0021-972X/00/$03.00/0
The Journal of Clinical Endocrinology & Metabolism

Vol. 85, No. 5
Printed in U.S.A.

Leptin in Postmenopausal Women: Influence of Hormone Therapy, Insulin, and Fat Distribution*

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ABSTRACT

Whether use of hormone-replacement therapy (HRT) influences menopause-related changes in body weight is unclear. HRT may affect energy balance by influencing synthesis of the adipocyte-derived hormone leptin. The objectives of this study were to: 1) identify factors influencing circulating leptin in postmenopausal women; 2) determine whether HRT influences serum leptin after adjusting for confounding factors; and, 3) identify potential independent effects of HRT or leptin on resting energy expenditure (REE). Subjects were 54 postmenopausal women, 45–55 yr old, 35 of whom used HRT (estrogen plus progestin). Total and regional body composition and fat distribution were determined by dual-energy x-ray absorptiometry and computed tomography; fasting serum leptin and insulin, by RIA; and REE, by indirect calorimetry. Stepwise multiple linear regression analysis indicated that serum leptin could best be predicted from total fat mass, fasting serum insulin, and total lean mass [log leptin = 1.08·log fat mass) + (0.46·log insulin) + (−1.25·log lean mass) + 1.88; model $R^2 = 0.78$, $P < 0.001$]. Multiple linear regression analysis indicated that visceral fat was independently related to leptin (parameter estimate = 0.23, $P < 0.05$), after adjusting for sc abdominal fat and leg fat, as well as lean mass and insulin. After adjusting for total fat mass, total lean mass, and fasting insulin, serum leptin did not differ between users and nonusers of HRT (21.7 ± 1.0 *vs.* 20.2 ± 1.3 ng/mL , $P = 0.369$, adjusted mean ± SE, respectively). Serum estradiol was inversely correlated with (adjusted) leptin in non-HRT users ($r = -0.50$), suggesting that ovarian senescence may lead to an increase in leptin. Multiple linear regression analysis indicated that REE (adjusted for fat mass, fat-free mass, and ethnicity) was not associated with leptin ($P = 0.298$) or hormone use status ($P = 0.999$; 1323 ± 31 *vs.* 1316 ± 42 kcal/day, adjusted mean ± SE for users and nonusers, respectively). These results indicate that, in postmenopausal women: 1) total fat mass, lean mass, and fasting insulin, but not HRT, are significant determinants of serum leptin; 2) visceral and sc fat contribute to serum leptin; and, 3) neither HRT nor leptin is independently related to REE. (*J Clin Endocrinol Metab* **85:** 1770–1775, 2000)

MENOPAUSE is a period during which women tend to gain body fat (1). If the increase in adiposity is a consequence of the decline in endogenous estrogen that occurs at this time, postmenopausal hormone-replacement therapy (HRT) might prevent or reduce body fat gain. However, existing clinical data addressing this issue are discordant. Data from the Postmenopausal Estrogen-Progestin Intervention Trial indicate that women who used hormones gained less weight, on average, over a 3-yr period (1.1 ± 0.4 kg) than those who did not (2.1 ± 0.4 kg) (2). However, data from the Pittsburgh Healthy Women's Study indicated that hormone users gained an average of 2.3 ± 0.8 kg over 2.5 yr, compared with 1.0 ± 0.6 kg in women who remained premenopausal (3). Other data suggest that, because of pharmacological effects, oral estrogen causes an increase in body fat, possibly by limiting lipid oxidation (4, 5). Thus, whether and how hormone therapy affects body composition in postmenopausal women is unclear.

Received September 2, 1999. Revision received January 6, 2000. Accepted January 12, 2000.

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* This work was supported by NIA K01-AG-00740 (to B.A.G.), DK-54918 (to T.R.N.), and General Clinical Research Center Grant M01-RR-00032.

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Ovarian hormones may influence body composition through several potential mechanisms. It has been suggested that estradiol inhibits the action of adipose tissue lipoprotein lipase, the enzyme that hydrolyzes circulating triglycerides, allowing for the uptake of fatty acids into adipocytes (6). Furthermore, data from rodent models indicate that estrogen acts as an anorectic, decreasing voluntary food intake (7), and that estrogen increases physical activity-related energy expenditure (8) and may increase resting energy expenditure (REE) (9). Thus, both menopause and exogenous hormones could alter body composition via energy intake, energy expenditure, and biochemical mechanisms.

