Age-Related Changes in Sex Hormones Affect the Sex Difference in Serum Leptin Independently of Changes in Body Fat

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Serum leptin concentrations are highly correlated with body fatness, but there is considerable variability among individuals after adjusting for differences in body fatness. Theoretically, sex hormone levels may influence serum leptin, since the levels are higher in women than in men independently of body fat. Increasing old age is associated with decreases in serum sex hormone concentrations and changes in body fatness that may independently alter serum leptin concentrations. In a cross-sectional sample of 106 men and 166 women aged 62 to 98 years, serum leptin adjusted for total body fat had a significant positive association with age in men and a nonsignificant negative association with age in women. Serum testosterone had a significant negative association with serum leptin in men after adjusting for total body fat, the fasting insulin resistance index (FIRI), and sex hormone–binding globulin (SHBG). In a longitudinal sample of 22 elderly men and 52 women, serum leptin levels increased significantly over a 14-year period in men, but not in women. Increases in serum leptin were significantly associated with decreases in serum testosterone but not with changes in the body mass index (BMI) in men. In contrast, changes in leptin were associated with changes in the BMI but not with changes in serum estrone in women. These results suggest that differences among men and changes with age in serum leptin are associated with circulating levels of testosterone. Elderly men become progressively "hyperleptinemic" with age regardless of changes in body fatness, possibly due to decreasing testosterone levels.

LEPTIN is a cytokine-like hormone that is secreted by adipose tissue in direct proportion to the amount of stored fat, and may therefore play an important role in the regulation of appetite, insulin action, glucose metabolism, and body composition.[1-4] In humans, serum leptin concentrations are positively associated with body fatness, suggesting that obese subjects may be relatively resistant to the effects of leptin.[1,5] However, an underlying basis for leptin resistance in humans has not been established.[6] There is considerable variability in serum leptin concentrations among individuals after adjusting for body fatness, which remains to be explained. Published data indicate that individuals may be relatively "hyperleptinemic" or "hypoleptinemic" at all levels of body fat. Some of this variability may be due to measurement errors and variation in the diurnal cycle and pulsatile secretion of leptin.[7] One important clue to this variability may be that serum leptin levels are systematically higher in women versus men regardless of body fatness.[8] This suggests that sex hormones may play a role in regulating leptin production, clearance, or action, and could explain not only the sex difference in serum leptin concentrations but also some of the variability in fat-adjusted leptin levels within each sex.

Another possible clue is the observation that body composition changes with age. Body fatness increases with age up to about 60 years, and then remains stable or decreases during old age. The distribution of adipose tissue also becomes more centralized with age, particularly in women during and following menopause. These age-related changes in body composition have been linked to changes in sex hormones and other hormones involved in the regulation of metabolism, in addition to changes in the balance between energy intake and expenditure. It is not clear to what extent these age-related changes in body composition and hormones are associated with changes in serum leptin concentrations. Several cross-sectional studies have reported no association of age with serum leptin independently of body fatness or the body mass index (BMI).[1,8-10] On the other hand, Ostlund et al[11] reported that serum leptin had a significant negative association with age in 204 men and women and was 53% lower in elderly subjects older than 60 years compared with younger subjects. Rosenbaum et al[8] reported that serum leptin levels were significantly lower in elderly postmenopausal compared with younger premenopausal women.

Results from a growing number of recent studies suggest that testosterone inhibits leptin production, whereas estrogens may either increase production or cause leptin resistance.[8-10,12-23] Testosterone decreases with age in men over 60 years, and the prevalence of low testosterone increases rapidly in old age.[24] Estrogens decrease in women during menopause, but the variability with age in postmenopausal women is most likely due to either differences in body fatness and conversion of androgens to estrone in adipose tissue or hormone replacement therapy (HRT). If testosterone inhibits leptin production, serum leptin concentrations could increase during old age in men, as testosterone levels decline independently of body fatness. If estrogens positively affect leptin levels, then postmenopausal women with low levels of estrone should have lower serum leptin concentrations, closer to those in men, and women on HRT should maintain higher leptin concentrations typical for younger women independently of body fatness. Taken together, these potential opposing effects of sex hormones should diminish the sex difference in fat-adjusted serum leptin concentrations in old age. Thus, age-related changes in serum leptin could

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occur due to changes in sex steroid hormones, particularly androgens, independently of changes in body composition.

The purpose of this study was to test the hypotheses that in elderly men and women (1) the sex difference in serum leptin decreases with age, (2) serum leptin is associated with serum sex hormones independently of age and body fatness, (3) serum leptin changes over time within individuals independently of changes in BMI, and (4) changes over time in serum leptin are associated with changes in serum sex hormone levels. To test these hypotheses, we used a combination of cross-sectional and longitudinal data for participants in the New Mexico Aging Process Study (NMAPS).

