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Circulating leptin and ghrelin are differentially influenced by estrogen/progestin therapy and raloxifene

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Abstract

Background: Leptin and ghrelin are increasingly being recognized as cardiotropic hormones, promoting or inhibiting the atherosclerotic process, respectively. Apoptosis may be one pathway through which the actions of these hormones are mediated. Sex hormones are reported to influence the secretion and action of ghrelin and leptin.
Objective: To evaluate (1) the association of circulating ghrelin and leptin with selected markers of receptor-mediated apoptosis and (2) the effect of estrogen monotherapy, low dose estrogen–progestin therapy, tibolone and raloxifene on serum ghrelin and leptin in healthy postmenopausal women.
Methods: Eighty eight postmenopausal women aged 44–62 years were randomly allocated to daily (1) conjugated equine estrogens 0.625 mg (CEE), (2) 17β-estradiol 1 mg plus norethisterone acetate 0.5 mg (E_2/NETA), (3) tibolone 2.5 mg, (4) raloxifene HCl 60 mg or (5) no treatment. Serum markers of apoptosis sFas, Fas-ligand (Fas-L) and caspase-1 were measured at baseline. Serum leptin and ghrelin were measured at baseline and at 3 months.
Results: Body Mass Index (BMI) and estradiol levels correlated positively, while FSH correlated negatively with serum leptin (BMI: $r = 0.646$, $p = 0.005$, estradiol: $r = 0.432$, $p = 0.001$, FSH: $r = -0.401$, $p = 0.002$). Insulin levels associated positively with circulating leptin ($r = 0.394$, $p = 0.011$) and negatively with circulating ghrelin ($r = -0.401$, $p = 0.009$). Serum leptin decreased significantly in E2/NETA group (baseline: 2.882 ± 0.76 ng/ml, 3 months: 2.687 ± 0.66 ng/ml, $p = 0.043$), while it increased significantly in the raloxifene group (baseline: 2.671 ± 0.54 ng/ml, 3 months: 2.839 ± 0.47 ng/ml). Ghrelin levels decreased significantly only in the raloxifene group (baseline: 1634 ± 592 pg/ml, 3 months: 1408 ± 534 pg/ml).
Conclusion: Apoptosis may be a pathway through which leptin exerts a pro-atherogenic effect. Low dose HT may act carioprotectively by decreasing letpin levels in healthy recently menopaused women.
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Keywords: Leptin; Ghrelin; Apoptosis; Postmenopausal; Hormone therapy; Estrogen; Tibolone; Raloxifene

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1. Introduction

Cardiovascular disease (CVD) is a leading cause of death in postmenopausal women [1]. The low prevalence of cardiovascular disease (CVD) in premenopausal women and the increase of CVD risk following menopause, has led to the hypothesis that estrogens have a cardioprotective effect in women [2]. Indeed, several observational epidemiological studies suggested that hormone therapy (HT) may decrease significantly the risk for CVD [3]. Recent randomized trials, however, (WHI, HERS) associated HT with an increased risk in early coronary events [4,5]. This discrepancy between observational and clinical studies has raised the question about the timing of estrogen administration in relation to the age and the menopause duration of the individual woman. Furthermore, clinical interest has shifted towards alternative therapies. Tibolone is a synthetic compound with estrogenic, progestogenic and androgenic properties, which is extensively used in Europe for the treatment of climacteric symptoms as well as for the prevention of postmenopausal osteoporosis. Tibolone is suggested to have a favorable effect on various markers of CVD and to have a lesser stimulative effect on coagulation compared to HT [6]. Raloxifene is a selective estrogen receptor modulator (SERM), indicated for the prevention and treatment of postmenopausal osteoporosis. Raloxifene has an estrogen-agonist effect on the skeleton, while it acts as an estrogen antagonist on the breast and the endometrium [7]. The effect of these commonly prescribed regimens on the cardiovascular system in postmenopausal women according to age and stage of atherosclerosis has not been thoroughly studied.