One means through which estrogen may affect energy balance is through influencing synthesis of the hormone leptin. Leptin is a hormone produced by adipocytes (10) that is thought to affect energy intake and expenditure (11–13). Leptin-deficient mice are obese and, when given exogenous leptin, lose body fat attributable to both an increase in REE and a decrease in food intake. Circumstantial evidence suggests that ovarian hormones affect leptin production. Leptin is reported to fluctuate with the menstrual cycle (14) [although results differ (15, 16)], and it increases with pubertal progression in girls (17). Additionally, in isolated adipocytes (18) and 3T3-L1 adipocytes (18), estradiol exposure increases leptin messenger RNA expression and leptin secretion, respectively, suggesting a direct effect of estradiol on adipose tissue.

Other factors that influence leptin production are circulating insulin and body fat distribution. Leptin production is

positively associated with insulin concentration (19–21) and increases after insulin administration (20). In addition, adipose tissue seems heterogeneous, with respect to leptin production. Based on *in vitro* data (22, 23), visceral fat seems to produce less leptin than sc fat. Likewise, results of several *in vivo* studies have indicated that visceral fat is less well correlated with leptin than sc fat and, in multiple regression modeling, is not positively associated with leptin after adjusting for other indices of adiposity (24–26). Thus, at any given amount of total body fat, individuals with more visceral fat are likely to have less circulating leptin.

Studies designed to examine the influence of HRT on circulating leptin have been limited in number (27–29). Despite the provocative *in vitro* data, no *in vivo* study has uncovered a significant relationship between hormone use and circulating leptin. However, in most cases, data were adjusted for body mass index. Recent data indicate that simultaneous modeling of fat mass and lean body mass may be preferable to use of body mass index or percent fat (25, 30, 31). Additionally, potential variance caused by differences in insulin concentration or body fat distribution were not considered.

Therefore, the objectives of this study were, in a population of early postmenopausal women, to: 1) identify factors influencing serum leptin; 2) determine whether HRT influences serum leptin after adjusting for confounding factors; and, 3) identify potential independent effects of HRT or leptin on REE. This study is unique in examining the interrelationships between leptin and REE in a group of postmenopausal women using or not using hormone therapy, and in employing multiple regression modeling of both body composition (fat mass and lean mass) and fat distribution for serum leptin in a group of postmenopausal women.

Subjects and Methods

Experimental subjects

Subjects were 54 postmenopausal women, 45–55 yr old (48 Caucasian and 6 African-American). Only women who experienced a natural menopause, with the time of menopause known to occur at least 6 months before contact, were recruited. Both women using HRT and women not using HRT were recruited. Among hormone users, only subjects using an oral estrogen/progestin combination were included (n = 35; predominantly conjugated equine estrogens, 0.625 mg/day; and medroxyprogesterone acetate, 2.5 mg/day). Only women using HRT for between 1 month and 6 yr were included in the study. In cases where usage was cyclic, testing was conducted during the combined (estrogen + progestin) portion of the cycle. The designation, No HRT use ($n = 19$), was defined as no current use and no use within the past 6 months. Eighteen nonusers had never used HRT. Only nonusers with FSH levels greater than 30 mIU/mL were included in the study. Data were collected over a 27-h period during an inpatient visit to the Department of Nutrition Sciences and the General Clinical Research Center (GCRC) at the University of Alabama at Birmingham. The protocol was approved by the Institutional Review Board for Human Use at the University of Alabama at Birmingham, and all subjects signed an informed consent before testing.

Materials and methods

Protocol. Subjects arrived at the Department of Nutrition Sciences at approximately 0900 h in the fasted condition (12-h fast). Body composition was determined by dual-energy x-ray absorptiometry (DXA). At approximately 1200 h, subjects were escorted to the University of Alabama at Birmingham's GCRC. Subjects remained at the GCRC for approximately 24 h, departing at noon the following day. At approximately 1900 h, subjects were escorted to Radiology for computed tomography scanning. While at the GCRC, all food was provided. The evening snack was consumed before 1900 h. Subjects then remained fasted until indirect calorimetry (~0500 h) and fasting blood draw (~0700 h) the following morning.

