adiponectin in PCOS women demonstrating IGT compared with those with normal glucose tolerance, they did not directly correlate adiponectin multimer status and S_i.

Glintborg and colleagues [6] reported lower total adiponectin levels in 30 obese women with PCOS compared with 14 age- and BMI-matched healthy women, but they did not find significant differences in either absolute HMW adiponectin levels or S_A between groups. Considering only women with PCOS, the authors did report significant negative linear relationships between HMW adiponectin and both WHR and S_i. However, unlike the study by Glintborg et al, we used multivariate analysis to determine

which anthropometric and biochemical variables independently predicted S_i.

Lastly, Barber and colleagues [31] compared adiponectin multimer distribution between 50 PCOS cases and 28 female controls, including 22 BMI- and fat mass–matched pairs, and found that total and HMW adiponectin levels were lower in PCOS women. However, after adjusting for fat mass and age, these differences were no longer significant. In light of these findings, the authors concluded that the observed differences in adiponectin multimers between PCOS and healthy women were attributable to differences in fat mass and rejected the hypothesis that adiponectin plays a primary role in the development of PCOS independent of adiposity. Of note, many (n = 21) of the PCOS women in the study by Barber et al [31] were not metformin naive. Although metformin was discontinued in these women 1 week before testing and significant differences in total and HMW adiponectin between metformin-naive and metformin-exposed women were not observed in their study, adiponectin levels may be altered by treatment with metformin [9].

Various methods for quantifying HMW adiponectin have been described in the literature, and each of the outlined studies investigating alterations in adiponectin multimers in women with PCOS used different techniques. Hence, differences in methodology make direct comparisons among studies difficult. Aroda and colleagues [32] determined adiponectin multimerization status using gel electrophoresis under nonreducing conditions followed by Western blotting, whereas Glintborg et al [6] used fast protein liquid chromatography to determine the distribution of adiponectin multimers. Absolute values for HMW adiponectin were not determined directly by either of these methods. Instead, the absolute HMW adiponectin level was calculated by multiplying total serum adiponectin levels (determined by immunofluorometric assay or radioimmunoassay) by the estimated HMW fraction. Barber et al [31] measured HMW adiponectin levels with an immunoassay using monoclonal antibodies.

In our study, HMW adiponectin levels were measured directly by a commercially available ELISA that used sample pretreatment with a protease that selectively digests non-HMW forms of adiponectin. Quantification of HMW adiponectin using this specific ELISA method has previously been shown to correlate closely with results from quantitative Western blot analysis [26] and has been subsequently used by several investigators [33-35]. Although direct measurement of HMW adiponectin using a standardized ELISA may represent a strength of our study, Bluher and colleagues [35] reported that measurement of HMW adiponectin using the same ELISA technique was not superior to total adiponectin in assessing S_i at baseline or in response to physical training in men and women with normal glucose tolerance, IGT, and overt type 2 diabetes mellitus. At least 2 other ELISA systems have also been developed that directly measure HMW adiponectin [36,37]; however, the precision and accuracy of each of the ELISA methods have not yet been directly compared. Consequently, further studies are needed to determine the most accurate and reliable method for measuring adiponectin multimers, particularly in large populations.

Another potential limitation of our study is the relatively small sample size. In this study, the differences in total and HMW adiponectin observed between groups were highly significant. Although it is unlikely that a larger sample size would change these relationships, a more robust sample may allow for the identification of other significant differences between groups. In particular, the sample size may limit the ability of multivariate regression analysis to identify independent variables or interactions that would be significant if a larger population were studied. In addition, it is important to emphasize that our cross-sectional study design can be used to identify alterations in adiponectin among PCOS women but cannot establish causality.

In summary, after controlling for age, weight status, and central adiposity, we found that women with PCOS have lower HMW adiponectin levels compared with healthy women. Furthermore, compared with healthy women, women with PCOS appear to have alterations in adiponectin multimerization, with HMW adiponectin comprising a smaller proportion of total circulating adiponectin levels. Levels of HMW adiponectin also correlated inversely with WHR and free testosterone. Although total adiponectin and the ratio of HMW to total adiponectin were found to correlate with S_i, absolute HMW adiponectin levels and WHR were identified as the strongest independent predictors of S_i. After controlling for differences in S_i, PCOS group status was no longer identified as an independent predictor of differences in HMW adiponectin levels. Although the limited number of previous studies assessing adiponectin multimers in women with PCOS have yielded conflicting results, our findings support the hypothesis that alterations in HMW adiponectin among PCOS women may contribute to the insulin resistance intrinsic to the syndrome.

Acknowledgment

This work was supported by the following research grants from the National Institutes of Health: K23-HD053742 (to EPW), K24-HD40237 (to JEN), R01-HD35629 (to JEN), K23-HD049454 (to KIC), and General Clinical Research Center MO1-RR00065.

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