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Effect of postmenopause and hormone replacement therapy on serum adiponectin levels

Lucyna Sieminska[a,*], Celina Wojciechowska[b], Danuta Niedziolka[a], Bogdan Marek[a], Beata Kos-Kudla[a], Dariusz Kajdaniuk[a], Mariusz Nowak[a]

[a]*Department of Pathophysiology and Endocrinology, Silesian Medical University, 41-800 Zabrze, Poland*
[b]*II Department and Clinic of Cardiology, Silesian Medical University, 41-800 Zabrze, Poland*

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Abstract

Little is known about the effects of menopause and hormone replacement therapy (HRT) on adiponectin production. The objectives of the study were to compare levels of serum adiponectin in post- and premenopausal women, to test whether adiponectin levels are related to endogenous estradiol and sex hormone–binding globulin (SHBG) levels, to determine whether HRT influences serum adiponectin, and to investigate relationships of adiponectin levels with cardiovascular risk factors. One hundred four women matched for body mass index were enrolled in this study, and among them were 34 postmenopausal HRT nonusers, 34 postmenopausal HRT users, and 36 premenopausal healthy women with regular menstrual cycles. We evaluated waist circumference and waist-to-hip ratio (WHR) in each women. Serum was assayed for adiponectin, estradiol, SHBG, triglycerides (TGs), total cholesterol, high-density lipoprotein cholesterol, and fasting glucose levels. Post- and premenopausal women showed no significant differences in adiponectin and SHBG concentrations. There were no differences in serum adiponectin levels between postmenopusal HRT nonusers and users; however, SHBG concentrations were higher in HRT users. The simple linear regression analyses of all studied women indicated that serum adiponectin was negatively correlated with body mass index, waist circumference, WHR, and TG levels. Positive correlation was observed between adiponectin and high-density lipoprotein cholesterol as well as between adiponectin and SHBG levels. There were no relationships between adiponectin and estradiol levels in all studied women and among subgroups. Multiple regression analysis showed that WHR and TG were significant independent predictors of serum adiponectin. In conclusion, serum adiponectin levels are not influenced by menopausal status or serum estradiol levels. Exogenous estrogen treatment does not significantly affect serum adiponectin concentrations.

1. Introduction

Before menopause, women appear to be protected from atherosclerotic diseases when compared with men of similar age. Postmenopausal women are at increased risk for atherosclerotic diseases [1]. This is caused, at least in part, by a higher prevalence of visceral obesity [2]. In postmenopausal time, many women gain weight and, often, fat redistribution occurs. It is associated with increases in body mass index (BMI), waist circumference, and waist-to-hip ratio (WHR) [2]. An abdominal fat distribution is connected with concomitant alterations in the metabolic risk profile. Postmenopausal women are characterized by insulin resistance, dyslipidemia, and increased levels of plasminogen activator inhibitor type 1. It has been suggested that changes in sex steroid levels, mainly marked decrease of estradiol production, contribute to an increase in visceral adiposity. It has been reported that estrogens influence lipoprotein lipase activity, which regulate the metabolism of triglyceride (TG)-rich lipoproteins [3,4]. After menopause, higher abdominal and gluteal lipoprotein lipase activity were observed [5], and the overload of abdominal adipocytes with TG appeared. Because of the decline of estradiol levels, reduction of sex hormone–binding globulin (SHBG) concentrations also occurs. The observational studies suggest that low levels

* Corresponding author. Tel.: +48 32 2786126; fax: +48 32 2786126.
E-mail address: lusiem@poczta.onet.pl (L. Sieminska).

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of SHBG are strong correlates of obesity and risk factors for cardiovascular disease in women after menopause [6].

Recently, it has become evident that adipose tissue is an origin of metabolically active peptides such as adiponectin. Obese humans have decreased levels of adiponectin [7,8], and low levels of this hormone are assumed to play a role in pathogenesis of insulin resistance [7], atherosclerosis [8], and hypertension [9].

