Relationship of Serum Leptin Levels With Body Composition and Sex Steroid and Insulin Levels in Men and Women

Thierry Thomas, Bartolome Burguera, L. Joseph Melton III, Elizabeth J. Atkinson, W.M. O'Fallon, B. Lawrence Riggs, and Sundeep Khosla

Whether the higher serum leptin levels in women are due to gender differences in fat mass or to other factors such as sex steroids remains unclear. In addition to sex steroids, serum insulin levels also appear to be related to leptin levels, although whether this effect is independent of the effects of body composition is unclear. The purpose of this study was to identify the major determinants of circulating serum leptin levels. We studied a large, population-based cohort of 345 men (23 to 90 years), 137 premenopausal women (21 to 54 years), and 212 postmenopausal women (34 to 94 years), including 47 women on hormone replacement therapy (HRT). Serum leptin levels were related to body composition as assessed by dual-energy x-ray absorptiometry (DEXA) and to circulating sex steroid and insulin levels. Serum leptin levels remained significantly higher in women versus men even after adjustment for fat mass, and leptin levels were significantly correlated with fat mass independently of age. By univariate analyses, logarithmically transformed serum leptin levels correlated positively with bioavailable estrogen ([E] estradiol plus estrone) in postmenopausal women not on HRT, and negatively with total and bioavailable testosterone (T) levels in men. Serum insulin levels were directly related to leptin levels regardless of gender and age. By multivariate analyses, fat mass, lean mass, and insulin levels were the strongest predictors of leptin levels in all groups. In addition, bioavailable E entered the model in the postmenopausal women not on HRT. These studies indicate that the fat mass, lean mass, and insulin level are the major determinants of the serum leptin level in adults. Moreover, after adjusting for these variables, bioavailable E also explains a significant proportion of the variance in leptin levels among postmenopausal women not on HRT.

LEPTIN, a 16-kilodalton protein encoded by the *ob* gene,[1] is produced mainly by white adipose tissue.[2] Mutation of the *ob* gene induces obesity and leptin replacement results in weight loss in the ob/ob (leptin-deficient) mouse model,[3-5] consistent with a key role for leptin in the maintenance of body weight.[6] In humans, serum leptin concentrations are highly correlated with body fat content.[7,8] These data also reveal a striking sexual dimorphism in serum leptin levels, with women having 2- to 3-fold higher levels than men. Although this was initially attributed to a higher relative fat mass in women,[7,8] more recent studies have found that serum leptin levels remain higher in women even after adjustment for the body mass index (BMI) or absolute fat mass.[9-12] This could be related, in part, to differences in fat distribution between men and women,[13,14] with visceral fat producing less leptin than subcutaneous fat.[15]

Despite these data, the factors responsible for gender differences in the regulation of leptin production by adipose tissue remain unclear at present. Although some studies have found a positive relationship between serum leptin and estrogen (E) levels in women,[16,17] as well as higher circulating serum leptin levels during the luteal phase of the menstrual cycle,[16,18,19] other investigators have failed to confirm these findings.[11,20,21] Menopause was associated with a decrease in serum leptin in one study[9] but no change was found in other studies,[11,20] and the effects of hormone replacement therapy (HRT) on serum leptin levels are conflicting.[20-24] Several reports have demonstrated an inverse relationship between serum testosterone (T) and serum leptin levels during pubertal development,[25-28] during T substitution therapy in hypogonadal men,[29,30] or in a normal population.[17,31-34] However, this relationship disappeared after adjustment for the BMI,[32,33] and other studies did not consider the changes in body composition and the fat mass to lean mass ratio induced by changes in serum T levels.[29-31] Furthermore, little information exists on the relationship between serum bioavailable steroid and leptin levels.

