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Adiponectin isoform distribution in women—relationship to female sex steroids and insulin sensitivity

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Abstract

Little is known about the associations between adiponectin and its oligomeric isoforms with female sex steroids, and the relevance of these relationships to insulin sensitivity in women. In a cross-sectional study of 32 healthy women (12 premenopausal, 10 postmenopausal, and 10 early pregnant), we investigated the correlations of total adiponectin and the high–, medium–, and low–molecular weight oligomers (HMW, MMW, and LMW, respectively) with estrogen, progesterone, adiposity, and insulin resistance. Fat mass and serum concentrations of estradiol, progesterone, insulin, glucose, and total and isoform adiponectin were measured. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated. Serum concentrations of total and HMW adiponectin were highest in postmenopausal women and lowest in pregnant women. Concentrations of the MMW and LMW isoforms were not significantly different between the 3 groups. Total adiponectin, HMW adiponectin, and MMW adiponectin were negatively associated with estradiol and progesterone; but no associations between the LMW isoform and female sex steroids were observed. Fat mass and HOMA-IR were highest in pregnant women and lowest in premenopausal women. The HOMA-IR was positively associated with fat mass, estradiol, and progesterone, and negatively associated with total, HMW, and MMW adiponectin. Multivariate stepwise regression analysis revealed that fat mass explained 34% of the variance in HOMA-IR and that total and isoform adiponectin contributed an additional 10% to 15%. In the multivariate linear regression analysis, there were significant interactions of estradiol and progesterone with adiponectin or fat mass in the associations with HOMA-IR. In conclusion, there are strong negative associations of serum adiponectin and some of its isoforms with estradiol and progesterone. Female sex steroids are likely to affect insulin sensitivity through modulation of adiponectin and body fat.

1. Introduction

In women, substantial changes in body composition and insulin responses occur at menopause and during pregnancy [1-3]. The fall of female sex steroids in postmenopausal women is associated with an increase in abdominal adiposity and insulin resistance [2], and these changes are reduced with hormone replacement therapy [4] despite unaltered body weight [5]. On the other hand, the elevation of female sex steroids during pregnancy, probably with a concomitant increase in tumor necrosis factor–α and cortisol [6], alters substrate utilization that leads to an accumulation of body fat and a progressive decrease in insulin sensitivity [1,7]. In both states, the changes in adiposity and insulin action may contribute to development of insulin resistance.

As fat accumulation, in particular in the abdominal region, is a high-risk factor for the development of insulin resistance [8], it has been suggested that female sex steroids may regulate insulin action through modulation of adipose

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tissue deposition and/or metabolism [9]. Recent studies show that adipose tissue secretes a number of metabolically active factors known as *adipokines* [10]. It is conceivable that estrogen and progesterone may regulate the adipokine action to affect insulin sensitivity.

Adiponectin is an adipokine, which exhibits insulin-sensitizing effects [11,12]. Hypoadiponectinemia has been linked to metabolic disorders, including hyperlipidemia, insulin resistance, and diabetes. Adiponectin circulates as multiple oligomers in the forms of low–molecular weight (LMW) trimer, medium–molecular weight (MMW) hexamer, and high–molecular weight (HMW) multimer (12-18mers) [15,16]. These isoforms possess different abilities to activate signaling pathways [13-15], namely, the 5′-adenosine monophosphate–activated protein kinase, which mediates the metabolic effects of adiponectin [16,17], suggesting that the adiponectin isoforms may have distinct roles in regulating substrate metabolism. Furthermore, the HMW isoform appears to be a better predictor of insulin sensitivity than total adiponectin [18]. Although there is evidence that adiponectin expression and secretion are regulated by glucocorticoids and tumor necrosis factor–α, and vice versa [19,20], little is known about the association between female sex steroids and adiponectin isoform expression.

We report here a cross-sectional study on the concentrations of serum adiponectin and its isoforms, estradiol, progesterone, body fat content, and insulin resistance in premenopausal, postmenopausal, and pregnant women. The aim of this study was to examine whether there are associations between adiponectin, female sex steroids, and body fat in relation to insulin sensitivity.