Leptin, a 146 amino acid polypeptide, is synthesized and secreted mainly from subcutaneous adipose tissue [8]. Leptin was initially considered as a satiety-signaling hormone, influencing body weight homeostasis through its effects on food intake and energy expenditure by negative feedback on the hypothalamic nuclei [9]. The widespread distribution of functioning leptin receptors on the endothelium [10] and on vascular smooth muscle cells [11] has indicated that the cardiovascular system may also be an alternative target of leptin activity. In general, leptin's effect on the cardiovascular system appears to be proatherogenic, by inducing oxidative stress, endothelial dysfunction, platelet aggregation, as well as the migration, hypertrophy and proliferation of smooth muscle cells [12].

Insulin is a potent stimulus of leptin secretion [13]. Sex steroids also modulate leptin production, although the exact mechanism remains unclear. Several studies evaluating the action of sex steroids on leptin levels showed a stimulatory effect of estrogens and an inhibitory action of androgens both in vitro and in vivo [14]. The effect of HT on leptin still remains controversial [15–17], while data regarding tibolone and raloxifene is even more sparse.

Ghrelin is a 28-amino acid identified as the natural ligand of the GH secretagogue (GHS) receptor and it is considered as the only circulating factor promoting appetite and food intake [18]. Although predominantly produced by the stomach and the gut, ghrelin is synthesized by multiple tissues throughout the human body. The spread of ghrelin receptors includes the cardiovascular system, implying a multifunctional role of this gastric hormone [19]. Ghrelin is suggested to exert a favorable cardiovascular effect by reducing inflammation, by decreasing blood pressure and by improving peripheral glucose disposal as well as vascular compliance [20]. Animal studies have suggested that estrogens are implicated in the regulation of ghrelin secretion [21]. To our knowledge, there are only few published clinical studies regarding the effect of oral HT on ghrelin levels [22,23], while there is no data on the effect of tibolone and raloxifene on serum ghrelin.

Apoptosis has a crucial role in the pathogenesis of atherosclerosis. In early stages apoptotic loss of inflammatory cells may delay the atherosclerotic process by inhibiting the transformation of subintimal monocytes to foam cells. However, once the atheromatous plaque is formed, apoptosis of smooth muscle cells within the plaque may cause its destabilization and subsequent rupture [24]. Fas ligand (Fas-L) is a membrane protein which has the ability to induce apoptosis in cells expressing the Fas receptor (Fas). Soluble Fas (sFas), the circulating form of membrane-bound Fas, has been associated with increased risk for CVD [25].

Given the widespread use of HT, tibolone and raloxifene in postmenopausal women and the lack of data concerning the cardiovascular implication of ghrelin and leptin in this population, we undertook this study in order to investigate: (1) the association of serum leptin and ghrelin with endogenous sex hormones, insulin resistance and circulating markers of receptor-

mediated apoptosis and (2) the effect of estrogen monotherapy, low-dose combined hormone therapy, tibolone and raloxifene on circulating ghrelin and leptin levels.

2. Methods

Subjects were recruited from the Menopause Clinic of the 2nd Department of Obstetrics and Gynecology, University of Athens, Aretaieion Hospital. Patients were at least 2 years menopausal. Women who were past users of HT, tibolone or raloxifene were not included in the study unless they had been off-therapy for at least 6 months. The present study is part of a research protocol conducted in our Clinic aiming to investigate the effect of various commonly prescribed regimens on selected markers of vascular inflammation and apoptosis as well as on adipocytokines [26]. Before recruitment in the study, patients had a gynecological and biochemical evaluation which included: bimanual examination, PAP smear and transvaginal sonography, breast examination and mammography, thyroid–liver–renal function as well as blood coagulation tests and bone densitometry. Criteria for inclusion in the study were the absence of climacteric complaints, an endometrial thickness $\leq$5 mm, the absence of a history of gynecological malignancy, ischemic heart disease, thromboembolism, diabetes mellitus, non-treated thyroid dysfunction and the intake of lipid-lowering or antihypertensive medication.