Similarly, data are conflicting on the relationship between aging and serum leptin levels. Although an age-related increase in leptin gene expression that was independent of increasing adiposity was found in rats,[35] one study in humans found that the relationship between serum leptin levels and age was dependent on the increase in the BMI with age.[31] In contrast, two other studies in humans demonstrated an inverse relationship between serum leptin levels and age,[10,36] and another report found no relationship between age and leptin levels.[37] Finally, a recent study reported that aging was associated with a disruption of the relationship between fat content and leptin levels.[38]

In addition to sex steroids, serum insulin levels also appear to be related to leptin levels,[36,39,40] although whether this effect is independent of the effects of body composition is unclear. Thus, to identify the major factors determining the circulating serum leptin level, we studied a large, population-based cohort of Rochester, Minnesota men and women, including premenopausal and postmenopausal women and postmenopausal women on HRT. The relationship of leptin to indices of body composition, bioavailable sex steroids, and insulin was assessed in univariate and multivariate analyses.

SUBJECTS AND METHODS

Subjects

Subjects were recruited from age-stratified random samples of men and women from Rochester, MN identified through the medical records linkage system of the Rochester Epidemiology Project.[41] Over half of

From the Endocrine Research Unit, Division of Endocrinology, Metabolism, and Nutrition, Department of Internal Medicine, and the Department of Health Sciences Research, Mayo Clinic and Mayo Foundation, Rochester, MN.

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Address reprint requests to Sundeep Khosla, MD, Mayo Clinic, 200 First St SW, 5-194 West Joseph, Rochester, MN 55905.

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the Rochester population is identified annually in this system and the majority are evaluated in any 3-year period. Thus, the enumerated population approximates the underlying population of the community, including both free-living and institutionalized individuals. Altogether, 1,138 men and 938 women aged 20 years and over were approached for the study, but 239 men and 126 women were ineligible (among the men, 109 were demented and could not give informed consent, 13 were radiation workers, 91 died before contact, 25 were debilitated due to terminal cancer, and 1 was unable to participate due to pending legal action; among the women, 89 were demented, 11 were pregnant, 9 were radiation workers, 8 were participants in an ongoing clinical trial of osteoporosis prophylaxis, and 9 died before they could be contacted). Of 899 eligible men, 345 participated and provided full study data (age range, 23 to 90 years; mean, 55), although 2 were excluded from this analysis because one was on T therapy and one had inexplicably high (into the range of premenopausal women) estradiol (E_2) and estrone (E_1) levels. Of 812 eligible women, 349 participated and provided full study data. These included 137 premenopausal women (age range, 21 to 54 years; mean, 35) and 212 postmenopausal women (age range, 34 to 93 years; mean, 68), including 47 receiving HRT. Menopause was defined by either documented bilateral oophorectomy or a duration longer than 6 months without a menstrual period. For the data presented in Table 1, a diagnosis of diabetes mellitus was based on the clinical diagnosis in the medical record, not on the basis of the single fasting glucose value obtained as part of the study. The total number of subjects included in this analysis was 692 (343 men and 349 women). All but 13 men and 2 women were Caucasian, reflecting the ethnic composition of the population (96% white in 1990).

Protocol

The fat mass (coefficient of variation [CV] = 2.0%) and lean mass (CV = 0.6%) were determined from the dual-energy x-ray absorptiometry (DEXA) whole-body scan using the Hologic QDR2000 instrument (Hologic, Waltham, MA) with software version 5.40. Fasting-state serum samples were obtained between 8 and 9 AM. Samples were obtained during the 2 weeks following menses for premenopausal women. All samples were stored at −70°C until analysis. All studies were approved by the Mayo Institutional Review Board, and all subjects provided informed consent prior to participation.