Body composition and fat distribution. Total and regional body composition (fat mass and lean body mass) were measured by DXA using a DPX-L densitometer (Lunar Corp., Madison, WI). Subjects were scanned in light clothing while lying flat on their backs with arms at their sides. DXA scans were performed and analyzed with adult software version 1.5g. Visceral (intraabdominal) and sc abdominal fat were analyzed by computed tomography scanning with a HiLight/Advantage Scanner (General Electric, Milwaukee, WI), as previously described (32). A scout scan was first performed to locate the L4-L5 intervertebral space. Subsequently, a 5-mm scan of this abdominal site was taken. Scans were later analyzed for cross-sectional area (cm^2) of adipose tissue using the density contour program with Hounsfield units for adipose tissue set at −190 to −30. We have shown the test-retest reliability for visceral fat to be 1.7 percent (33). All scans were analyzed by the same investigator (T. R. Nagy). Scans were not available on four of the subjects (two HRT-users and two nonusers).

REE. REE was determined upon awakening, after an overnight fast, by indirect calorimetry (Deltatrac, Sensormedics, Yorba Linda, CA) in the GCRC. The instrument was calibrated before each test, against standard gases. During testing, all subjects were instructed to lie as still as possible. A canopy hood was used to collect expired air for 20 min after a 10-min equilibration period, and oxygen consumption and carbon dioxide production were measured continuously during this time. Energy expenditure was calculated using the equation of de Weir (34).

Assay of glucose, insulin, leptin, and estrogens. Serum was obtained after an overnight fast. Glucose was measured in 10 μL sera using an Ektachem DT II System (Johnson & Johnson Clinical Diagnostics). In our laboratory, this analysis has a mean intraassay coefficient of variation (c.v.) of 0.61% and a mean interassay c.v. of 1.45%. Insulin was assayed in duplicate 200-μL aliquots with Coat-A-Count kits (Diagnostic Products Corporation, Los Angeles, CA). According to the supplier, cross-reactivity of this assay with proinsulin is approximately 40% at midcurve; C-peptide is not detected. In our laboratory, this assay has a sensitivity of 1.9 μIU/mL , a mean intraassay c.v. of 5%, and a mean interassay c.v. of 6%. Commercial quality control sera of low, medium, and high insulin concentration (Lyphochek, Bio-Rad Laboratories, Inc., Anaheim, CA) are included in every assay, to monitor variation over time. Serum leptin was measured in duplicate 100-μL aliquots using a double-antibody RIA (Linco Research, Inc., St. Charles, MO). In our laboratory, this assay has a sensitivity of 0.4 ng/mL , a mean intraassay c.v. of 5%, and a mean interassay c.v. of 6%. Serum estradiol was measured using a double-antibody RIA (Diagnostic Products). Sera were first extracted in diethyl ether. Assay sensitivity was determined to be 4.2 pg/mL, intraassay c.v. to be 5.3%, and interassay c.v. to be 6.0%. Serum estrone sulfate was measured in duplicate 100-μL aliquots using a double-antibody RIA (Diagnostic Systems Laboratories, Inc., Webster, TX). Assay sensitivity was determined to be 0.47 ng/mL, intraassay c.v. to be 6.2%, and interassay c.v. to be 8.0%.

Statistics. For all analyses, values for body composition variables and serum analytes were log-transformed to produce a normal distribution. Pearson correlation analysis was used to examine associations between fat distribution variables and serum leptin. Stepwise multiple linear regression analysis was used to identify variables that best predicted serum leptin. Multiple linear regression analysis was used to identify independent effects of visceral fat, sc abdominal fat, and leg fat on serum leptin. Leg fat was selected to serve as an index of peripheral sc fat, which is metabolically unique from abdominal sc fat and visceral fat (35). Partial correlation analysis was used to examine the association between circulating estrogen concentrations and serum leptin, after adjusting for variables that were significant predictors of serum leptin (total fat, fasting insulin, and total lean mass). Multiple linear regression was used to determine whether HRT or leptin were independently related to REE after adjusting for fat mass, total lean mass, and ethnicity (36). Analysis of covariance (ANCOVA) was used to determine the mean values for leptin and REE for nonusers and users of HRT after adjusting for co-

variates (fat mass, lean mass, and insulin for serum leptin; fat mass, fat-free mass, and ethnicity for REE). All data were analyzed with SAS for Windows version 6.12 (SAS Institute Inc., Cary, NC).