One hundred postmenopausal women aged 44–62 years were randomly assigned to one of the following regimens: (1) conjugated equine estrogens 0.625 mg (CEE, Premarin, Wyeth–Ayerst Lab, Philadelphia, PA), (2) 17β-estradiol 1 mg plus norethisterone acetate 0.5 mg (E_2/NETA, Activelle, Novo–Nordisk, Copenhagen, Denmark), (3) tibolone 2.5 mg (Livial, Organon, Oss, The Netherlands), (4) raloxifene HCl 60 mg (Evista, Lilly, Indianapolis, USA) or (5) no treatment. All subjects signed an informed consent and Institutional Review Board approval was obtained by the Ethics Committee of Aretaieion Hospital.

The study period was 3 months. This time period was considered sufficient for hormones to take effect without associating with endometrial hyperplasia in the case of estrogen monotherapy. Blood pressure, weight and height were recorded in the morning in light clothing and Body Mass Index (BMI) was computed at each visit. Fasting blood samples were drawn in the morning, centrifuged and serum was stored at −80 °C until assayed.

Serum leptin was measured by the commercial ELISA kit: DPL00, R&D Systems Inc., Minneapolis, USA. The sensitivity was 0.5 ng/ml, the intra-assay coefficient of variation (CV) was 3.3% and the inter-assay CV was 5.4%. Serum total ghrelin was measured by the commercial RIA kit: GHRT-89HK, Linco Research, MO, USA. The sensitivity was 93 pg/ml, the intra-assay CV was 10% and the inter-assay CV was 14.7%. Soluble Fas and Fas-L were measured by commercial kits: R&D Systems Inc., catalog number: DFS00 and DFL00, respectively. The intra-assay coefficient of variation (CV) was 4.6% and 5.4%, the inter-assay CV was 6.7% and 8.8%, the sensitivity was 20 pg/ml and 2.66 pg/ml and the measuring range was 31.2–2000 pg/ml and 15.6–1000 pg/ml, respectively. Serum caspase-1 was measured on ELISA kit: R&D Systems Inc., catalog number: DCA100. The intra-assay coefficient of variation (CV) was 6.1%, the inter-assay CV was 9.4% and the sensitivity was 0.68 pg/ml. The measuring range was 6.25–400 pg/ml.

Estradiol was measured with the commercial Enzyme Immunoassay kit: DSK-10-4300, Diagnostic Systems Laboratories Inc. The total CV Ranged from 4.3% to 6.1% and the sensitivity was 8 pg/ml. Total Testosterone was measured with the DPC kit: "Total Testosterone" on Immulite analyzer (Diagnostic Products Corporation, Los Angeles, USA). The total %CV ranged from 8.0% to 16.0% and 8.1% to 15%, respectively. Sex Hormone Binding Globulin (SHBG) concentrations were measured with the chemiluminescent enzyme immunometric assay kit: DPC, Immulite SHBG on Immulite analyzer (Diagnostic Products Corporation). The total coefficient of variation ranged from 4.1% to 9.2%. Free Androgen Index (FAI) was calculated using total testosterone and SHBG values by the following equation: FAI = testosterone (ng/ml) × 3.47 × 100/SHBG (nmol/l). Insulin was measured by the Abbott kit "Insulin" on the Imx analyzer (Abbott Laboratories, USA). The total coefficient of variation (CV) % ranged from 4.4 to 6.0.

Statistical analysis was performed by SPSS Version 8.0 (Statistical Package for the Social Sciences, Chicago, IL). Variables that were not normally

distributed were log-transformed for further analysis. Baseline characteristics were compared between therapy groups by analysis of variance (ANOVA) for continuous variable and by chi-square for categorical variables. Pearson correlation analysis was used for associations between baseline continuous variables. Baseline and follow-up mean levels of leptin and ghrelin were compared across the same therapy group by paired *t*-test. Percental changes of these parameters between groups were assessed by ANOVA. Statistical significance was set at the 0.05 level.

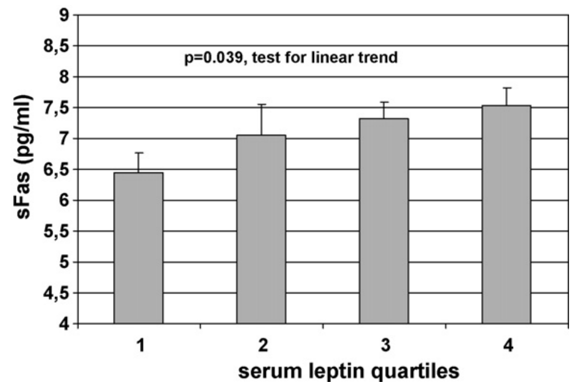

Fig. 1. Mean circulating soluble Fas antigen (pg/ml) across increasing serum leptin quartiles in 88 healthy recently menopaused women.