Laboratory Methods

Fasting serum samples were analyzed by radioimmunoassay (RIA) for total T (Diagnostic Products, Los Angeles, CA; interassay CV = 11%), E_2 (Diagnostic Products; interassay CV = 11%), E_1 (Diagnostic Systems Laboratories, Webster, TX; interassay CV = 9%), and sex hormone–binding globulin ([SHBG] Wien Laboratories, Succasunna, NJ; interassay CV = 7%). In addition, the non–SHBG-bound (bioavailable) fraction of total T, E_2, and E_1 was measured using a modification of the technique of O'Connor et al[42] and Tremblay and Dube,[43] as previously described.[44] Briefly, tracer amounts of [^{3}H]-labeled T, E_2, or E_1 were added to serum aliquots. An equal volume of saturated solution of ammonium sulfate (final concentration, 50%) was added to precipitate SHBG with its bound steroid. Separation of the SHBG fraction was achieved by centrifugation at 1,100 × *g* for 30 minutes at 4°C. The percentage of labeled steroid remaining in the supernatant (free and albumin-bound fractions) was then calculated. The bioavailable steroid concentration was then obtained by multiplying the total steroid concentration, determined by RIA, by the fraction that was non–SHBG-bound. The serum level of dehydroepiandrosterone sulfate (DHEAS) was measured by RIA (Diagnostic Products; interassay CV < 8%). Serum insulin was determined by an immunoenzymatic assay on the Beckman Access Immunoassay system (Beckman Instruments, Chaska, MN; interassay CV < 5%). The serum leptin level was measured using a commercial RIA for human leptin (Linco Research, St. Charles, MO; interassay CV = 8.3%).

Statistical Analysis

Pearson correlation analysis was used to summarize relationships between the various continuous variables. The logarithmic transformation was used for serum leptin levels because of skewness. The S-Plus lowess smoother function[45] was used to fit smoothed lines to the data points. Stepwise linear regression was used to determine which variables best predicted log (leptin). Higher-order terms and interactions were considered in the model once the main effects were selected. Finally, model assumptions were checked by examination of the model residuals. The SAS statistical package (SAS Institute, Cary, NC) was used for the analyses.

RESULTS

Relationship Between Serum Leptin, Fat Mass, and Age

Table 1 delineates the relevant anthropometric and biochemical variables in the study population. Figure 1 shows serum leptin levels as a function of age in the men and the various subgroups of women. By simple regression analysis, age and serum leptin were significantly correlated only in men ($r = .17$, $P < .01$). However, due to the skewness of the leptin values,

Table 1. Anthropometric and Biochemical Variables Among the Age-Stratified Sample of Rochester, MN Men and Women (mean ± SD)

Variable	Premenopausal Women	Postmenopausal Women		Men
		Not on HRT	On HRT	
No. of subjects	137	165	47	345
Age (yr)	35.0 ± 8.6	69.8 ± 13.1	61.0 ± 11.4	55.4 ± 19.6
Weight (kg)	69.2 ± 16.6	68.4 ± 14.2	63.9 ± 9.3	84.3 ± 15.5
Height (cm)	164.4 ± 6.8	159.6 ± 6.8	160.1 ± 5.7	175.5 ± 7.2
BMI (kg/m^2)	25.6 ± 5.9	26.9 ± 5.5	24.7 ± 3.3	27.3 ± 4.4
Fat mass (kg)	26.0 ± 13.4	29.9 ± 11.4	25.1 ± 7.3	23.6 ± 9.9
Lean mass (kg)	40.3 ± 5.2	36.0 ± 5.0	36.0 ± 4.3	56.9 ± 7.8
Bioavailable E (pg/mL)	60.5 ± 37.9	18.3 ± 19.7	34.5 ± 23.7	33.9 ± 16.5
Bioavailable T (ng/dL)	7.3 ± 4.7	5.9 ± 4.6	3.7 ± 2.7	125.8 ± 64.2
DHEAS (μg/dL)	150.8 ± 83.9	65.1 ± 43.9	58.9 ± 39.7	149.5 ± 121.4
Glucose (mg/dL)	94.4 ± 14.1	105.9 ± 24.0	96.3 ± 21.4	104.6 ± 29.2
Insulin (μU/mL)	8.1 ± 6.6	7.9 ± 4.9	6.0 ± 4.7	10.1 ± 11.8
% diabetic	0.0	1.8	2.1	7.5