To the best of our knowledge, interactions between estrogens and adiponectin have not yet been fully characterized. Higher concentrations of adiponectin in women than in men of comparable age and BMI [8,10,11] suggest that estrogens might have a stimulatory impact on production of adiponectin by adipocytes. However, the results of existing studies are contradictory. Experimental study by Combs et al [12] showed that estrogens exert a negative impact on adiponectin levels. Other studies on animals seem to indicate that estrogens do not affect adiponectin secretion [10]. However, Gui et al [13] proved that estrogen replacement significantly increased adiponectin messenger RNA levels. Recently, the connections between adiponectin and lipoprotein lipase have been actively investigated; examples are studies by von Eynatten et al [14] and Bullo et al [15]. Unfortunately, the relationship between adiponectin and estrogens with respect to lipoprotein lipase remains uncertain.

There is also limited information about the influence of postmenopausal hypoestrogenism on serum adiponectin in women, and a few studies on this subjects are inconsistent [10,16-18]. We hypothesized that the weight gain and accumulation of body fat, with preferential storage in the intra-abdominal depot, that occur after menopause should decrease the production of adiponectin.

Hormone replacement therapy (HRT) after menopause prevents the weight gain [19] and has beneficial influence on fat distribution and lipid profile [20]; therefore, we also hypothesized that adiponectin concentrations in postmenopausal women using HRT could be higher than in women without HRT.

The objective of the study was to analyze relationships among adiponectin, endogenous estrogen status, exogenous estrogen treatment, and cardiovascular risk factors. To reach these goals we estimated levels of serum adiponectin in postmenopausal women and compared them with concentrations in premenopausal women; tested if estradiol and SHBG levels are related to adiponectin concentration; and determined whether HRT influences serum adiponectin and investigated relationships between adiponectin levels and various risk factors.

2. Subjects and methods

One hundred four pre- and postmenopausal women, matched for BMI, were enrolled in this study, which was approved by the Ethics Committee of Silesian Medical University (Katowice, Poland). They were recruited from women visiting the endocrinologic and gynecologic clinics for routine checkup. All women were healthy, overweight (BMI >25), and aged between 42 and 58 years. Women with diabetes mellitus, coronary artery disease, and hypertension were excluded from the study. The subjects were not taking medications, except one group that was on HRT. Anthropometric measurements were taken for each woman: height, weight, waist and hip circumferences, BMI, and WHR. Eligible subjects were divided into 3 groups.

Group 1 (HRT nonusers) consisted of 34 postmenopausal women who had never used HRT. Postmenopausal status was defined by amenorrhea for at least 1 year and confirmed by serum estradiol (<20 pg/mL) and follicle-stimulating hormone levels (>40 IU/L) or the age of at least 55 years without natural menses for at least 5 years.

Group 2 (HRT users) consisted of 34 postmenopausal healthy women who had used combined estrogen/progestogen HRT continuously for at least 6 months, but no longer than 5 years. The average therapy lasted 3 years. Women were administered orally 1 tablet daily of Activelle (NovoNordisk A/S, Bagsvaerd, Denmark, estradiol 1.0 mg plus norethisterone acetate 0.5 mg).

Group 3 (control) comprised premenopausal healthy women with regular menstrual cycles and no previous problems with cycle regularities, without hirsutism, and who have never taken contraceptives under any form (n = 36).

Blood pressure was measured at rest in lying position. Fasting blood samples were drawn, and serum samples were stored at −80°C for subsequent assays. In premenopausal women, blood was sampled during the early follicular phase of their cycle.

2.1. Biochemistry

Adiponectin concentrations were measured with a commercial radioimmunoassay kit (Linco Research, St Charles, Mo). Circulating SHBG and estradiol levels were determined using immunoradiometric assay (Diagnostic Systems Laboratories, Webster, Tex). The inter- and intra-assay coefficients of variation were less than 10% across the range of measured results. Total cholesterol (Chol), high-density lipoprotein cholesterol (HDL-C), and TG were measured according to standard enzymatic methods.

We evaluated in each women the following risk factors: BMI, waist circumference, WHR, Chol, TG, HDL-C, and fasting glucose levels.

