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Research Paper

Normal menstrual cycle steroid hormones variation does not affect the blood levels of total adiponectin and its multimer forms

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ABSTRACT

Objective: Plasma total adiponectin reveals a sexual dimorphism indicating that gonadal steroids may be involved in its secretion and/or metabolism. However, results from previous reports are conflicting and data regarding the influence of ovarian steroids on adiponectin's multimer forms are scarce. The objective of the study was to assess if total adiponectin and its isoforms are affected by the changes of estradiol and progesterone during the normal menstrual cycle and the association of total adiponectin and its isoforms with the gonadal steroid levels.

Materials/methods: Quantitative determination of plasma adiponectin and its multimers was conducted in the three phases of an ovulatory cycle in 13 premenopausal women, in the follicular phase of 10 more premenopausal women, in 20 postmenopausal women and in 21 men. Moreover, serum levels of FSH, LH, prolactin, estradiol, progesterone, and testosterone, sex hormone binding globulin, glucose, and insulin were measured.

Results: The circulating levels of total adiponectin and its multimers were not affected by the normal variation of estradiol and progesterone across the ovulatory menstrual cycle. In the whole number of participants, the total adiponectin and high molecular weight adiponectin levels were significantly different between genders and associated positively with age and sex hormone binding globulin levels, and negatively with testosterone and progesterone levels and the waist/hip ratio. In the multiple logistic regression analysis, after adjustment for age, gender, and sex hormone binding globulin and progesterone levels, significant predictors of total adiponectin levels were the waist/hip ratio and testosterone levels, and of high molecular weight adiponectin the testosterone levels.

Conclusions: Normal menstrual cycle ovarian steroids are not involved directly in the regulation of secretion and/or metabolism of total adiponectin and its multimers. Testosterone seems to be responsible for the adiponectin's sexual dimorphism.

Introduction

Adiponectin is produced by the adipose tissue and is secreted into the bloodstream where it accounts for up to 0.05% of total serum protein and circulates as a low molecular weight (LMW) trimer (~65 kDa), mean molecular weight (MMW) hexamer (~150 kDa) and high molecular weight (HMW) multimers (~280 and ~420 kDa) [1]. HMW isoforms appear to be the most biologically active form of adiponectin, being related to reduced abdominal fat and high basal lipid oxidation [2]. Recent studies also suggest that HMW adiponectin and the ratio of HMW to TA are associated with insulin sensitivity, antiatherogenic activities, metabolic syndrome and the prediction of cardiovascular disease [3].

Plasma adiponectin reveals a sexual dimorphism, with females having significantly higher circulating levels of TA and HMW

Abbreviations: BMI, body mass index; Bio-T, bioavailable testosterone; CRP, C reactive protein; E2, estradiol; FAI, free androgen index; FSH, follicle stimulating hormone; FT, free testosterone; FT4, free thyroxine; HOMA-R, homeostasis model assessment of insulin resistance; HMW, high molecular weight; LH, luteinizing hormone; LMW, low molecular weight; MBP, mean blood pressure; MMW, mean molecular weight; PCOS, polycystic ovary syndrome; SHBG, sex hormone binding globulin; TSH, Thyroid stimulating hormone; TA, total adiponectin; TT, total testosterone; WC, waist circumference.

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isoforms than males in both humans [4,5] and rodents [6–8], whereas the levels of MMW and LMW forms are comparable between sexes [7]. This sexual dimorphism leads to the hypothesis that adiponectin secretion and/or metabolism are regulated by gonadal steroids. However, previous reports, applying a diversity of study models to investigate the potential effect of sex steroids exerted on adiponectin secretion, are conflicting.

Previous studies [9,10], but not all [4,11], have shown significantly higher circulating adiponectin concentrations in postmenopausal than in premenopausal and pregnant women. Specifically, serum concentrations of TA and HMW adiponectin were highest in postmenopausal women and lowest in pregnant women, whereas between the three groups MMW and LMW isoforms were comparable [10]. Existing data regarding the association between estradiol (E2) and adiponectin levels are conflicting [11,12], indicating that in women, besides E2, other factors such as age [6] and alterations in the androgen-to-estrogen ratio [5] may contribute to the mentioned differences. These might be the reason for the low adiponectin levels reported in women with polycystic ovary syndrome (PCOS) and elevated total testosterone (TT) level, although coexistent obesity and/or insulin resistance cannot be excluded [13]. Androgens also seem to influence plasma TA levels. Hypogonadal men, compared to eugonadal, have significantly higher plasma adiponectin levels, which are reduced by testosterone replacement therapy [14]. Similarly, in normal men experimental testosterone deficiency increased plasma TA levels, an effect that was suppressed when testosterone replacement therapy was also given [15]. However, supraphysiologic testosterone administration resulted in decreased plasma adiponectin levels [15].