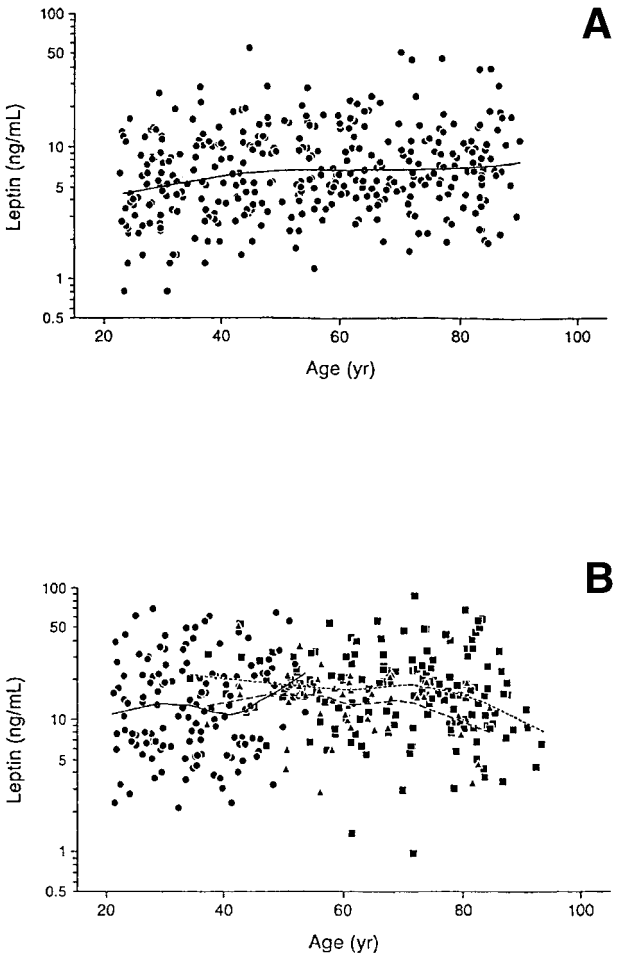

Fig 1. Serum leptin levels as a function of age among an age-stratified sample of (A) Rochester, MN men and (B) Rochester, MN premenopausal women (●—●), postmenopausal women on HRT (▲---▲), and untreated postmenopausal women (■---■).

log (leptin) was used in all subsequent analyses. Serum log (leptin) levels were directly related to fat mass in men ($r = .82$, $P < .001$; Fig 2A), premenopausal women ($r = .85$, $P < .001$), and postmenopausal women either on HRT ($r = .84$, $P < .001$) or untreated ($r = .76$, $P < .001$; Fig 2B). The sexual dimorphism of leptin levels was still observed after adjustment for fat mass, with approximately 2-fold higher levels in women compared with men ($P < .001$). In addition, serum leptin normalized to fat mass was still directly related to fat mass in all groups (data not shown), and the variability of leptin levels increased with increasing fat mass (Fig 2). Finally, to evaluate any age-induced alteration of the relationship between leptin levels and fat mass, we split our population into 10-year sextiles and found that leptin levels and fat mass remained significantly correlated in both sexes at any age (Figs 3 and 4).

Relationship Between Leptin Levels and Body Composition

Table 2 shows the results of univariate analyses relating log (leptin) levels to the body weight, BMI, total and regional (ie, truncal *v* extremity) fat mass, and lean mass. In both men and women, leptin levels were highly correlated with fat content regardless of the site of fat accumulation. Because total fat was more strongly related to leptin levels than any specific site, serum log (leptin) levels were related to total fat in further single and multiple regression analyses. Lean mass was relatively weakly associated with log (leptin) levels in premenopausal women and postmenopausal women not on HRT, but not in postmenopausal women on HRT or in the men.