3. Results

From the 100 women originally enrolled in the study, 88 completed the 3-month study period and were entered in the statistical analysis. Baseline demographic characteristics according to treatment assignment are presented in Table 1. No differences with respect to age, menopausal age, BMI, lipids or hormones were detected.

Pearson correlation coefficients between baseline serum leptin and ghrelin and demographic characteristics and serum hormones are presented in Table 2. Serum insulin was negatively correlated with ghrelin, while leptin exhibited a positive correlation with this parameter (ghrelin: $r = -0.401$, $p = 0.009$; leptin: $r = 0.394$, $p = 0.011$). Body Mass Index (BMI) and estradiol levels correlated positively, while FSH correlated negatively with serum leptin (BMI: $r = 0.646$, $p = 0.005$, estradiol: $r = 0.432$, $p = 0.001$, FSH: $r = -0.401$, $p = 0.002$). Serum sFas increased linearly with increasing leptin quartiles (Fig. 1). No association either with serum ghrelin or leptin was detected with sFasL or caspase-1.

Baseline and 3-month mean values of serum leptin and ghrelin are presented in Table 3). Serum leptin decreased significantly in the E_2/NETA group (baseline: 2.882 ± 0.76 ng/ml, 3 months: 2.687 ± 0.66 ng/ml, $p = 0.043$), while it increased significantly in the raloxifene group (baseline: 2.671 ± 0.54 ng/ml, 3 months: 2.839 ± 0.47 ng/ml).

Table 1
Baseline deomographic, anthropometric and hormonal characteristics of the 88 healthy postmenopausal characteristics who participated in the study

Continuous variables	Controls ($n = 19$) mean (S.D.)	CEE ($n = 16$) mean (S.D.)	E_2/NETA ($n = 15$) mean (S.D.)	Tibolone ($n = 18$) mean (S.D.)	Raloxifene ($n = 20$) mean (S.D.)	p^{**}
Age (years)	52.9 (3.3)	52.2 (4.0)	51.2 (5.0)	53.9 (3.2)	53.0 (4.3)	0.48
Years since menopause	4.1 (2.0)	3.9 (2.7)	3.2 (3.1)	4.2 (4.6)	3.4 (2.1)	0.36
BMI (kg/m^2)	25.8 (2.9)	24.9 (3.4)	23.6 (3.5)	26.2 (1.0)	24.4 (1.0)	0.21
FSH (mIU/ml)	60.9 (35.5)	66.7 (38.5)	62.3 (32.8)	82.9 (35.4)	90.6 (29.8)	0.11
Estradiol (pg/ml)[a]	19.0 (14.8)	18.8 (18.2)	14.5 (12.2)	17.0 (8.1)	21.0 (10.1)	0.27
Testosterone (ng/dl)[a]	0.50 (0.49)	0.63 (0.295)	0.48 (0.285)	0.40 (0.21)	0.45 (0.68)	0.59
Sex hormone binding globulin (nmol/l)[a]	39.9 (18.3)	66.0 (36.3)	60.2 (29.9)	43.7 (34.4)	58.1 (29.6)	0.14
Δ4*A* (ng/dl)[a]	112.9 (44.5)	110.7 (95.5)	133.4 (63.1)	117.3 (58.0)	170.4 (90.9)	0.33
DHEAS (ng/dl)[a]	1107.5 (747.3)	1626.7 (915.7)	902.0 (515.6)	1011.9 (616.6)	1005.3 (636.6)	0.52
Free androgen index	5.18 (5.486)	2.84 (1.649)	3.57 (1.52)	4.76 (5.21)	3.18 (6.22)	0.39
Insulin (iIU/ml)[a]	7.3 (5.1)	8.6 (10.4)	6.9 (2.5)	9.6 (2.5)	9.0 (4.4)	0.25

[a] Variables with skewed distribution. Median is presented instead of mean.
[**] *p*: comparison between groups by ANOVA for continuous variables.