2. Materials and methods

2.1. Subjects

The present study was part of a larger study comparing the effects of female sex steroids on substrate metabolism and insulin action in premenopausal, postmenopausal, and early pregnant women, in which the metabolic findings had been published [1]. A total of 32 healthy women were recruited from the general population at Sydney, including 12 premenopausal, 10 postmenopausal, and 10 pregnant women. The premenopausal subjects were studied in the follicular stage of the menstrual cycle. The pregnant subjects were studied at 19 ± 1 (mean ± SEM) weeks gestation; all underwent a glucose tolerance tests at 26 to 28 weeks, and subjects with gestational diabetes mellitus were excluded. All subjects did not take any medications, including oral contraceptive steroids and hormone replacement therapy. The study protocol was approved by the Research Ethics Committee of South Eastern Sydney Area Health Service Southern Section (97/123) and the Committee on Experimental Procedures Involving Human Subjects of the University of New South Wales (98015). Written informed consent was obtained from each subject.

2.2. Clinical and laboratory studies

Details of the study design have been reported previously [1]. Briefly, all subjects attended the Clinical Research Room in St George Hospital, Sydney, at 8:00 AM after a 12-hour overnight fast. After weight and height measurement, the subjects underwent metabolic studies to assess energy expenditure and substrate oxidation before and after a standardized mixed meal, with frequent blood sampling over a period of 3 hours. In the present study, baseline blood samples were used for measurement of insulin, glucose, estradiol, progesterone, and total and isoform adiponectin. Body composition was measured by bioimpedance analysis (Bodystat 1500; Bodystat, Douglas, Isle of Man, United Kingdom), from which percentage body fat (%BF) and fat mass (FM) were determined as previously reported [1].

Serum concentrations of total adiponectin and the 3 oligomeric isoforms prepared by gel filtration chromatography were measured using an in-house enzyme-linked immunosorbent assay as previously described [21]. The intra- and interassay coefficients of variance (CVs) of the adiponectin enzyme-linked immunosorbent assay were 5% to 6% and 6% to 8%, respectively. The monoclonal antibodies used in this assay had similar affinities to all the isoforms (A Xu, unpublished observations).

Estradiol was measured using the Clinical Assays Estradiol-2 radioimmunoassay kit (Sorin Biomedica, Saluggia, Italy), with intra- and interassay CVs of 8% and 12%, respectively. Progesterone and insulin were measured by solid-phase 2-site chemiluminescent assays (IMMULITE 2000; Siemens Healthcare Diagnostics, Los Angeles, CA). The intra- and interassay CVs for the progesterone assay were 7% and 13%, respectively; and those for insulin were 6% and 7%, respectively. The insulin assay had 8% cross-reactivity with proinsulin and did not detect C-peptide. In the postmenopausal group, 8 subjects had progesterone concentrations less than the assay detection limit of 0.6 nmol/L; and a value of 0.5 nmol/L was assigned to these samples for analytical purposes. Blood glucose concentrations were measured using a glucose analyzer (Model 23AM; Yellow Springs Instrument, Yellow Springs, OH). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as follows: HOMA-IR = fasting insulin (milliunits per liter) × fasting glucose (millimoles per liter)/22.5.

2.3. Statistical analysis

All results are expressed as mean ± SEM. Comparison of demographic and biochemical variables and adiponectin concentrations of the 3 study groups was performed using analysis of variance. Univariate linear regression analysis was performed to investigate the relationship between concentrations of adiponectin (total and isoforms), estradiol or progesterone, FM, and HOMA-IR. Multivariate regression analyses were used to assess the relative contribution of FM, female sex steroids, adiponectin, and their interactions to the variation of HOMA-IR. Log_{10}-transformed concentrations of

estradiol (logE), progesterone (logP), and adiponectin (logTotal, logHMW, logMMW, and logLMW) and isoforms as percentages of total adiponectin (log%HMW, log%MMW, and log%LMW) were used for the analysis because these values were not normally distributed. Statistical analyses were performed with StatView 4.5 (Abacus Concepts, Berkeley, CA) and STATA Version 10 (StataCorp, College Station, TX), with significance defined as P less than .05.