Results

Descriptive statistics for hormone users and nonusers are given in Table 1. Subjects reported last experiencing a menstrual cycle between 0.5 and 6.0 yr before testing. HRT-users had been using hormone therapy for an average of 2.3 yr (range, 0.25–6 yr). HRT-users and nonusers did not differ, with respect to age, weight, body composition, fasting glucose, fasting insulin, leptin, REE, or years since menopause ($P > 0.05$). Users of HRT had lower amounts of sc abdominal fat and visceral fat ($P < 0.05$) and had higher serum concentrations of both estradiol and estrone sulfate ($P < 0.001$).

Stepwise multiple linear regression analysis indicated that serum leptin could best be predicted from total fat mass (positive relationship), fasting serum insulin (positive relationship), and total lean mass (inverse relationship; model $R^2 = 0.78$; Table 2); HRT use status did not enter the model. Correlation analysis indicated that all body fat variables were highly correlated with serum leptin. Correlation coefficients were 0.79 for total fat, 0.67 for leg fat, 0.64 for visceral fat, and 0.66 for sc abdominal fat ($P < 0.001$ for all). Multiple linear regression analysis indicated that visceral fat was independently related to leptin ($P < 0.01$) after adjusting for sc abdominal fat and leg fat (Table 3). The relationship between serum estrogens (estradiol and estrone sulfate) and leptin differed with HRT use status; although no significant correlations were observed among HRT users, estradiol was inversely correlated with leptin among nonusers (partial $r = -0.50$, $P < 0.05$, after adjusting for fat mass, insulin, and lean body mass; Table 4, Fig. 1). Inclusion of estradiol and estrone sulfate in the selection of independent variables in the stepwise multiple regression model (Table 2) did not alter the results. Multiple linear regression analysis indicated that REE (adjusted for fat mass, fat-free mass, and ethnicity) was not associated with leptin ($P = 0.298$) or hormone use status ($P = 0.999$; Table 5). Adjusted means for leptin and REE in hormone users and nonusers are shown in Figs. 2 and 3, respectively; neither leptin nor REE differed between groups.

Discussion

The first objective of the study was to identify factors influencing leptin in postmenopausal women. We found that leptin could best be predicted from a model containing total fat mass, fasting insulin concentration, and total lean body mass, all of which made independent contributions to leptin. Additionally, visceral fat was associated with leptin independent of leg fat and sc abdominal fat. Regarding the second study objective, we determined that HRT was not independently associated with leptin after adjusting for confounding factors. Thus, despite results from *in vitro* studies indicating that estradiol increases leptin production, exogenous estrogen in the form of conjugated equine estrogens does not seem to affect circulating leptin concentration *in vivo*. With respect to the third objective, we determined that neither HRT nor serum leptin was independently associated with REE. This observation suggests that, unlike what is observed with administration of estradiol or leptin to rodent models: 1) administration of HRT to postmenopausal women does not influence REE; and 2) endogenous leptin is not associated with energy expenditure in postmenopausal women.

TABLE 1. Descriptive statistics; mean ± SD (range)

	Nonusers (n = 19)	HRT users (n = 35)
Age (yr)	50.2 ± 2.7 (46–55)	50.2 ± 2.5 (45–55)
Years since menopause	2.13 ± 1.15 (0.5–4.0)	2.86 ± 1.40 (0.5–6.0)
Weight (kg)	70.9 ± 12.0 (54.5–101.7)	67.2 ± 10.2 (47.3–99.3)
% Fat	40.6 ± 5.1 (31.5–52.3)	37.4 ± 7.1 (25.9–52.0)
Total fat (kg)	28.8 ± 8.5 (18.0–51.6)	25.3 ± 7.9 (13.9–40.4)
Leg fat (kg)	11.5 ± 3.4 (5.5–20.1)	10.7 ± 3.5 (4.9–19.2)
Visceral fat (cm^2)[a]	125.3 ± 55.7 (57.0–266.3)	93.0 ± 38.6 (28.8–183.4)[b]
sc abdominal fat (cm^2)[a]	353.7 ± 130.9 (175.3–674.0)	281.8 ± 102.5 (106.4–526.8)[b]
Lean body mass (kg)	38.7 ± 4.0 (32.9–46.8)	38.6 ± 4.7 (30.3–56.0)
Fasting glucose (mg/dL)	94 ± 7 (80–108)	92 ± 7 (78–106)
Fasting insulin (μIU/mL)	11 ± 7 (4–36)	9 ± 4 (4–19)
Serum leptin (ng/mL)	27.3 ± 14.6 (6.8–55.2)	22.6 ± 11.0 (4.3–53.9)
Serum estradiol (pg/mL)	14.7 ± 18.0 (4.2–79.9)	38.2 ± 29.2 (5.5–167.7)[c]
Serum estrone sulfate (ng/mL)	1.5 ± 0.7 (0.6–3.1)	8.8 ± 6.0 (1.4–31.1)[c]
Resting metabolic rate (kcal/day)	1342 ± 252 (950–1810)	1309 ± 176 (1010–1870)