2.2. Statistical analysis

Statistical calculations were performed using Statistica 6.0 package made by StatSoft, Tulsa, OK. Comparison of normally distributed variables was performed using unpaired Student *t* test. However, many variables (serum adiponectin, SHBG, estradiol, TG) were not normally distributed in Shapiro-Wilk test, and nonparametric statistical test was used. Groups were compared with Mann-Whitney *U* test. The relations between particular parameters were estimated by calculating the correlation coefficient *r*

Table 1
The clinical and metabolic parameters of studied women and comparison between the groups

Parameter	Group 1: postmenopausal women without HRT (n = 34)	Group 2: postmenopausal HRT users (n = 34)	Group 3: premenopausal women (n = 36)	Differences between groups 1 and 2 (*P*)	Differences between groups 1 and 3 (*P*)
BMI	28.6 ± 2.8	27.6 ± 2.1	27.8 ± 1.1	NS	NS
Waist circumference (cm)	90.2 ± 4.0	85.4 ± 3.7	84.2 ± 4.7	<.01	<.01
WHR	0.86 ± 0.05	0.82 ± 0.04	0.82 ± 0.05	<.01	<.01
Age	54.6 ± 2.9	53.5 ± 3.5	46.5 ± 2.5	NS	<.01
Adiponectin (μg/mL)	14.9 (10.6; 18.0)	15.2 (10.2; 17.7)	14.4 (12.2; 18.6)	NS	NS
Estradiol (pg/mL)	13.4 (7.0; 19.3)	39.6 (12.5; 71.6)	51.5 (25.0; 130.3)	<.001	<.001
SHBG (nmol/L)	24.3 (12.3; 32.7)	39.2 (26.0; 56.5)	26.9 (16.1; 51.7)	<.001	NS
Chol (mmol/L)	6.0 ± 1.0	5.6 ± 0.8	5.6 ± 0.7	NS	NS
HDL-C (mmol/L)	1.3 ± 0.2	1.3 ± 0.3	1.4 ± 0.2	NS	.05
TG (mmol/L)	1.9 (1.5; 2.1)	1.8 (1.4; 1.7)	1.1 (0.8; 1.4)	NS	<.001
Fasting glucose (mmol/L)	5.5 ± 0.6	5.4 ± 0.5	5.6 ± 0.4	NS	NS

Skewed data are given as median plus (25th; 75th) percentiles. Normally distributed data are given as mean ± SD. The significance tests used are the Mann-Whitney *U* test for normally distributed variables and unpaired *t* test for normally distributed variables. NS indicates not significant.

by the Spearman method. Analysis of covariance was used to examine differences between groups after statistically removing the effect of selected covariates. Multiple regression analysis was performed with serum adiponectin and SHBG level as dependent variables, respectively.

3. Results

3.1. Comparison of post- and premenopausal women

Table 1 shows the clinical and biochemical characteristics of the studied groups.

As expected, postmenopausal women were significantly older than premenopausal women, and they showed lower estradiol concentrations. There was no significant difference in BMI; however, waist circumference and WHR were significantly higher in postmenopausal compared with premenopausal women. Pre- and postmenopausal women showed no significant differences in adiponectin and SHBG levels. No differences were also found when data were statistically adjusted for age or for WHR, or for waist circumference in analysis of covariance. In postmenopausal women, serum TG was significantly higher and HDL-C tended to be decreased. No differences were seen in the Chol and in the fasting glucose.

3.2. Comparison of postmenopausal HRT users and HRT nonusers

Although there were no differences in age and BMI between women taking HRT and those not on the HRT, postmenopausal HRT users were characterized by statistically lower WHR and waist circumferences. However, there were no differences in serum adiponectin levels (Table 1). No differences were also found when data were statistically adjusted for WHR or for waist circumference in analysis of covariance. Among women using HRT, median estradiol and SHBG levels were significantly higher than in women not using HRT; however, there were no differences in lipids and fasting glucose.