SUBJECTS AND METHODS

The data are for elderly participants aged 62 to 98 years in the NMAPS, a longitudinal study of elderly men and women that began in 1980. A detailed description of the NMAPS can be found elsewhere.[25] All subjects were Caucasian, with about 3% claiming Hispanic ancestry. Two different data sets were analyzed. The first consisted of cross-sectional data for 106 male and 166 female participants collected in 1995 that included measurements of body composition from dual-energy x-ray absorptiometry, as well as serum leptin, fasting insulin and glucose, and sex hormone concentrations. This cross-sectional data set was used to examine the effects of sex- and age-related differences in body composition, hormones, and insulin resistance on serum leptin. The second data set consisted of longitudinal data for 22 male and 52 female participants who entered the study between 1980 and 1982 for whom the BMI, serum leptin, and sex hormone data were available at entry and for 1994. This longitudinal data set was used to determine whether serum leptin changes over time within elderly individuals in association with changes in the BMI and sex hormones.

Entrance criteria for the NMAPS have been constant since 1980, and exclude those with serious clinical conditions such as recent myocardial infarction, significant peripheral vascular disease, insulin-dependent diabetes, hepatic disease, history of internal cancer requiring surgery, x-ray or chemotherapy in the past 10 years, a positive test for hepatitis, and untreated hypertension (systolic blood pressure [BP] > 180 mm Hg, diastolic BP > 100 mm Hg) and those taking prescription medications, except thyroid and estrogen replacement or minor antihypertensives. Men taking anabolic steroid medications were excluded from both cross-sectional and longitudinal data sets.

In the cross-sectional data set, 20% of the women and 29% of the men had coronary heart or cardiovascular disease, 37% of women and 31% of men had cancer diagnosed subsequent to entry (mostly skin cancer), 52% of men and 67% of women had osteoarthritis, and 24% of men and 29% of women had hypertension controlled by medications. None had diagnosed non–insulin-dependent diabetes mellitus (NIDDM). Twenty-eight percent of the women were taking HRT. None of the men and less than 5% of the women were smokers, and alcohol intake was reported to be light to moderate. All subjects were weight-stable at the time of examination and did not report significant weight loss or gain in the 6 months prior to the study. None reported recent acute illness or current use of significant medications other than antihypertensives or HRT in the women. None of the participants in the longitudinal cohort were diagnosed with incident NIDDM during the follow-up period.

Weight was measured to the nearest 0.1 kg using a beam-balance scale. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. The BMI was calculated as weight (kilograms) divided by height (meters) squared. Body composition was measured during 1995 using dual-energy x-ray absorptiometry (Lunar DPX, version 3.6z software; Lunar Radiation, Madison, WI). Medium-length scans (20 minutes) were used, except in subjects with greater than 27-cm anteroposterior thickness, for whom the slow (40-minute) scan speed was used. Fat and fat-free soft tissue masses were measured for the whole body, arms, and legs using the manufacturer's definitions.[26] The precision is about 1.5% for fat mass and about 1% for fat-free mass.

Fasting blood samples (~50 mL) were obtained in all study years by venipuncture between 7:30 and 9:00 AM, placed by aliquots into Nalgene (Fisher Scientific, Houston, TX) cryovials, and stored at −70°C. For the 1995 cross-sectional sample, the fasting serum insulin level was measured using a solid-phase ^{125}I-radioimmunoassay (Diagnostic Products, Los Angeles, CA) at the General Clinical Research Center laboratory at the University of New Mexico. Fasting serum glucose was assayed by the New Mexico Medical Reference Laboratory on blood samples on the same day they were drawn. All other assays were performed in the Clinical Nutrition Program Laboratory. The leptin level was measured using a radioimmunoassay (Linco Research, St Louis, MO). The interassay coefficient of variation (CV) for this assay was 6.2%. Estrone assays were performed by Quest Laboratories (San Juan Capistrano, CA) using extraction, chromatography, and a ^{125}I-radioimmunoassay with a reported sensitivity of 10 pg/mL and interassay CV of 15%. Testosterone was assayed using a ^{125}I-coated tube radioimmunoassay (Incstar, Stillwater, MN) with a sensitivity of 0.059 ng/mL. The intraassay CV range was 13.8% at low levels to 4% at concentrations greater than 5 ng/mL. The sex hormone–binding globulin (SHBG) level was measured using a radioimmunoassay (Radim Group, Wein Laboratories, Succasunna, NJ) with a sensitivity of 6 nmol/L and interassay CV of 8%.

