

The Influence of Hormonal Changes During Menstrual Cycle on Serum Adiponectin Concentrations in Healthy Women

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Summary

Adiponectin is an adipocyte-derived hormone involved in the regulation of carbohydrate and lipid metabolism. Its concentrations are decreased in patients with obesity, type 2 diabetes and atherosclerosis and are higher in females than in males. Gender differences of adiponectin levels raise the possibility that sex hormones directly regulate its serum concentrations, which may in turn influence insulin sensitivity in different phases of the menstrual cycle. To test this hypothesis we measured serum adiponectin, estradiol, progesterone, luteinizing hormone and follicle-stimulating hormone concentrations daily throughout the menstrual cycle in six healthy women. Mean adiponectin levels strongly positively correlated with serum cortisol concentrations [$R=0.94286$; $p=0.0048$ (Spearman correlation test)], but were not significantly related to other anthropometric, biochemical and hormonal characteristics of the subjects (BMI, blood glucose, insulin, testosterone, prolactin, cholesterol, HDL cholesterol, LDL cholesterol, triglycerides concentrations, or atherogenic index). Furthermore, no significant changes of serum adiponectin levels were found throughout the menstrual cycle. We conclude that changes in sex hormones during the menstrual cycle do not affect total circulating adiponectin levels in healthy women. Therefore, the differences in insulin sensitivity in various phases of the menstrual cycle are not due to changes of circulating adiponectin levels.

Key words

Adiponectin • Menstrual cycle • Cortisol • Insulin sensitivity

Introduction

Adiponectin (OMIM 605441; also known as Acrp30, GBP28, AdipoQ and APM-1), the protein secreted predominantly by adipocytes (Maeda *et al.* 1996,

Saito *et al.* 1999) was described independently by Scherer *et al.* (1995) and Hu *et al.* (1996). Numerous functions of adiponectin have been reported including its insulin-sensitizing, anti-atherosclerotic and anti-inflammatory properties (Berg *et al.* 2002, Haluzik *et al.* 2004). In

contrast to most other adipose tissue-derived hormones adiponectin levels are decreased in patients with obesity, type 2 diabetes and/or atherosclerosis suggesting the possible role of its deficiency in the etiopathogenesis of these diseases (Arita *et al.* 1999). Interestingly, total plasma adiponectin concentrations are markedly higher than those of other hormones reaching quantities of mg/l. Adiponectin circulates in several isoforms that may have different target tissues and even distinct biological effects (Pajvani *et al.* 2004).

Plasma adiponectin concentrations are influenced by numerous factors and metabolic parameters. In addition to decreased adiponectin levels in obesity, type 2 diabetes and atherosclerosis a positive correlation of adiponectin with HDL-cholesterol was described in several studies (Arita *et al.* 1999, Kazumi *et al.* 2004). Glucose clamp studies have shown that plasma adiponectin concentrations are strongly positively associated with whole body glucose uptake – the measure of muscle insulin sensitivity (Weyer *et al.* 2001). Adiponectin levels are also gender-dependent being approximately 2-3 times higher in females compared to males (Bottner *et al.* 2004). The reason for these gender-related differences is not entirely clear. Experimental studies demonstrated that both androgens and estrogens decreased adiponectin levels (Combs *et al.* 2003, Nishizawa *et al.* 2002). Furthermore, decreased adiponectin levels were also found in the last trimester of physiological pregnancy and in patients with gestational diabetes (Mazaki-Tovi *et al.* 2005).

Physiological hormonal changes during the menstrual cycle are, among others, accompanied by changes of insulin sensitivity and the mechanism of these changes is not completely understood. Here we tested the hypothesis that hormonal fluctuations during menstrual cycle may affect serum adiponectin levels that in turn could modulate insulin sensitivity. To this end we performed daily measurements of adiponectin levels in six healthy women throughout the menstrual cycle and analyzed its changes in different menstrual cycle phases.

Methods

Six lean healthy Caucasian women (age 27.17 ± 5.19 years; BMI 23.44 ± 2.71 kg.m⁻²) with regular menstrual cycle (28 ± 1 day) without any use of hormonal contraception for at least six months were enrolled into the study. The subjects were informed about the purpose of the study and signed an informed consent approved by

the local ethical committee. The baseline clinical characteristics were obtained at the beginning of the study and biochemical analyses were assessed on the first day of menstruation (Table 1). Peripheral venous blood samples were withdrawn daily after overnight fasting between 7:30 and 8:30 h for one-month period. The serum was separated by centrifugation and stored at -80 °C until analysis.