3.3. Relationships between adiponectin and other parameters

To assess which factors affect concentration of circulating adiponectin, associations with anthropometric measures, estradiol, SHBG, lipids, and fasting glucose were calculated. A simple linear regression analyses of all studied women indicated that serum adiponectin was negatively correlated with BMI, waist circumference, WHR, and with TG levels (Table 2). Positive correlation was observed between adiponectin and HDL-C, as well as between adiponectin and SHBG levels. However, the relation between adiponectin and SHBG disappeared after including obesity parameters: waist circumferences, WHR, and BMI in the regression model. A simple linear regression analysis revealed that serum SHBG was negatively correlated with BMI, waist circumference, WHR, and with TG levels. Positive correlation was observed between SHBG and HDL-C (Table 2). There were no connections between

Table 2
Spearman coefficients of the relationships among adiponectin, SHBG, and other variables in all studied women

Variable	Adiponectin		SHBG	
	r	*P*	*r*	*P*
BMI	−0.35	<.001	−0.40	<.001
Waist circumference	−0.42	<.001	−0.38	<.001
WHR	−0.41	<.001	−0.35	<.001
Age	−0.21	NS	−0.11	NS
Adiponectin level			0.29	<.01
Estradiol level	−0.15	NS	−0.21	NS
SHBG level	0.29	<.01		
Chol level	0.09	NS	0.15	NS
HDL-C level	0.34	<.01	0.28	<.01
TG level	−0.41	<.001	−0.36	<.01
Glucose level	−0.10	NS	−0.15	NS

adiponectin and estradiol levels in all studied women and among subgroups (the post- and premenopausal). Variables in simple linear regression analyses that were found to be correlated with serum adiponectin were used in multiple regression analysis. In a model including adiponectin as dependent variable and BMI, WHR, waist circumference, SHBG, TG, and HDL-C levels as independent variables, WHR and TG remained as parameters independently related to adiponectin level ($R^2 = 0.24$, $P < .001$). Using a multiple regression analysis with SHBG as dependent variable and a model including BMI, waist circumference, WHR, HDL-C, TG, and the presence of HRT (yes/no) as independent variables, only HRT was significantly associated with SHBG level ($R^2 = 0.30$, $P < .001$).

4. Discussion

Several hormonal factors have recently been reported to be involved in the regulation of adiponectin synthesis. Nishizawa et al [10] and Lanfranco et al [21] demonstrated that testosterone suppressed the secretion of adiponectin. Combs et al [12] observed that prolactin leads to a reduction of adiponectin production. It is known that hyperinsulinemia and/or insulin resistance predisposes to a decreased adiponectin production [7,11]. It has been also shown that catecholamines have inhibitory effect on adiponectin expression [22]. However, the influence of estrogens, estradiol in particular, is not fully understood. Estrogen deficiency in postmenopausal women had been proposed as an explanation for the rise in cardiovascular risk associated with menopause. On the other hand, low levels of adiponectin are assumed to be a risk factor for insulin resistance and cardiovascular risk [7,8,11,16]. Therefore, we initially assumed that postmenopausal women with low levels of estradiol could have lower adiponectin levels than premenopausal women with higher estradiol levels. However, as this study indicates, adiponectin levels are similar for both pre- and postmenopausal women. Our results are concordant with the findings of Nishizawa et al [10], but appear to contradict results of Gavrila et al [18] who reported that postmenopausal women have higher adiponectin levels than premenopausal subjects. Moreover, the age-related increases in adiponectin levels in women have been reported [11,16]. Discrepancies can be attributed to differences in body composition of the subjects. In the study of Gavrila et al [18], the premenopausal subjects were characterized by an increased adiposity compared with their postmenopausal counterparts. After Matsubara and colleagues [16] adjusted their data for body fat mass, they also obtained results that were in agreement with our conclusions. These authors found that there was a positive correlation between age and adiponectin concentration; however, after body mass factor had been included in the analysis, there was no association between age and adiponectin.