3. Results

3.1. Study groups

Table 1 shows the demographic and biochemical characteristics of the study groups. Age was significantly different between the 3 groups. The premenopausal group had the lowest FM and %BF. As expected, serum concentrations of estradiol and progesterone were lowest in the postmenopausal group and highest in the pregnant group. Fasting glucose concentrations were not different between the 3 groups, whereas fasting insulin concentrations and HOMA-IR were highest in the pregnant group and lowest in the premenopausal group.

3.2. Total and isoform adiponectin

The total adiponectin concentration was highest in the postmenopausal group and lowest in the pregnant group (Fig. 1). A similar pattern was observed with the HMW isoform. Concentrations of the MMW and LMW isoforms were not significantly different between the 3 groups. The HMW oligomer was the most abundant adiponectin isoform. This was followed by the MMW isoform, and the LMW isoform was the least abundant. Among the 3 study groups, %HMW was highest in postmenopausal women and lowest in pregnant women. In contrast, %MMW was highest in the pregnant group and lowest in the postmenopausal group. Percentage of LMW was significantly lower in the postmenopausal group.

Table 1
Demographic and biochemical characteristics of the study groups

	Premenopausal	Postmenopausal	Pregnant
n	12	10	10
Age (y)	30 ± 1	66 ± 1†	36 ± 1†,§
Weight (kg)	62.4 ± 1.8	68.5 ± 2.7	80.8 ± 2.5†,§
Height (cm)	168 ± 2	161 ± 2*	166 ± 3
BMI (kg/m^2)	22.2 ± 0.5	26.6 ± 1.2†	29.7 ± 1.4†
Fat mass (kg)	15.8 ± 1.0	27.7 ± 2.8†	30.4 ± 2.1†
Body fat (%)	25.3 ± 1.4	41.0 ± 2.5†	37.3 ± 1.6†
Estradiol (pmol/L)	200 ± 24	99 ± 20†	66295 ± 4398†
Progesterone (pmol/L)	3.4 ± 1.6	0.66 ± 0.14	443 ± 109†
Fasting glucose (mmol/L)	5.0 ± 0.2	5.5 ± 0.3	4.9 ± 0.1‡
Fasting insulin (mU/L)	5.0 ± 0.6	6.5 ± 1.1	10.2 ± 1.2†,‡
HOMA-IR	1.13 ± 0.15	1.61 ± 0.29	2.22 ± 0.31†

Data are mean ± SE and were compared using the Student unpaired t test.
*$P < .05$, $^{\dagger}P < .005$ vs premenopausal.
$^{\ddagger}P < .05$, $^{\S}P < .005$ vs postmenopausal.

To investigate the relationships of total and isoform adiponectin with the demographic and biochemical variables, univariate linear regression analysis was performed. LogTotal was associated negatively with logE ($\beta = -0.06$, $r^2 = 0.20$, $P = .01$) and logP ($\beta = -0.07$, $r^2 = 0.22$, $P = .007$) and positively with age ($\beta = 0.005$, $r^2 = 0.23$, $P = .005$), and there was no association with FM or %BF. Similar findings were observed with logHMW and logMMW, except that the association between logMMW and logE did not reach statistical significance ($P = .07$). The LMW isoform was not significantly associated with any of the variables. When individual groups were studied, total adiponectin, HMW adiponectin, and MMW adiponectin were significantly related to age (β = 0.02-0.03, r^2 = 0.41-0.53, $P < .05$), but not to other variables, in postmenopausal women. No associations of adiponectin with any of these variables were observed in premenopausal and pregnant women.