Serum adiponectin concentrations were measured in duplicate by Human Adiponectin ELISA kit (Linco Research, USA) with intra- and inter-assay variability of (1.0-7.4 %) and (2.4-8.4 %), respectively. The menstrual cycle phase determination was based on the profiles of luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol and progesterone (PRG). Estradiol, FSH and LH serum concentrations were measured by two-step immunoassays using Chemiluminiscent Microparticle Immunoassay (CMIA) technology with flexible assay protocols, referred to as Chemiflex™. System: Architect i2000 (ABBOTT Laboratories). The Progesterone assay is a competitive immunoassay using direct chemiluminiscent technology, ADVIA Centaur System (BAYER Diagnostics).

The other biochemical parameters of initial investigations were measured at the Department of Clinical Biochemistry and Laboratory Medicine, General University Hospital and First Faculty of Medicine, Charles University in Prague, by routine laboratory methods. Serum glucose concentration was assessed by photometric enzyme assay GOD/POD (Pliva – Lachema), serum insulin concentration was measured by electro-chemiluminiscent immunoanalysis, serum concentration of triglycerides was measured by photometric enzyme assay GPO/PAP (Human Gesellschaft für Biochemica und Diagnostica mbH), serum concentrations of cholesterol and HDL cholesterol were studied by photometric enzyme assay CHOD/PAP (Human Gesellschaft für Biochemica und Diagnostica mbH) – all of them on the System MODULAR (ROCHE Diagnostics). Testosterone, prolactin and cortisol were assessed by chemiluminiscent immunoanalysis, ADVIA Centaur System (BAYER Diagnostics). LDL cholesterol serum concentration and the atherogenic index were calculated.

Statistical analysis was performed using S.A.S. (Statistical Analysis Software) release 8.02 and STATISTICA98 Edition software (StatSoft Inc., Tulsa, OK). According to an experienced biostatistician, the method of 5-points moving average was used for smoothing of curves of all daily measured parameters

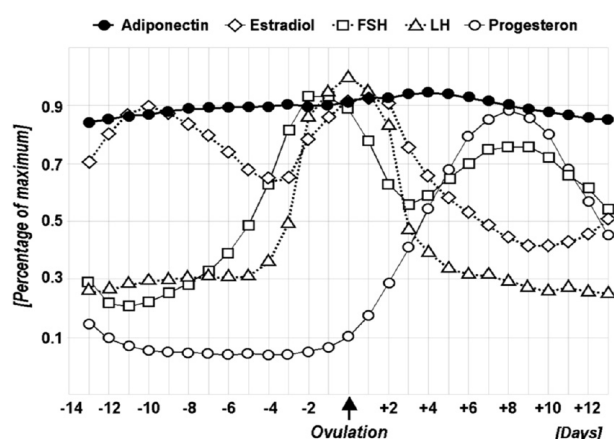


Fig. 1. Daily mean values (5-points moving average of the values on five consecutive days) of adiponectin, estradiol, FSH, LH and PRG in six analyzed subjects during their menstrual cycle (values at the beginning and at the end of menstrual cycle used for calculation of mean values are overlapping; day 0 = ovulation).

(adiponectin, estradiol, PRG, FSH and LH). This adjusting did not affect the direction and trend of the examined markers. The Spearman correlation test was used for analysis of correlations between adiponectin concentrations and the initial clinical/biochemical characteristics of the subjects. Analysis of changes in adiponectin concentration during menstrual cycle compared to all daily measured sexual hormones (estradiol, progesterone, LH and FSH) was performed using cross-correlation analysis. The cross-correlation coefficient represents the relationship between two series of data, where one variable lags behind a certain interval of the second variable. We examined the „best“ correlation between the variables at a given time (time delay). In other words, we have an independent or explanatory variable that affects the dependent variable with some time lag. This method allows us to investigate these lags and relations. We examined the time lags and correlations of adiponectin in relation by the other markers.