We also hypothesized that adiponectin concentrations in postmenopausal women using estrogens could be higher than in women without HRT. Consistent with another study [18], we found similar levels of adiponectin in postmenopausal women, whether or not they had participated in the HRT. Recently, it was also found that 3- and 6-month-long hormonal therapy did not affect the adiponectin level [17]. Lack of differences in adiponectin concentrations is not the argument for positive influence of HRT on cardiovascular system.

In our study, we found no correlation between adiponectin and estradiol serum levels, neither among postmenopausal nor premenopausal women, which contradicts the results of Gavrila et al [18] and Tanko et al [23]. Those results suggesting suppressed influence of estradiol on adiponectin level are surprising because adiponectin concentrations are greater in females than in males. The relatively small size of all above-mentioned studies, as well as differences in metabolic statuses of studied populations, may explain discrepancies.

Menopause is associated with decreased production of SHBG [6]. Because the low SHBG level is a characteristic marker for insulin resistance [24] and cardiovascular risk [25], we also measured the serum concentration of SHBG and its relationships with adiponectin. Consistent with earlier reports [26], low levels of SHBG were associated with low HDL-C, high TG, and abdominal obesity. We found that adiponectin concentrations were also associated with SHBG. In agreement with these results, we previously reported positive correlations between serum adiponectin and SHBG in women with polycystic ovarian syndrome [27]. These associations, however, appeared to be explained by associations of obesity (as assessed by BMI, waist circumference, and WHR) with adiponectin and SHBG. It has been shown that hyperinsulinemia inhibit production of SHBG in liver, and low SHBG levels may indicate the presence of insulin resistance [25]. Furthermore, low SHBG levels are related to increased androgenicity in women or to relative increase of androgen to estrogen balance [24]. Thus, we speculate that adiponectinemia in women could be related to the androgen-estrogen ratio. Unfortunately, in the present study, androgen levels were not measured, and this hypothesis needs to be verified.

We confirmed that WHR and TG were the major determinants of adiponectin concentration. It strongly suggests that there is a close association between intra-abdominal fat and adiponectin level in women.

Our results clearly indicated that adiponectin levels in women of different menopausal status and those on HRT were not different, a finding suggesting that adiponectin levels are not regulated by estrogens. The absence of menopause-related differences in our study could be associated with the particular, cross-sectional design. Thus, time-dependent studies that examine changes in adiponectin as women transition from the premenopausal to the postmenopausal stage are planned. Fortunately, the patients in the region are not very mobile, and there is chance that we

will able to follow the same set of patients over the next 10 years. We showed positive correlation between adiponectin and SHBG levels and explained these results in terms of their association with obesity. Larger studies of the relationships between all sex steroid hormones, fat distribution, and adiponectin in postmenopausal women are needed to elucidate physiological and pathophysiological associations of adipose tissue and menopausal status.