When relative abundance of the adiponectin isoforms was examined, log%HMW was associated negatively with logE ($\beta = -0.02$, $r^2 = 0.29$, $P = .001$) and logP ($\beta = -0.03$, $r^2 = 0.33$, $P < .001$), and positively with age ($\beta = 0.002$, $r^2 = 0.31$, $P < .001$). In contrast, log%MMW was related positively to logE and logP (both with $\beta = 0.03$, $r^2 = 0.24$, $P = .005$), and negatively to age ($\beta = -0.002$, $r^2 = 0.18$, $P = .02$). A negative correlation between log%LMW and age ($\beta = -0.004$, $r^2 = 0.17$, $P = .02$) was also observed. When the 3 groups were analyzed individually, none of the relative isoform abundance was significantly associated with these variables (data not shown).

After adjusting for age, logTotal, logHMW, logMMW, and log%HMW remained negatively associated with logE ($\beta = -0.009$ to -0.04, $r^2 = 0.16$-0.17, $P < .05$) and logP ($\beta = -0.01$ to -0.05, $r^2 = 0.20$-0.21, $P < .01$). Log%MMW was positively associated with logE ($\beta = 0.009$, $r^2 = 0.17$, $P = .02$) and logP ($\beta = 0.01$, $r^2 = 0.21$, $P = .009$). There was no significant association of age-adjusted logLMW and log%LMW with logE or logP.

When compared between the 3 groups, age-adjusted logTotal, logHMW, logMMW, and log%HMW remained significantly higher in the postmenopausal group and lower in the pregnant group than the premenopausal group ($P < .001$). Similar results were observed with adjustment for logE or logP (both with $P < .001$).

3.3. Relationships with insulin resistance

The associations between insulin resistance and demographic variables, estradiol, progesterone, and adiponectin were next examined. The HOMA-IR was associated positively with logE ($\beta = 0.29$, $r^2 = 0.17$, $P = .02$), logP ($\beta = 0.31$, $r^2 = 0.16$, $P = .02$), and FM ($\beta = 0.06$, $r^2 = 0.34$, $P < .001$), and negatively with logTotal, logHMW, logMMW ($\beta = -1.94$ to -2.78, $r^2 = 0.21$-0.23, $P < .01$), and log%HMW ($\beta = -7.18$, $r^2 = 0.16$, $P = .02$). The HOMA-IR was not significantly associated with age, logLMW, log%MMW, and log%LMW. In the analysis of individual groups, HOMA-