[a] n = 17 for nonusers, and 33 for HRT users.
[b] $P < 0.05$.
[c] $P < 0.001$.

TABLE 2. Stepwise multiple linear regression analysis to determine the best model for predicting (log) serum leptin

Step and independent variable selected[a]	Regression equation	Model R^2
1. Total fat mass	(1.35 · total fat mass) − 4.61	0.60
2. Insulin	(0.90 · total fat mass) + (0.47 · insulin) − 3.08	0.72
3. Total lean mass	(1.08 · total fat mass) + (0.46 · insulin) + (−1.25 · total lean mass) + 1.88	0.78

[a] Independent variables that did not enter the model were leg fat, visceral fat, sc abdominal fat, HRT use status, and ethnicity; all continuous variables were log transformed for analysis.

TABLE 3. Multiple linear regression model for the dependent variable serum leptin; $P < 0.001$

Independent variable[a]	Parameter estimate ± SEE	P	Partial R^2	Model R^2
Intercept	3.36 ± 1.44	<0.05		
Leg fat	0.63 ± 0.18	<0.001	0.45	0.45
Insulin	0.53 ± 0.09	<0.001	0.21	0.66
Lean body mass	−1.30 ± 0.33	<0.001	0.08	0.74
Visceral fat	0.23 ± 0.11	<0.05	0.06	0.80
sc abdominal fat	0.18 ± 0.15	0.249	0.00	0.80

SEE, Standard error of the estimate.
[a] All variables were log transformed for analysis.

TABLE 4. Partial correlations between serum leptin and serum estrogens

	Nonusers	HRT users	Combined
Estradiol	−0.50[a]	0.02	−0.06
Estrone sulfate	−0.32	0.24	0.17

Leptin was adjusted for total fat, fasting insulin, and total lean mass; all variables were log transformed for analysis.
[a] $P < 0.05$.

Results indicated that, in this group of postmenopausal women, leptin could best be predicted from total fat mass, fasting serum insulin, and total lean mass. Whereas fat mass and insulin were positively related to leptin, lean mass was inversely related. This observation indicates that it was the relative proportion of fat mass to lean mass, as well as the absolute amount of body fat, that influenced circulating leptin concentration. Similar observations regarding the negative association between lean mass and leptin have been observed in children (25) and adolescents (30, 31).

Results from a number of studies have suggested that leptin production is not uniform across different adipose tissue depots. In general, sc adipose tissue seems to produce more leptin that visceral adipose tissue. Among prepubertal children (genders combined), visceral fat is not independently related to leptin after adjusting for other body composition/fat distribution indices, gender, and ethnicity (25). Likewise, in a group of children and adolescents (genders combined), the association between visceral fat and leptin was not significant after adjusting for sc fat (24). A study with Asian Indian men (mean age, 38.6 ± 10 yr) indicated a significant correlation between leptin and sc abdominal fat (0.89, $P < 0.001$) but not between leptin and visceral fat ($r = 0.37$, $P > 0.05$) (26). These observations agree with *in vitro* data suggesting that visceral fat produces less leptin than sc fat (23).

In the present study, simple correlation analysis indicated that leptin was correlated with all individual body fat regions examined ($r = 0.64–0.67$). However, multiple linear regression analysis indicated that, of these three regions, only leg fat and visceral fat were independently associated with serum leptin (sc abdominal fat was not independently related to leptin). The independent association of leptin with visceral fat is both a unique finding and a finding at odds with the studies cited above. However, all of the cited studies were conducted in relatively young subjects. If the subjects of the present study are representative of postmenopausal women in general, then the present results may suggest that leptin production by sc abdominal fat decreases, or production by visceral fat increases, after menopause. Some support for this hypothesis comes from a study of elderly subjects in whom the association between visceral fat and leptin was strong in both men ($R^2 = 0.57$) and women ($R^2 = 0.38$) (21). Taken together, data suggest that the relative contribution of visceral fat to circulating leptin concentration may increase with age.