Relationship Between Leptin Levels and Sex Steroid and Insulin Levels

Table 3 shows the relationship between serum sex steroid, insulin, and leptin levels in the men and women. Total and bioavailable sex steroids and insulin were related to both log (leptin) and log (leptin) adjusted for fat mass. For simplicity, we have presented the data for total and bioavailable E (E_2 plus E_1), although E_2 and E_1 individually showed similar changes. In the men, total and bioavailable T and SHBG were inversely related to log (leptin) levels, and insulin was positively related. After adjustment for total fat mass, total and bioavailable T and insulin remained significantly related to log (leptin) levels. In the premenopausal women or postmeno-

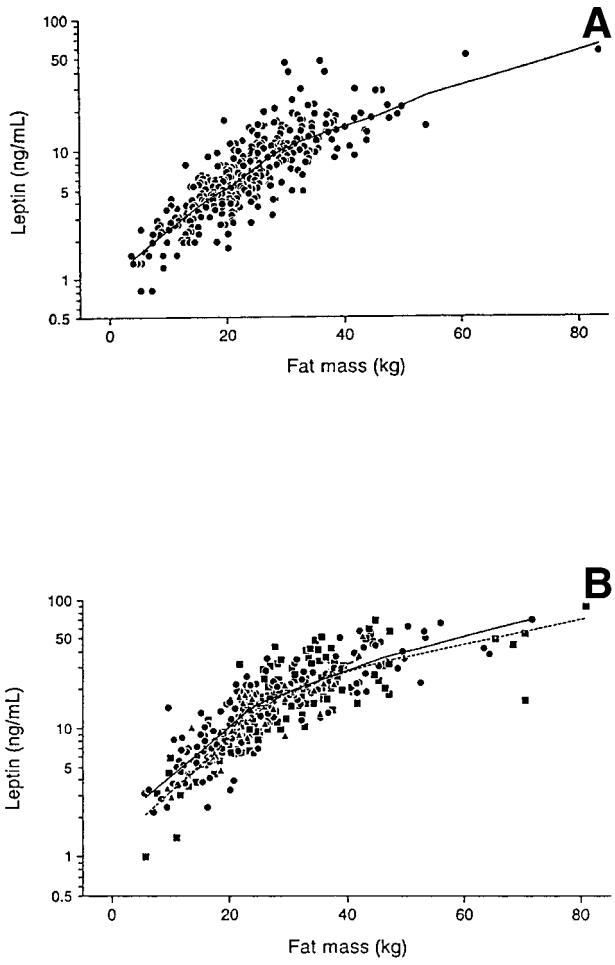

Fig 2. Serum leptin levels as a function of fat mass among an age-stratified sample of (A) Rochester, MN men and (B) Rochester, MN premenopausal women (●—●), postmenopausal women on HRT (▲---▲), and untreated postmenopausal women (■---■).

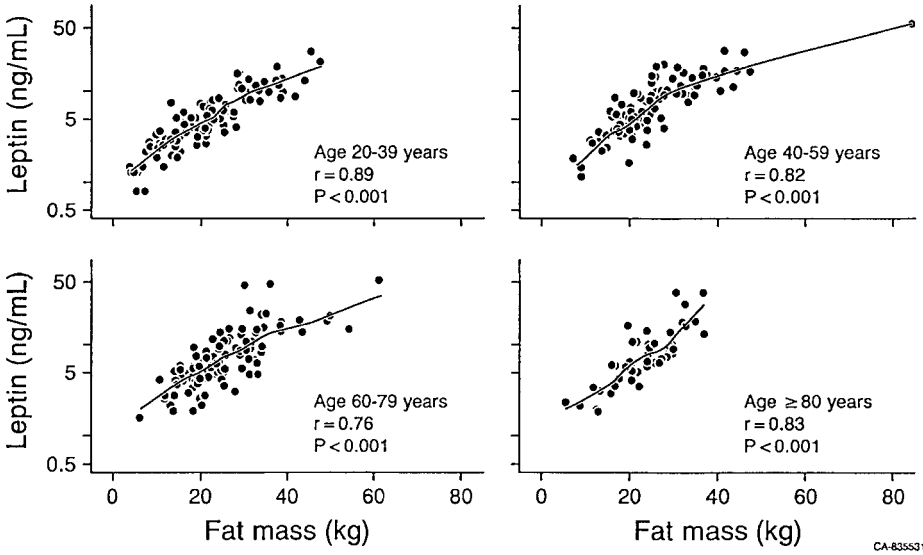

Fig 3. Serum leptin levels as a function of fat mass among Rochester, MN men of different ages.

pausal women on HRT, neither total or bioavailable sex steroids nor SHBG were significantly related to log (leptin) levels after adjustment for fat mass. However, in the untreated postmenopausal women, total and bioavailable E and SHBG levels were directly related to log (leptin) levels, even after adjusting for fat mass. Serum insulin levels were also associated with log (leptin) levels in all groups, although these remained significant only in the postmenopausal women on HRT and in the men after adjusting for fat mass.