Table 2
Pearson correlation coefficients of demographic, anthropometric and hormonal parameters with serum leptin and ghrelin in 88 healthy postmenopausal women

	Leptin		Ghrelin	
	r	*p*	*r*	*p*
Age (years)	0.130	0.280	−0.050	0.677
Years since menopause	−0.063	0.608	0.107	0.382
BMI (kg/m^2)	0.646	0.005	−0.143	0.585
FSH (mIU/ml)	−0.401	0.002	0.160	0.239
Estradiol (pg/ml)[a]	0.432	0.001	−0.090	0.519
Testosterone (ng/dl)[a]	−0.122	0.418	−0.126	0.405
Sex hormone binding globulin (nmol/l)[a]	−0.277	0.069	0.170	0.269
Δ4*A* (ng/dl)[a]	−0.152	0.320	0.096	0.530
DHEAS (ng/dl)[a]	−0.101	0.501	0.083	0.581
Free Androgen Index[a]	0.139	0.376	−0.222	0.153
Insulin (iIU/ml)[a]	0.394	0.011	−0.401	0.009

[a] Logarithmically transformed variable.

Table 3
Baseline and follow-up serum leptin and ghrelin levels (mean, S.D.) in 88 healthy postmenopausal women according to treatment assignment

Months	Controls (*n* = 19)	CEE (*n* = 16)	E_2/NETA (*n* = 15)	Tibolone (*n* = 18)	Raloxifene (*n* = 20)
Leptin (ng/ml)					
0	21.48 (10.89)	14.86 (8.68)	22.57 (14.80)	20.10 (11.97)	17.09 (13.20)
3	21.00 (9.58)	12.50 (9.42)	17.70 (10.56)	18.44 (7.03)	19.15 (10.33)
p[*]	0.770	0.455	0.023	0.628	0.039
Ghrelin (pg/ml)					
0	1257.6 (596.7)	1912.0 (340.7)	1515.3 (498.1)	1472.7 (739.3)	1634.3 (591.9)
3	1246.4 (627.2)	2169.6 (1015.0)	1467.0 (543.6)	1420.6 (685.6)	1408.4 (534.3)
p[*]	0.915	0.480	0.487	0.383	0.004

[*] *p*: ANOVA for repeated measures: therapy effect within the same group.

No difference was detected between baseline and post-treatment levels in the other groups. Ghrelin levels decreased significantly in the raloxifene group (baseline: 1634 ± 592 pg/ml, 3 months: 1408 ± 534 pg/ml), while they remained unchanged at the end of the study in all other groups of treatment.

4. Discussion

Serum leptin associated in our study positively with BMI, insulin and endogenous estradiol and negatively with FSH and SHBG, although the last association did not reach statistical significance. Furthermore serum sFas increased linearly across increasing leptin quartiles. On the contrary serum ghrelin exhibited an inverse correlation with serum insulin. Different postmenopausal therapy had a different effect on circulating adipocytokines: raloxifene increased serum leptin and decreased serum ghrelin, while E_2/NETA had a decreasing effect on leptin only. CEE and tibolone did not affect either leptin or ghrelin.

Although originally described as a weight-controlling hormone, leptin is increasingly being recognized as a pro-atherogenic mediator. In experimental models, leptin has been shown to stimulate smooth muscle cell proliferation [11] and vascular wall calcification [27] as well as to increase oxidative stress [28]. Furthermore, leptin may induce a pro-thrombotic state through stimulation of platelet aggregation and may contribute to a non-beneficial shift in the coagulation–fibrinolysis balance observed in the metabolic syndrome [29]. Additionally, leptin has been suggested to mediate the proinflammatory state associated with obesity, as it has been demonstrated to correlate with acute phase reactants, such as

CRP and serum amyloid A in both normal weight and obese patients [30,31].