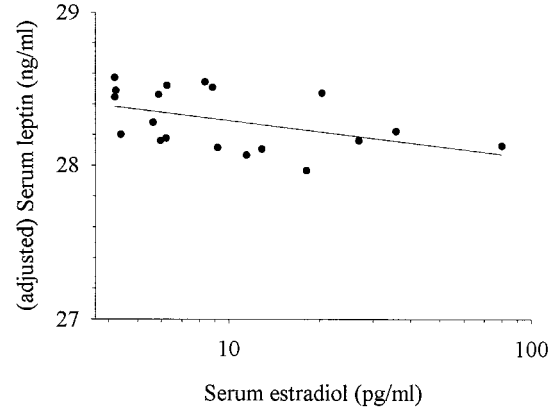

FIG. 1. Adjusted serum leptin *vs.* estradiol in women who did not use hormone therapy (slope = −0.24, intercept = 28.5, $r = 0.50$, $P < 0.05$); leptin was adjusted for total fat mass, fasting insulin concentration, and total lean mass.

TABLE 5. Multiple linear regression model for the dependent variable REE; model $R^2 = 0.30$, $P < 0.01$

Independent variable[a]	Parameter estimate ± SEE	P
Intercept (kcal/day)	−6933 ± 2587	0.010
Total fat mass (kg)	236 ± 395	0.552
Total lean mass (kg)	1509 ± 691	0.034
Ethnicity[b]	10.1 ± 88.9	0.910
HRT use[b]	−0.03 ± 26.7	0.999
Leptin (ng/mL)	214 ± 203	0.298

[a] All continuous variables were log transformed for analysis.
[b] Dummy coded such that 0 = African-American and 1 = Caucasian, and 0 = no HRT and 2 = estrogen and progestin use.

The positive association between leptin and fasting insulin observed in this group of healthy postmenopausal women is consistent with observations in other populations (19–21). One limitation of the current study is the use of a single, fasting blood draw for measurements of insulin and leptin; in the fasted state, the influence of insulin on leptin may be less apparent. Nonetheless, because of the significant independent relationship between insulin and leptin, we chose to use insulin as a covariate when examining the influence on HRT on serum leptin.

Hormone use status did not influence serum leptin after adjusting for fat mass, fasting insulin concentration, and lean mass (Fig. 2). This observation agrees with other data showing no relationship between hormone use and circulating leptin (21, 27–29). Thus, if menopause is associated with a decrease in circulating leptin [as suggested by some (14), but not all (28, 29), studies], this decrease does not seem to be reversed by treatment with exogenous hormones. However,

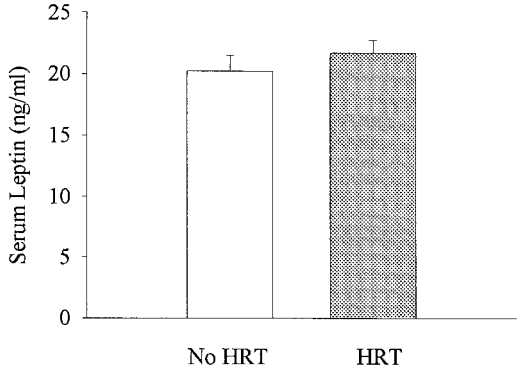

FIG. 2. Serum leptin concentration in nonusers (20.21 ± 1.32) and users (21.72 ± 1.02) of HRT. Data were adjusted for fat mass, fasting insulin concentration, and total lean mass. $P = 0.369$.