Multivariate Models Determining Leptin Levels

To assess the relative contribution of body composition indices, androgens, estrogens, and insulin in determining serum leptin levels, multivariate models were constructed in which log (leptin) was the dependent variable (Table 4). Models were fit separately for men, premenopausal women, postmenopausal women currently taking HRT, and untreated postmenopausal women. In each of the 4 different groups, the variables allowed to enter the model included total fat mass, total lean mass, bioavailable E, bioavailable T, DHEAS, and insulin. In all 4 groups, fat mass was the single most important predictor of serum log (leptin) levels. After adjusting for the effects of fat mass, lean mass was a significant negative predictor of leptin levels. Serum insulin was a positive predictor of serum log (leptin) levels in all groups except postmenopausal women not on HRT. In contrast, in this group, the serum bioavailable E level was a positive predictor of the leptin level. DHEAS was a significant determinant of serum log (leptin) in postmenopausal women on HRT. To test if differences in body size had an impact on our analyses, we repeated the entire analysis by forcing height into the model, which turned out not to be a significant predictor and had no impact on the models. Forcing weight into the models essentially eliminated fat mass but otherwise did not change the results (data not shown). We also repeated the analysis after excluding lean mass (since fat mass and lean mass were correlated in all of the groups), with essentially the same parameters entering the model (data not shown). Finally, Table 4 shows that most of these relationships were nonlinear, as

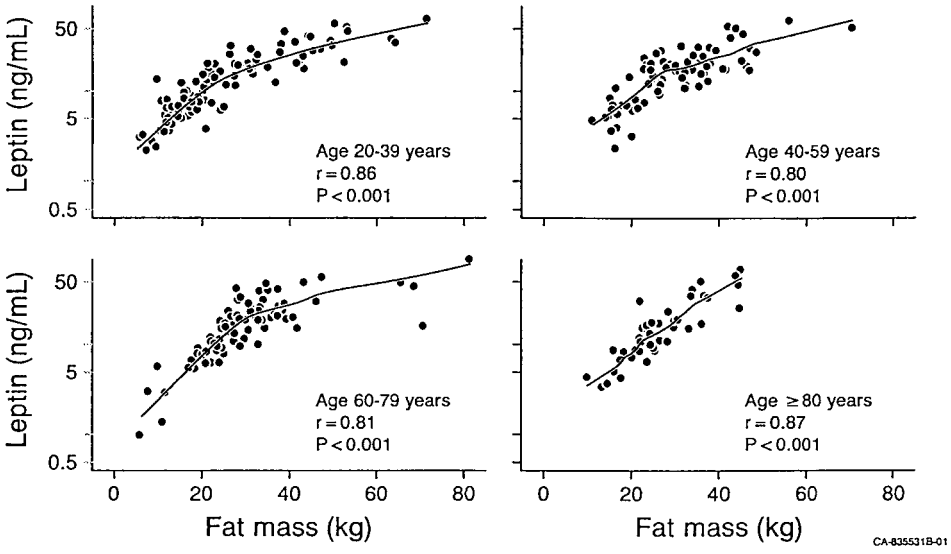

Fig 4. Serum leptin levels as a function of fat mass among Rochester, MN women of different ages. For this analysis examining the effects of age on the relationship between leptin levels and fat mass, premenopausal women and untreated postmenopausal women were merged and analyzed as a group; postmenopausal women on HRT were excluded.