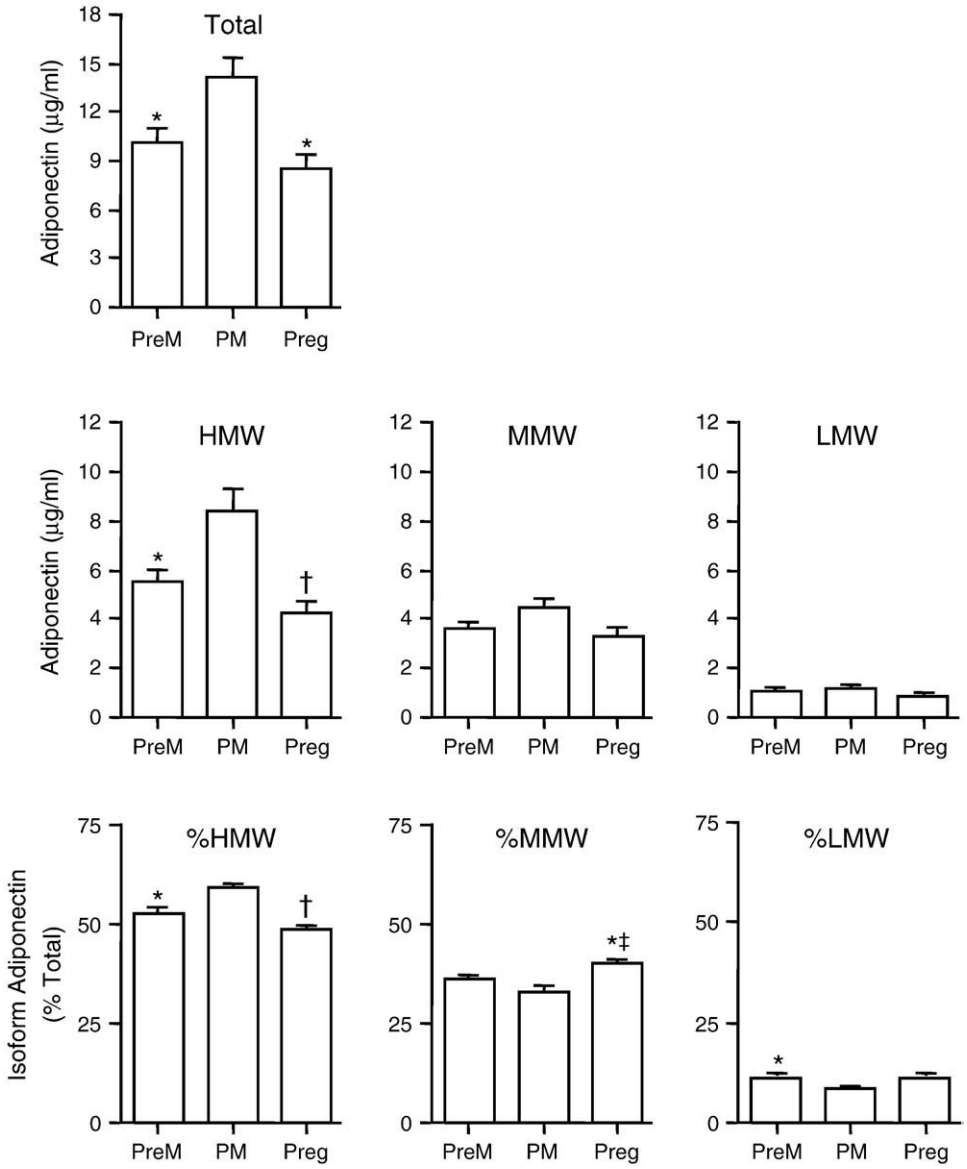

Fig. 1. Concentrations (mean ± SE) of total and isoform (HMW, MMW, and LMW) adiponectin, as well as relative abundance of the isoforms (%HMW, %MMW, and %LMW), in the premenopausal, postmenopausal, and pregnant groups. *$P < .05$, †$P < .005$ vs postmenopausal. ‡$P < .05$ vs premenopausal. PreM indicates premenopausal; PM, postmenopausal; Preg, pregnant.

IR was associated positively with FM ($\beta = 0.11$, $r^2 = 0.58$, $P = .02$) and negatively with logTotal, logHMW, logMMW, and log%HMW ($\beta = -3.56$ to -19.07, $r^2 = 0.56$-0.67, $P < .01$) in pregnant women. The HOMA-IR was not significantly associated with any of the variables in the premenopausal and postmenopausal groups.

To estimate the relative contributions of FM, female sex steroids, and adiponectin (total, HMW, MMW, or %HMW) to the variance in HOMA-IR, we conducted multivariate stepwise regression analysis with these variables as covariates. Fat mass explained 34% of the variance in HOMA-IR ($P < .001$); and total, HMW, MMW, or %HMW adiponectin each contributed a further 11% to 15% of the variance ($P < .001$). No significant associations were observed with logE and logP in this model.

To examine the relationships between FM, female sex steroids, and adiponectin with HOMA-IR, multivariate linear regression analysis was performed, with interaction terms included in the models. As shown in Table 2, FM, logE or logP, and the interactions between total adiponectin and female sex steroids were significantly associated with HOMA-IR, contributing more than 60% of the variance. The HOMA-IR was no longer independently associated with adiponectin after adjustment for these other covariates, but the interaction between adiponectin and female sex steroids was significant.

The multivariate linear regression analysis in individual groups demonstrated that the strongest negative association between HOMA-IR and female sex steroids was found in the pregnant women (data not shown). The interactions between

Table 2
Multivariate linear regression analysis of HOMA-IR with FM, adiponectin, and female sex steroids as covariates

	β Coefficient	95% Confidence interval	P value	r^2
*LogE**				
FM	0.05	0.03, 0.07	<.001	0.61
LogTotal*	1.63	−0.99, 4.25	.2	
LogE	1.05	0.34, 1.77	.006	
LogTotal − logE	−1.08	−1.84, −0.32	.007	
*LogP**				
FM	0.05	0.03, 0.07	<.001	0.63
LogTotal	−0.39	−1.78, 1.01	.6	
LogP	1.18	0.46, 1.90	.002	
LogTotal − logP	−1.21	−2.01, −0.42	.004	

* Log_{10}-transformed concentrations of estradiol, total adiponectin, and progesterone.

female sex steroids and adiponectin were also significantly associated with HOMA-IR in this model.