Results

In the entire cohort of six healthy women serum adiponectin levels showed 38.1 % variability of its mean concentrations throughout the menstrual cycle. The mean monthly adiponectin profile did not show significant changes with respect to the menstrual cycle or sexual hormone patterns (Fig. 1). However, the individual adiponectin concentrations fluctuated markedly in each subject throughout the cycle (Fig. 2) – the individual adiponectin variability ranged between 24.1 % and

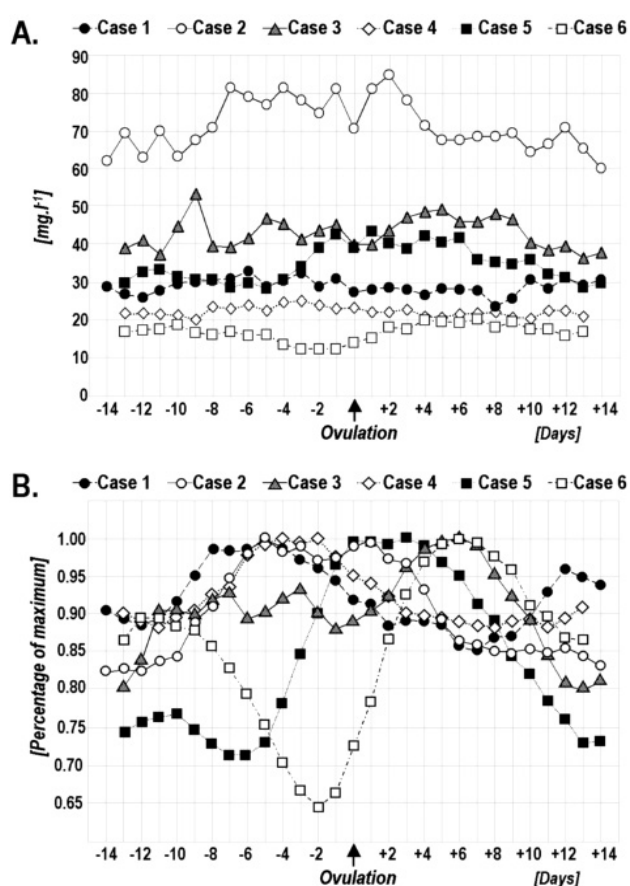


Fig. 2. Individual serum adiponectin fluctuations during menstrual cycle: (A.) absolute serum adiponectin concentrations in mg.l^{-1} ; (B.) relative changes (maximal serum adiponectin concentration represents 100 %; day 0 = ovulation).

50.3 % of individual mean adiponectin concentrations (Table 1). These individual changes were inconsistent and they did not cluster in any particular phase of the menstrual cycle (Table 1).

The baseline adiponectin levels were correlated with entrance characteristics of analyzed subjects (body mass index (BMI), serum concentrations of glucose, insulin, testosterone, prolactin, cortisol, triglycerides, total and HDL cholesterol, LDL cholesterol, and atherogenic index).

The only statistically significant positive correlation was found between cortisol and minimal, maximal, mean and median serum adiponectin values [(all) $R=0.94286$; $p=0.0048$. Similarly, the positive correlation was found between the range of adiponectin values (maximal – minimal) and serum cortisol concentration [$R=0.8857$; $p=0.0188$ (Spearman correlation test)]. No statistically significant relationship was found between adiponectin and other clinical and biochemical characteristics of analyzed subjects.

Table 1. Baseline anthropometric, biochemical and hormonal parameters of healthy females obtained on the first day of menstruation and characteristics of adiponectin concentrations obtained from daily measurements throughout the menstrual cycle.

Parameter	Subject no.					
	1	2	3	4	5	6
Entrance clinical and biochemical characteristics of the analyzed subjects						
Age [years]	36	25	28	27	20	27
High [m]	1.65	1.75	1.74	1.82	1.58	1.7
Weight [kg]	67	76	60	67	62	76
BMI [kg.m ⁻²]	24.6	24.8	19.8	20.2	24.8	26.3
Glucose [mmol.l ⁻¹]	4.7	5.0	4.4	4.3	4.8	4.5
Insulin [mIU.l ⁻¹]	2.6	4.5	4.4	2.7	10.3	5.3
Testosterone [nmol.l ⁻¹]	2.6	3.6	4.5	1.1	2.7	3.0
Prolactin [µg.l ⁻¹]	8.6	22.2	11.9	17.5	14.2	11.9
Cortisol [nmol.l ⁻¹]	475.2	607.3	480.6	443.3	488.3	406.1
Cholesterol [mmol.l ⁻¹]	5.0	4.4	5.4	3.7	4.1	5.7
HDL cholesterol [mmol.l ⁻¹]	2.0	1.7	2.0	1.9	1.8	2.2
LDL cholesterol [mmol.l ⁻¹]	2.5	2.2	3.0	1.5	2.0	3.1
Triglycerides [mmol.l ⁻¹]	0.9	1.0	0.8	0.8	0.7	1.0
Atherogenic index	1.5	1.5	1.7	1.0	1.3	1.6
Adiponectin values during follow-up period						
Acrp30 mean [mg.l ⁻¹]	28.2	71.7	42.6	21.6	34.1	15.9
Acrp30 median [mg.l ⁻¹]	28.3	70.4	42.2	21.5	33.1	16.1
Acrp30 minimum [mg.l ⁻¹] in day*	23.0 in +8	59.6 in +14	35.8 in +13	19.3 in -9	27.6 in -5	11.4 in -2
Acrp30 maximum [mg.l ⁻¹] in day*	32.5 in -6	84.9 in +2	53.0 in -9	24.5 in -3	43.0 in +1	19.4 in +7
Acrp30 fluctuation [%]**	82-115	83-118	84-124	89-113	81-126	72-122