Previous studies investigating variations of circulating adiponectin concentrations during the menstrual cycle are limited and have shown contradictory data. Moreover, only total circulating adiponectin levels were measured and the speculation that mainly the HMW isoform of adiponectin is sensitive to female sex steroids changes [12] has not been further investigated. As far as we know, there is only one study where circulating adiponectin multimer forms concentrations were measured at the early phase of the menstrual cycle [16]. Therefore, we investigated whether sex hormones affect not only plasma TA levels but also adiponectin multimer forms in all phases of a normal menstrual cycle. A normal menstrual cycle corresponds to a three-step model with low E2 levels in the follicular phase, increased E2 levels in the preovulatory phase and increased E2 plus progesterone levels in the luteal phase with the androgens remaining unchanged. Moreover, we assessed the associations between blood testosterone and estradiol with total adiponectin, HMW adiponectin and the HMW/total adiponectin ratio in healthy pre- and postmenopausal women and men.

Subjects and methods

Subjects

In the study, twenty-three premenopausal women were enrolled with mean (±SD) age 34.0 ± 4.7 and range 27–40 years with normal body weight (BMI 22.3 ± 2.2) and regular menstrual cycle. All women were healthy and none of them had received hormonal or any other medical treatment for at least 6 months prior to this study. Fasting blood samples were collected between 8:00 and 10:00 am. From thirteen out of the twenty-three premenopausal women blood was obtained at the three phases of a 28-days menstrual cycle, defined by the menses onset: follicular (4th–5th day), ovulatory (11th–12th day) and luteal phase (20th–21st day). From the other ten premenopausal women blood was obtained only at the follicular phase of the menstrual cycle (4th–5th day). Five of them were withdrawn from the study, in four the studied menstrual cycle was anovulatory and in one the last time point serum samples were lost.

Blood was also obtained from twenty postmenopausal women (more than 1 year from the last menstrual period) and twenty-one men. All were healthy and none of them had received any medication during the last six months prior to this study. Postmenopausal women and men had mean age 56.0 ± 3.0 (range 51–63) and 37.0 ± 3.4 (range 32–44) years, respectively. Before blood sampling each subject underwent a thorough physical examination. Body weight and height, waist circumference (WC) and blood pressure were measured as previously described [17]. Mean arterial blood pressure (MBP) was calculated as follows: MBP = diastolic BP + (1/3 systolic BP).

Laboratory parameters measurement

After sampling in EDTA or serum tubes, blood was centrifuged at 1465 g for 7 min and aliquots were immediately frozen at −86 °C until assayed. Blood samples were analyzed for FSH, LH, E_2, TSH, FT_4, PRL, PRG, testosterone, SHBG, albumin, glucose, insulin and HbA1c by an auto analyzer (Olympus 600; Medicon, Athens, Greece) using standard techniques. Serum free testosterone (FT) and bioavailable testosterone (bio-T) were calculated from serum total testosterone, SHBG and albumin concentrations as previously described [17]. Free androgen index (FAI) was computed as the ratio of total testosterone (in nmol/L) to SHBG (in nmol/L) concentration. The homeostasis model assessment of insulin resistance (HOMA-R) index was calculated as plasma insulin (in l U/mL) × plasma glucose (in mg/dL) divided by 22.5 × 18. Total adiponectin and multimers' concentration were quantified by ELISA (Bühlmann Labs, Switzerland) in the same assay. The amount of HMW, MMW and LMW adiponectin was calculated according to the manufacturer's instructions. The intra-assay CV was 5% for total adiponectin, 6% for MMW + HMW, and 5.7% for HMW adiponectin.

Written informed consent was obtained from all participants and the study protocol was reviewed and approved by the Scientific and Ethics Committee of the School of Medicine, University of Thessaly.

Statistical analysis

Results for quantitative variables are expressed as mean ± standard deviation (SD) unless otherwise indicated. Data for qualitative variables were described as numbers and/or percentages. Student *t* test or the Mann–Whitney *U*-test was used to estimate differences between mean values, as appropriate. Comparison of frequencies was performed using X^2 or Fisher's exact test. One way ANOVA with Bonferroni correction was used to determine trends of the repeated measures on the 13 premenopausal women during the menstrual cycle and differences across the groups. Spearman's coefficient was used to test for bivariate correlations. Multiple logistic regressions were used to examine the association between total adiponectin, HMW adiponectin or HMW/TA ratio as a dependent variable and age, gender, abdominal obesity, SHBG, progesterone, and FT or TT levels as independent variables. A probability value of $p < 0.05$ was considered statistically significant. Analyses were performed using SPSS for windows version 17.0 (SPSS Inc Chicago, IL).

Results

In the thirteen women studied at the three phases of their menstrual cycle, serum prolactin was normal (20.8 ± 8.2) and the concentrations of E2 and P showed the typical patterns of an ovulatory cycle (Table 1). The duration of the study cycle ranged between 25 and 28 days (26.9 ± 1.3 days). In contrast to the