The angiogenetic properties of leptin have been considered as one of the plausible mechanisms through which leptin exerts its pro-atherogenic action [10]. Leptin receptor-deficient mice are unable to synthesize numerous angiogenic-related proteins, such as matrix metalloproteinases (MMPs) and vascular endothelial growth factor (VEGF) [32]. On the other hand, leptin induces endothelial cell proliferation and expression of MMPs in vivo and in vitro [33] and promotes angiogenesis [10]. This proliferative effect of leptin, however, may not only relate to the stimulation of cell proliferation, but also to the inhibition of cell death. Leptin is shown to have bcl-2 dependent anti-apoptotic action in human endothelial cells [34]. Furthermore, leptin exerts Fas-L mediated anti-apoptotic activity in mice hepatic stellate cells [35] and mice lymphocytes [36]. In our study, serum levels of sFas increased linearly with increasing leptin concentration. Our observation may imply that leptin, by increasing sFas concentration induces the inactivation of Fas-L and thus inhibits Fas-mediated apoptosis. One may further speculate that the positive association observed in previous studies between sFas and cardiovascular risk [25,37–39] may be mediated by leptin.

Menopause is usually related to an increase in body weight and in total and percent fat mass as well as to the centralization of body fat with a shift toward an android distribution [40]. These pro-atherogenic metabolic changes may in part be responsible for the increase in CVD incidence following menopause [41]. Leptin has been suggested as a possible mediator of the cardiovascular impact of menopause-associated metabolic changes. In our study, as well as in previous studies BMI and fasting serum insulin correlate positively with circulating leptin. Furthermore, lean body mass, independently of BMI, exhibits a negative correlation with leptin [42]. Sex steroids also seem to affect leptin secretion, although the exact mechanism remains unclear. Several studies evaluating the action of sex steroids on leptin levels showed a stimulatory effect of estrogens and an inhibitory action of androgens in animals both in vitro and in vivo [43].

Estrogen/progestin hormone therapy (HT) seems to prevent the body composition changes characteristic of the climacteric period [43]. The effect, however, of HT on leptin levels remains unclear. Numerous studies reported no effect of HT on leptin in subjects of varying BMI and percentage body fat [16,44,45]. On the other hand, some studies have showed an increase in serum leptin, after 1 month of therapy with transdermal estradiol alone or in combination with transvaginal progesterone [46], after 2 months of treatment with 2 mg of oral estradiol [47] as well as after 6 months of oral estrogen therapy or HT [48]. On the contrary, Di Carlo et al. [49] showed a decrease of serum leptin to premenopausal levels after transdermal HRT, a finding confirmed by the same group in a subsequent study [50]. Further to differences in study design and regimens used, this discrepancy may be explained by the indirect lowering effect of HT on leptin through the reduction of adipose tissue content, as opposed to a direct estrogen-mediated stimulatory effect on leptin secretion [14]. To our knowledge, our study is the first to evaluate the effect of low dose E_2/NETA on serum leptin. The leptin-lowering effect of this regimen might be explained by the body composition changes towards restoration of lean body mass, independently of body weight changes associated with HT. On the other hand, a net steroid effect on serum leptin levels could not have been demonstrated in our study, due to the mild androgenic effect of the progestin component of E_2/NETA, which could have counterbalanced the stimulatory effect of estradiol on leptin secretion [51].

Data regarding the effect of tibolone on serum leptin is sparse. The lack of an effect of tibolone treatment on leptin concentration observed in this study, as well as in our previous study [44], is in accordance with existing literature [52,53]. Panidis et al. [54] showed that long-term tibolone administration in postmenopausal normal weight and obese women does not significantly influence leptin levels. Taking into account that tibolone's active metabolites have both estrogenic and androgenic properties, the absence of an effect on serum leptin could be explained by a neutralizing effect of the two steroid actions on leptin secretion.

Data on raloxifene's effect on leptin is even more sparse. The increase in serum leptin following raloxifene administration observed in our study is in line with the findings of Cakmak et al. [55], who reported that raloxifene therapy for 6 months associated with an increase in serum leptin both in obese and non-obese postmenopausal women. On the other hand, Tommaselli et al. [56] reported no effect of raloxifene

therapy on serum leptin in postmenopausal women, along with an absence of an effect on body composition. Although our study is limited by the fact that fat and lean mass were not assessed, the increase in serum leptin under raloxifene therapy, given the neutral effect of raloxifene on fat and lean mass [56] may indicate a direct estrogen-like effect of raloxifene on leptin secretion.