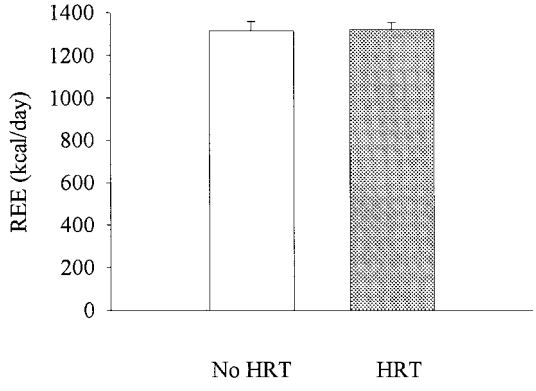

FIG. 3. Resting energy expenditure in nonusers (1316 ± 42) and users (1323 ± 31) of HRT. Data were adjusted for fat mass, total lean mass, and ethnicity. $P = 0.899$.

an intervention study is needed to definitively determine whether HRT affects leptin production.

In contrast, analysis of serum estrogen concentrations indicated a relationship between circulating estradiol and serum leptin. Among subjects not using hormones, serum estradiol was inversely associated with serum leptin, after adjusting for fat mass, lean body mass, and insulin (Table 5, Fig. 1). The same was not true with hormone users, among whom neither the circulating concentration of the native, active estrogen, estradiol, nor that of the estrogen metabolite, estrone sulfate, was significantly correlated with serum leptin. Estrone sulfate is a major constituent of oral conjugated equine estrogens (~45%) and is the major circulating estrogen in humans. It can be converted to more active estrogens, such as estradiol. Previous studies have either failed to observe a relationship between circulating estrogen concentrations and serum leptin (15, 16) or have observed a positive correlation between estradiol and leptin (37), and estrone and leptin (21). However, in the latter case, adjustment for body fat eliminated the relationship. Differences between the present study and previous studies may be attributable to differences in aspects of the subject population (age, menopausal status) or to the method of statistical adjustment. In the present study, leptin was adjusted for the major confounding variables (total body fat, lean body mass, and insulin). This method of adjustment may have allowed for the relationship between estradiol and leptin to be observed. The observed inverse relationship between estradiol and leptin in nonusers implies that, as ovarian hormone production declines, leptin production increases. However, this observation awaits confirmation.

The third objective of the study was to determine whether HRT or leptin were independently associated with REE. Estrogen is thought to increase REE in animal models (38–40), although most evidence is circumstantial. Few studies have used indirect calorimetry to measure REE in estrogen-treated animals, and conclusions differ; results of two studies indicate a stimulatory effect of estradiol on REE (9, 40), whereas results of another indicate no effect (39). Although REE in women declines over the menopause transition, this decline is associated with a loss of lean body mass (1), leaving it unclear as to whether mass-specific REE is altered by menopause (and the associated decline in estrogen). In the present study, REE did not differ with hormone use after adjusting for body composition and ethnicity (Fig. 2). However, all subjects on HRT used a combined estrogen-progestin regimen and were tested during the progestin phase. Progesterone can block or antagonize metabolic effects of estrogen (38). Thus, whether treatment with unopposed estrogen, or testing of subjects during the estrogen-only phase, would yield different results regarding the influence of HRT on REE remains to be determined.

Leptin did not explain any additional variance in REE. This result is in agreement with findings from most (41–45), but not all (46–48), other clinical studies examining relationships between circulating leptin and REE. In cases where a positive association was observed between leptin and REE (46, 48), fat mass was not included in the regression model. In the present study, both fat mass and fat-free mass were included in the regression model. If the present data were analyzed as in References 46 and 48 (*i.e.* without fat mass as an independent variable), then leptin would be significantly and positively associated with REE (data not shown). Because the correlation coefficient between fat mass and leptin was 0.79 in the present study, the two variables cannot be considered colinear [$r \geq 0.95$ (49)], and it was appropriate to put both in the model for REE. Thus, our data would suggest that if leptin influences REE in humans, the effect is likely small in magnitude and difficult to detect in cross-sectional observations.

In conclusion, fat mass, fasting insulin concentration, and lean body mass, but not HRT, were significant determinants of serum leptin in this group of postmenopausal women. Visceral fat was associated with leptin independent of sc fat (both leg fat and abdominal fat), suggesting that the contribution of visceral fat to leptin may increase with age or transition through the menopause. Serum estradiol was inversely correlated with (adjusted) leptin in non-HRT users, suggesting that ovarian senescence may lead to an increase in leptin. Neither HRT nor leptin was independently related to REE. Further research is needed to clarify the role of circulating leptin in determining energy balance in postmenopausal women.

Acknowledgments

We acknowledge Kangmei Ren for hormone determinations, and the help of the GCRC staff.

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