4. Discussion

To our knowledge, this is the first human study demonstrating a negative association between serum adiponectin, as well as its HMW and MMW isoforms, with estradiol and progesterone. We further demonstrated that HOMA-IR was associated positively with FM, estradiol, and progesterone, and negatively with adiponectin and the 2 higher-order isoforms. The multivariate stepwise regression analysis showed that FM contributed one third of the variance in HOMA-IR, whereas adiponectin and its isoforms explained around one sixth of the variance. No independent association of estradiol or progesterone with HOMA-IR was observed in this model. However, in the multivariate linear regression analysis, there were significant interactions of female sex steroids with adiponectin and FM in affecting HOMA-IR. The collective findings thus suggest that the associations between female sex steroids and insulin sensitivity vary with adiponectin concentration and adiposity.

Only a few studies examining the adiponectin status at menopause and pregnancy have been published, and the results are inconsistent. Some groups have detected higher adiponectin concentrations in postmenopausal than premenopausal women [22,23], whereas one study found no difference between the 2 groups [24]. On the other hand, early pregnant women were shown to have lower serum adiponectin concentrations than premenopausal women in some [9,25] but not other studies [26,27]. There is also evidence that prepregnant body weight may affect the response of adiponectin to pregnancy. Serum adiponectin concentrations at early pregnancy were lower in overweight women than those with normal BMI, but this difference disappeared with advancing gestational age [28].

The present study showed that adiponectin concentrations increased after menopause and decreased at early pregnancy, with strong negative associations of adiponectin with estradiol and progesterone. An inverse relationship between adiponectin and estrogen has been reported [22], whereas its association with progesterone was not known before. However, our findings are at odds with a previous report in which no significant changes in serum adiponectin concentrations were detected throughout the menstrual cycle [29]. One possible explanation for the discrepancies is that the estrogen and progesterone concentrations peak at different stages in the cycle, and there may be continuous negative effects from the female sex steroids on circulating adiponectin.

Similar to total adiponectin, the HMW and MMW isoforms were negatively associated with female sex steroids. Interestingly, a different pattern of associations was observed with the relative abundance, with %HMW inversely related to estradiol and progesterone, as opposed to the positive association of %MMW. The LMW isoform, in either the absolute or the relative amount, was not associated with estradiol and progesterone. These findings suggest that female sex steroids exert diverse effects on expression of the adiponectin isoforms. The physiologic significance of these observations is not clear and warrants further investigations.

One striking finding in this study is the lack of an association between adiponectin and FM, an observation similar to that of Lara-Castro et al [30], but different to some reports that adiponectin concentration decreases with increasing adiposity [22,31]. These discrepancies may be due to the different patterns of fat distribution in the 3 study groups. Changes in hormonal status alter fat storage in the way that postmenopausal women have increased visceral fat, whereas pregnant women store more fat in the subcutaneous compartment. As regulation of adiponectin secretion is fat depot dependent [32], the heterogeneous nature of fat depots in the 3 study groups appears to have confounding effects on the analysis of the relationship between adiponectin and FM.

In addition to fat depots, potential confounding factors would include age, glucocorticoids, and cytokines (such as tumor necrosis factor–α and interleukin-18). Although the sample size in this study has restrained our investigation into the effects of all these confounders, we showed that adiponectin adjusted for age (a significant covariate of total adiponectin and most isoforms) remained significantly associated with estradiol and progesterone. These findings are in accordance with a recent study with a larger cohort (42 premenopausal and 111 postmenopausal women) in which a strong correlation between adiponectin and age was observed after adjusting for menopausal status and FM [23]. Previous animal studies have also shown that ovariectomized mice had increased concentrations of plasma adiponectin compared with the age-matched, sham-operated control; and these changes were reversed with estrogen treatment [33]. Taken together, the collective data suggest that estrogen exerts a negative effect on adiponectin.