* Day of menstrual period (day 0 = ovulation), ** Adiponectin fluctuation during menstrual cycle in individual subjects: expressed as minimum (%) – maximum (%); mean represents 100 %. Reference intervals in human serum: Glucose [4.2- 6.0 mmol.l⁻¹]; Insulin [2.5-24.0 mIU.l⁻¹]; Testosterone [0.3-0.8 nmol.l⁻¹]; Prolactin [3.6-13.4 µg.l⁻¹]; Cortisol [118-618 nmol.l⁻¹]; Cholesterol [3.1-5.2 mmol.l⁻¹]; HDL cholesterol [1.3-2.3 mmol.l⁻¹]; LDL cholesterol [1.5-4.2 mmol.l⁻¹]; Triglycerides [0.68-1.69 mmol.l⁻¹]; Atherogenic index [0-3.0]

Discussion

The most important finding of this study is that total circulating adiponectin levels are not significantly affected by hormonal changes during the menstrual cycle in a group of healthy women. Furthermore, total adiponectin concentrations did not correlate with sex hormone profiles. Analysis of individual variability of adiponectin concentrations showed relatively high intra-individual variability, but these changes were not consistently related to a particular phase of the menstrual cycle.

To our best knowledge this is the first study that evaluated the possible influence of the menstrual cycle on serum adiponectin levels. The changes of sex hormone

levels during the menstrual cycle affect numerous parameters including concentrations of some hormones. For example, the most extensively characterized adipose tissue-derived hormone leptin is markedly affected by the menstrual cycle being higher in the luteal relative to follicular phase (Hardie *et al.* 1997). Moreover, leptin levels are similarly to adiponectin affected by gender, being higher in females than in males (Maffei *et al.* 1995). The results of our study demonstrate that the regulation of serum adiponectin levels differs from leptin regulation with respect to the influence of the menstrual cycle. This finding is somewhat surprising in view of both *in vitro* and experimental data that demonstrated significant influence of both androgens and estrogens on circulating adiponectin levels (Nishizawa *et al.* 2002,

Combs *et al.* 2003)

One explanation for the negative results of our study may be related to the fact that measuring total adiponectin levels as performed in this study may not necessarily reflect the changes in its different circulating fractions. Previous studies have shown that adiponectin circulates in several isoforms such as low molecular weight (LMW) hexameric isoform and a high molecular weight (HMW) isoform consisting of two or three hexamers (Pajvani *et al.* 2004). The relative abundance of respective isoforms in the circulation may in turn affect the adiponectin biological activity without the changes of its total concentration (Pajvani *et al.* 2003). These authors have described sexual dimorphism in terms of circulating adiponectin complexes in mice: females had relatively balanced distribution of both LMW and HMW serum adiponectin isoforms, whereas predominantly the LMW form was found in males. Since only total adiponectin levels were measured in our study, the possible fluctuation in respective circulating isoforms of adiponectin may have been missed.

Different phases of the menstrual cycle can also affect insulin sensitivity (Bruns and Kemnitz 2004). It

has been demonstrated that insulin sensitivity decreases significantly during the luteal phase of the menstrual cycle. This decrease is at least partially the result of the changes of circulating sex hormones and possibly also of variations in food intake during the menstrual cycle. Our results do not support the hypothesis that changes in circulating total adiponectin levels are involved in this process. It should be noted that the changes in insulin sensitivity were not directly measured in our study and therefore our conclusion is based on the combination of our and previously published data (Bruns and Kemnitz 2004). Furthermore, we are aware of the relative low number of subjects included into our study, which may have partially affected the power of statistical analysis. Further studies are necessary to decide whether metabolic changes during the menstrual cycle might be mediated by modulations in various circulating adiponectin isoforms.

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