Table 2. Correlation Between Log (leptin) Levels and Body Weight, BMI, Total and Regional Fat Mass, and Lean Mass Among an Age-Stratified Sample of Rochester, MN Men and Women

Group	Body Weight	BMI	Total Fat Mass	Truncal Fat Mass	Appendicular Fat Mass	Lean Mass
Women						
Premenopausal	.77†	.82†	.85†	.84†	.82†	.26*
Postmenopausal						
Not on HRT	.68†	.72†	.76†	.74†	.71†	.23*
On HRT	.63†	.63†	.84†	.79†	.76†	−.07
Men	.58†	.67†	.82†	.82†	.74†	.08

*$P < .01$.
†$P < .001$.

illustrated in Fig 2 for the relationship between fat mass and leptin. There were also significant interactions in the models between fat mass and insulin, as well as lean mass. Overall, these models predicted 78% to 83% of the variance in serum log (leptin) levels. If we eliminate the higher-order terms and interactions from the models, the R^2 values are reduced from .81 to .75 in premenopausal women, from .78 to .66 in postmenopausal women not on HRT, and from .79 to .73 in the men. There were no higher-order terms or interactions for the analysis in postmenopausal women on HRT.

DISCUSSION

In the present population-based study, we sought to determine the major anthropometric and hormonal factors that influence serum leptin levels. We did not find a significant independent effect of aging on serum leptin, nor did we confirm the observation of Moller et al[38] that aging alters the relationship between leptin levels and fat content. In fact, we found that in both genders and in any age group of our population between 23 and 90 years, serum leptin and fat mass were highly and consistently related. Differences in the size of the respective study populations, and therefore in statistical power, could explain part of the discrepancies since each subgroup was 4 times larger in our study than in the study by Moller et al.[38] Whether a 5-day diet including 3 days as inpatients in the latter study could have altered serum leptin levels is also an issue. Two studies previously reported an inverse relationship between age and serum leptin.[10,36] Neither used log (leptin) as the dependent variable, even though their data showed the same high skewness of serum leptin levels with increasing fat mass that we found. Moreover, Ryan and Elahi[36] conducted their study in highly trained women athletes, while Ostlund et al[10] did not find any increase in body fat content with age in their population. This is the opposite of what our group[46] and others[47] have reported. These discrepancies suggest differences in the representativeness of the respective populations.

In accordance with recent studies,[9-12] we did find a sexual dimorphism for serum leptin levels; this persisted after adjustment for total fat mass, with 2-fold higher leptin levels in women compared with men. Furthermore, leptin adjusted for total fat mass was still directly related to fat mass, in accordance with the observation that larger adipocytes associated with increasing fat accumulation express leptin more abundantly.[48] Because there is a strong gender difference in fat distribution and because leptin expression is lower in visceral compared with subcutaneous fat,[15] fat distribution has been suggested as a potential mechanism for the sexual dimorphism in serum leptin levels. We observed comparable relationships between serum leptin and truncal or peripheral fat content, and total fat mass was a better predictor than any specific fat content site. Although truncal fat measured by DEXA is strongly correlated with intraabdominal adipose tissue,[49] this method does not differentiate subcutaneous and visceral fat and has been considered only a surrogate for the latter. Thus, based on our data, we cannot definitely address whether the subcutaneous/visceral truncal fat ratio plays a role in the gender-related difference in leptin levels.

Next, we investigated whether sex steroids are related to serum leptin and subsequently whether they could play a role in explaining its sexual dimorphism. We found that in postmenopausal women not on HRT, bioavailable E levels were significantly and directly related to serum leptin levels. These data, together with the study by Hassink et al[50] showing an increase in serum leptin independent of adiposity in girls with increased Tanner stage suggest that estrogens may modulate leptin expression. Indeed, recent in vitro studies have found that E increases leptin mRNA levels in rat adipocytes.[51] However, at higher E levels, ie, in premenopausal women or in women on HRT, variations in E levels were not associated with leptin levels, suggesting a possible threshold effect.