In contrast to leptin, ghrelin appears to have an anti-atherogenic effect on the cardiovascular system. Intravenous administration of ghrelin decreased mean arterial pressure of healthy volunteers [57] suggesting a vasodilatory effect of ghrelin [58,59]. This effect appears to be independent of the IGF-1 pathway [58] and may be mediated through the antagonism of endothelin-1 vasoconstrictory effect [60]. Ghrelin has a potent anti-inflammatory effect on human endothelial cells through inhibiting proinflammatory cytokine production and mononuclear cell binding in human endothelial cells in vitro [61] and inhibiting leptin- and endotoxin-induced proinflammatory cytokine production in vivo [62], possibly through inhibition of tumor necrosis factor-a (TNF-a) induced nuclear factor-Kb activation (nFkB). Moreover, decreased levels of active ghrelin are associated with increased oxidative stress, a factor contributing to the development of atherosclerosis [62]. Finally, ghrelin has been recently demonstrated to reverse endothelial dysfunction in patients with metabolic syndrome by increasing NO bioactivity [63], while decreased ghrelin levels have been reported to be related to carotid atherosclerosis among older male and female subjects with metabolic syndrome [64].

Ghrelin levels increase in conditions characterized by negative energy balance due to low food intake [65,66], while decreased levels have been associated with obesity and states of insulin resistance [67,68]. In line with our study, Suematsu et al. [62] demonstrated a strong negative association of fasting serum insulin with circulating ghrelin in normal weight and obese subjects. Sex hormones may be also involved in the regulation of ghrelin secretion. Hyperandrogenism in women with polycystic ovary disease associates with low ghrelin levels [69], while antiandrogen therapy in these subjects increases circulating ghrelin [70]. Ghrelin's association to estrogens has also been suggested in animal studies [21], although the exact mechanism remains unclear.

Our study has demonstrated a decreasing effect of raloxifene therapy on circulating ghrelin, while estrogen-only or combined HT had no effect on this hormone. Data on the potential relationship between HT and ghrelin in postmenopausal women is conflicting. Purnell et al. reported no changes in total ghrelin levels between users and non-users of hormone therapy [71]. Furthermore, Veldhuis et al. [72] reported no alterations in total ghrelin levels after transdermally administering E2 in escalating doses to mimic late follicular-phase E2 concentrations in 10 postmenopausal women compared to placebo group. In contrast, Chu et al. [23] found a decrease in total ghrelin levels following oral or transdermal estrogen therapy in obese postmenopausal women with metabolic syndrome. Kellokoski et al. [22], on the other hand, recently reported that estrogen therapy in hysterectomized women increases plasma active ghrelin levels by 14% especially following oral administration as compared to transdermal. The higher estrogen dose as well as the measurement of active ghrelin as opposed to total ghrelin in our study may account for the discrepant results.

To our knowledge, this is the first report to investigate an effect of raloxifene on serum ghrelin. The decreasing effect of raloxifene on ghrelin, along with the increase in serum leptin may imply a negative impact of raloxifene. However, this finding may have no bearing on raloxifene's overall effect on the cardiovascular system. As with other drug regimens, the effect of raloxifene on CVD outcomes may be influenced by various parameters and risk factors differentially affected by this drug. Raloxifene has been reported to modulate beneficially a series of CVD risk markers, such as lipids–lipoproteins [73], NO/endothelin-1 ratio [74], homocysteine [75], VCAMs [76], VE-cadherin [77] and Regulated upon Activation Normal T cell Expressed and Secreted (RANTES) [26]. Recently however, the results of the RUTH trial showed that raloxifene therapy for 5 years has no effect on primary coronary events in postmenopausal women [78].

Concluding, baseline serum leptin correlated positively with circulating sFas, suggesting a possible proatherogenic action of this hormone in postmenopausal women. The decreasing effect of low dose HT on serum leptin may associate with an atheroprotective action of this regimen, at least in young, recently

menopaused women. Finally, raloxifene's negative impact on circulating ghrelin and leptin may not necessarily translate into increased clinical risk, since this SERM has been reported to have a beneficial effect on a series of cardiovascular risk factors.

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