The mechanism underlying the regulation of adiponectin by female sex steroids is largely unknown. As adiponectin is produced primarily by adipocytes [34], estrogen and progesterone may affect the adiponectin concentration by controlling adipose tissue metabolism [35]. Estrogen has been shown to alter the action of lipoprotein lipase and hormone-sensitive lipase in adipocytes [36]. However, this possibility is considered unlikely because we did not detect an association of adiponectin with FM or %BF. Alternatively, age, being an independent covariate of adiponectin, might have a confounding effect on the relationship between adiponectin and female sex steroids. This possibility, however, can also be excluded because adiponectin adjusted for age remained significantly associated with estradiol and progesterone. Finally, there is in vitro evidence that estrogen decreased adiponectin expression in adipocytes [33]. It is conceivable that female sex steroids may inhibit adiponectin production.

Both menopause and pregnancy are characterized by a decrease in insulin sensitivity, although the female sex steroid concentrations are very different between the 2 groups. To investigate how estradiol and progesterone may affect insulin sensitivity, we examined the associations between adiponectin and female sex steroids to insulin resistance. As expected, HOMA-IR was higher in postmenopausal and pregnant women than in premenopausal women. This pattern was similar to that of FM, but not to those of adiponectin, estradiol, and progesterone, suggesting that FM was a more important determinant of insulin sensitivity than adiponectin or female sex steroids. This view is supported by the findings of the multivariate stepwise regression analysis that FM was the first entry in the analysis, accounting for over one third of the variance in HOMA-IR. In this context, other adipocyte-secreted proteins, such as tumor necrosis factor–α, may have a confounding role in the development of insulin resistance [6].

In the same analysis, total adiponectin and isoform adiponectin were independent covariates of HOMA-IR. The observation that adiponectin was related to insulin resistance in an FM-independent manner has also been reported by Tschritter et al [37]. Most interestingly, there were highly significant interactions of estradiol or progesterone with adiponectin in their relationship with HOMA-IR in the multivariate linear regression analysis. These findings together suggest that the effects of female sex steroids on insulin sensitivity are likely to be mediated indirectly through their effects on adiponectin and adiposity.

There has been an intense interest in the oligomeric isoform distribution of adiponectin since Pajvani et al [18] reported that the relative abundance of the HMW isoform was a much better predictor of insulin sensitivity than total adiponectin. However, this initial finding was replicated in some [38,39] but not all recent studies [30,40,41]. Our results showed that the HMW and MMW isoforms and %HMW were associated with insulin resistance in a similar manner to that of total adiponectin. The reasons for the discrepancies are not obvious. It is noteworthy that most of the observations supporting the view of the HMW isoform being a better predictor were made in diabetic or obese subjects, whereas many of the studies that do not support this view, including the present study, were conducted with nondiabetic subjects or a mixed population. More work is needed to investigate whether a closer association between the HMW adiponectin isoform and insulin sensitivity is present only in certain metabolic conditions.

Given the cross-sectional design of this study and the relatively small sample size in each group, it is reasonable to interpret the present findings as preliminary data regarding the interplay between female sex steroids, adiponectin, and FM and their role in insulin sensitivity in women. These observations provide the basis for further investigations with larger groups that allow a comprehensive assessment of other potential confounders (such as pregnant body weight, glucocorticoids, and cytokines). Despite that, our study is sufficiently powered as demonstrated by the strength of the associations found between female sex steroids, adiponectin, and HOMA-IR.

In summary, circulating adiponectin and its major oligomeric isoforms were negatively associated with estradiol and progesterone. Although there was a significant, positive association between insulin resistance and female sex steroids, the effects of estrogen and progesterone appeared to be mediated indirectly through their interactions with adiponectin and FM. These findings have provided novel insights into the complex interplay between female sex steroids, adiponectin, and adiposity in affecting insulin function in women.

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