In women, T or bioavailable T levels were not related to serum leptin levels after adjustment for fat mass, suggesting no independent role of this hormone in the regulation of leptin

Table 3. Unadjusted and Fat Mass–Adjusted Correlations Between Log (leptin) and Sex Steroid and Insulin Levels Among an Age-Stratified Sample of Rochester, MN Men and Women

	Premenopausal Women		Postmenopausal Women Not on HRT		Postmenopausal Women on HRT		Men	
Variable	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
E	−.01	.11	.26†	.17*	−.09	−.08	.02	−.03
T	.23†	.09	.13	.09	.39†	.34	−.49‡	−.26‡
SHBG	−.31‡	−.01	−.48‡	−.19*	−.40†	−.31	−.19‡	−.04
Bioavailable E	.15	.15	.31‡	.19*	.06	.15	.07	−.09
Bioavailable T	.33‡	.10	.23†	.15	.40†	.31	−.23‡	−.15*
DHEAS	.10	.08	.07	−.02	.41†	.27	−.10	.11*
Insulin	.57‡	.15	.45‡	.14	.42†	.28*	.39‡	.23‡

*$P < .05$.
†$P < .01$.
‡$P < .001$.

Table 4. Multivariate Models With Log (leptin) as the Dependent Variable and Age, Total Fat Mass, Total Lean Mass, Bioavailable E, Bioavailable T, DHEAS, and Insulin as the Independent Variables Among Rochester, MN Men and Women

Independent Variable	Premenopausal Women			Postmenopausal Women Not on HRT			Postmenopausal Women on HRT			Men		
	β	SE	*P*	β	SE	*P*	β	SE	*P*	β	SE	*P*
Fat mass	0.099	0.010	<.001	0.124	0.008	<.001	0.061	0.006	<.001	0.120	0.014	<.001
Lean mass	−0.018	0.007	.019	−0.033	0.007	<.001	−0.039	0.009	<.001	−0.091	0.025	<.001
Insulin	0.055	0.013	<.001				0.025	0.008	.004	0.013	0.006	<.001
DHEAS							0.002	0.001	.02			
Bioavailable E				0.014	0.004	<.001						
Fat mass^2	−0.0008	0.0002	<.001	−0.001	0.0001	<.001				−0.0006	0.0001	<.001
Lean mass^2										0.0007	0.0002	<.001
Insulin^2	−0.0007	0.0003	.02							−0.0002	0.00003	.001
Bioavailable E^2				−0.0001	0.00003	.025						
Fat mass × insulin										0.0005	0.0002	.019
Lean mass × fat mass										−0.0006	0.0003	.036
Model R^2			.81			.78			.83			.79

expression in women. In men, total and bioavailable T levels were inversely related to log (leptin) levels even after adjustment for fat mass. Similar findings were reported by Haffner et al,[32] who found an association between leptin and free T, although this was no longer significant after adjusting for the BMI. In contrast, Paolisso et al[17] noted a persistent relationship between T and leptin even after adjusting for fat mass. Collectively, these data suggest a significant role for T in mediating the sexual dimorphism in serum leptin levels in adults. These findings are also consistent with the results of studies in pubertal children,[25-28] in hypogonadal men,[29,30] and in vitro in rat adipocytes,[51] which strongly suggest that T inhibits leptin production.

In an attempt to define which parameters have the most significant role in modulating serum leptin levels independent of changes in fat mass, we performed a stepwise selection of the different variables. These models were able to predict approximately 80% of the variance in serum leptin. Fat mass, lean mass, and insulin, in decreasing order, were consistently found as the variables significantly predicting serum leptin levels. Of note, after adjusting for fat mass, lean mass was negatively associated with leptin levels. In addition, in postmenopausal women not on HRT, bioavailable E levels were also present in the models, supporting a role for residual estrogens in predicting serum leptin levels.

In summary, this cross-sectional study in a large, population-based group of women and men shows that in addition to fat mass, lean mass and insulin are the strongest predictors of serum leptin levels. It also indicates that bioavailable E may participate in the regulation of leptin production in postmenopausal women not on HRT. While our study has identified significant predictors of circulating leptin levels, further studies are clearly needed to better define the mechanism(s) by which these factors modulate leptin levels.

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