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References

[1] Barrett-Connor E. Epidemiology and the menopause: a global overview. Int J Fertil Menopausal Stud 1993;38:6-14.
[2] Colombel A, Charbonnel B. Weight gain and cardiovascular risk factors in the postmenopausal women. Hum Reprod 1997;1:134-45.
[3] Palin SL, McTernan PG, Anderson LA, Sturdee DW, Barnett AH, Kumar S. 17 beta-estradiol and anti-estrogen ICI: compound 182,780 regulate expression of lipoprotein lipase and hormone-sensitive lipase in isolated subcutaneous abdominal adipocytes. Metabolism 2003;52:383-8.
[4] Price TM, O'Brien SN, Welter BH, George R, Anandjiwala J, Kilgore M. Estrogen regulation of adipose tissue lipoprotein lipase-possible mechanism of body fat distribution. Am J Obstet Gynecol 1998; 178:101-7.
[5] Tchernof A, Desmeules A, Richard C, et al. Ovarian hormone status and abdominal visceral adipose tissue metabolism. J Clin Endocrinol Metab 2004;89:3425-30.
[6] Tchernof A, Despres JP. Sex steroid hormones, sex hormone–binding globulin, and obesity in men and women. Horm Metab Res 2000; 32:526-36.
[7] Weyer C, Funahashi T, Tanaka S, et al. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. J Clin Endocrinol Metab 2001;86:1930-5.
[8] Hotta K, Funahashi T, Arita Y, et al. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol 2000;20:1595-9.
[9] Adamczak M, Wiecek A, Funahashi T, Chudek J, Kokot F, Matsuzawa Y. Decreased plasma adiponectin concentration in patients with essential hypertension. Am J Hypertens 2003;16:72-5.
[10] Nishizawa H, Shimomura I, Kishida K, et al. Androgens decrease plasma adiponectin, an insulin-sensitizing adipocyte-derived protein. Diabetes 2002;51:2734-41.
[11] Cnop M, Havel J, Utzschneider KM, et al. Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. Diabetologia 2004;46:459-69.
[12] Combs TP, Berg AH, Rajala MW, et al. Sexual differentiation, pregnancy, calorie restriction, and aging affect the adipocyte-specific secretory protein adiponectin. Diabetes 2003;52:268-76.
[13] Gui Y, Silha GY, Murphy LJ. Sexual dimorphism and regulation of resistin, adiponectin, and leptin expression in the mouse. Obes Res 2004;12:1481-91.
[14] von Eynatten M, Schneider JG, Humpert PM, et al. Decreased plasma lipoprotein lipase in hypoadiponectinemia: association independent of systemic inflammation and insulin resistance. Diabetes Care 2004; 27:2925-9.
[15] Bullo M, Salas-Salvado J, Garcia-Lorda P. Adiponectin expression and adipose tissue lipolytic activity in lean and obese women. Obes Surg 2005;15:382-6.
[16] Matsubara M, Maruoka S, Katayose S. Decreased plasma adiponectin concentrations in women with dyslipidemia. J Clin Endocrinol Metab 2002;87:2764-9.
[17] Sumino H, Takahashi T, Itoh T, et al. Plasma adiponectin levels in post-menopausal women receiving hormone replacement therapy. J Int Med Res 2004:639-45.
[18] Gavrila A, Chan JL, Yiannakouris N, et al. Serum adiponectin levels are inversely associated with overall and central fat distribution but not directly regulated by acute fasting or leptin administration in humans: cross-sectional and interventional studies. J Clin Endocrinol Metab 2003;88:4823-31.
[19] Matthews KA, Abrams B, Crawford S, et al. Body mass index in mid-life women: relative influence of menopause, hormone use, and ethnicity. Int J Obes Relat Metab Disord 2001;25:863-73.
[20] Tchernof A, Poehlman ET, Despres JP. Body fat distribution, the menopause transition, and hormone replacement therapy. Diabetes Metab 2000;26:12-20.
[21] Lanfranco F, Zitzmann M, Simoni M, Nieschlag E. Serum adiponectin levels in hypogonadal males: influence of testosterone replacement therapy. Clin Endocrinol 2004;60:500-7.
[22] Fasshauer M, Klein J, Neumann S, Eszlinger M, Paschke R. Adiponectin gene expression is inhibited by beta-adrenergic stimulation via protein kinase A in 3T3-L1 adipocytes. FEBS Lett 2001;507:142-6.
[23] Tanko LB, Bruun JM, Alexandersen P, et al. Novel associations between bioavailable estradiol and adipokines in elderly women with different phenotypes of obesity. Circulation 2004;110:2246-52.
[24] Hautanen A. Synthesis and regulation of sex hormone–binding globulin in obesity. Int J Obes Relat Metab Disord 2000;24s: 64-70.
[25] Rexrode KM, Manson JE, Lee IM, et al. Sex hormone levels and risk of cardiovascular events in postmenopausal women. Circulation 2003;108:1688-93.
[26] Tchernof A, Toth MJ, Poehlman ET. Sex hormone–binding globulin levels in middle-aged premenopausal women. Association with visceral obesity and metabolic profile. Diabetes Care 1999;22:1875-81.
[27] Sieminska L, Marek B, Kos-Kudla B, et al. Serum adiponectin in women with polycystic ovarian syndrome and its relation to clinical, metabolic and endocrine parameters. J Endocrinol Invest 2004;28: 528-34.