For the longitudinal data set, serum leptin, estrone, and testosterone were assayed by the Geriatric Research, Education, and Clinical Center (GRECC), St Louis, MO, as part of a study of long-term changes in hormone levels within NMAPS participants.[24] SHBG could not be measured in most samples due to an insufficient volume remaining after assays for the other hormones. The serum leptin level was measured with the same commercial radioimmunoassay kit (Linco Research, St Louis, MO) used by the Clinical Nutrition Program (CNP). The level of serum estrone was also measured using the same method as in the cross-sectional study. Serum testosterone was determined using the DPC (Los Angeles, CA) radioimmunoassay kit. The intraassay CV was 5.8% and the interassay CV was 10.4%, as reported previously.[24] Because the CNP and GRECC used different assays for testosterone, data from the different assays were not mixed in either the cross-sectional or longitudinal analyses. There was a statistically significant difference between 1994 and 1995 testosterone levels determined using different assays at the two laboratories (mean difference, 1995-1994 = −92.2 ± 22.0 ng/dL, $P < .001$), but it was not clear if this was due to a decrease in testosterone levels or a systematic difference between assays. The correlation between 1994 and 1995 testosterone concentrations by the different assays was .87. There was no significant difference between laboratories for 1994 and 1995 determinations of serum leptin (mean difference, 0.45 ± 0.60 ng/mL, $P = .46$), and the correlation between 1994 and 1995 values was .84.

Data analyses were performed using the JMP statistical package (SAS Institute, Cary, NC). The distributions for insulin, leptin, estrone, and SHBG were positively skewed and were transformed using natural logarithms to approximate normal distribution. The fasting insulin resistance index (FIRI) was calculated as described by Duncan et al.[27] This index was derived as the product of fasting glucose (millimolars) and fasting insulin (milliunits per liter), normalized by dividing by 25 (5 mmol/L glucose × 5 mU/L insulin). The index was significantly skewed, and was consequently transformed using natural logarithms to approximate normality. Multiple linear regression analysis was used to analyze the associations of sex, age, body fatness, fasting serum insulin and glucose, and sex hormones with serum leptin. Paired t tests were used to determine statistically significant changes between the baseline and follow-up periods in serum leptin, sex hormones, and the BMI within each sex. Multiple linear regression analysis was used to test the association between the change in serum leptin and the change in sex

hormones controlling for age and change in the BMI. All comparisons or regression parameters were considered statistically significant at a P level less than .05.

All participants in the study provided informed consent for all procedures. The study was approved by the Human Research Review Committee of the University of New Mexico School of Medicine.

RESULTS

Cross-Sectional Analyses

Descriptive statistics for study variables in the cross-sectional sample are shown in Table 1. There was no statistically significant difference between men and women for age, BMI, or fasting serum insulin or glucose. Women had significantly higher total body fat and serum leptin concentrations than men. SHBG was significantly higher in women versus men; however, there was no difference between the sexes when women (n = 39) on HRT were excluded. Serum estrone was significantly increased in women taking HRT, but there was no statistically significant difference in serum leptin between women taking HRT (mean ± SE, 22.4 ± 2.1 ng/mL) and those not taking HRT (mean ± SE, 19.5 ± 1.2 ng/mL).

Total body fat had a strong linear association with log-transformed serum leptin in men ($r = .75$, $P < .0001$) and women ($r = .82$, $P < .0001$). Figure 1 shows the association of age with serum leptin adjusted for total body fat in each sex by regression analysis. Age had a statistically significant positive correlation with fat-adjusted leptin in men ($r = .23$, $P < .02$) and a nonsignificant negative correlation in women ($r = -.13$, $P < .10$). As a result, the regression lines for men and women converged with increasing age. This convergence with age was even more pronounced when serum leptin was expressed as nanograms per milliliter per kilogram of fat mass (data not shown). Notably, even in very old age (>90 years), women were predicted to have higher serum leptin concentrations than men after adjusting for body fat.

The interaction of age and sex was statistically significant ($P < .003$; Table 2). This model indicates that when maintaining body fat constant, there is a slight statistically nonsignificant decrease with age in serum leptin in women, but a significant increase with age in men. For example, if body fat is kept constant at 22 kg in both sexes, the model predicts that serum leptin will be 11% lower in women aged 80 years versus those aged 65 years, but 31% greater in men aged 80 years versus those aged 65 years.

Table 1. Descriptive Statistics for Cross-Sectional Sample for 1995

Parameter	Men (n = 106)	Women (n = 166)
Age (yr)	78.6 ± 5.8	77.6 ± 6.4
BMI (kg/m²)	25.3 ± 3.5	25.2 ± 3.9
Waist to hip ratio	0.97 ± 0.06	0.86 ± 0.06
Total fat mass (kg)	19.8 ± 7.6	23.6 ± 8.6
Fasting insulin (pmol/L)	68.9 ± 43.5	61.2 ± 35.2
Fasting glucose (mmol/L)	5.3 ± 0.6	5.1 ± 0.7
Estrone (pmol/L)	—	177.8 ± 215.5*
Testosterone (nmol/L)	13.2 ± 4.8	—
SHBG (nmol/L)	46.3 ± 33.7	66.6 ± 38.1
Leptin	7.7 ± 5.1	20.1 ± 13.2

*Thirty-nine women were taking HRT: serum estrone levels were significantly ($P < .0001$) higher in women taking HRT *v* those not taking HRT.

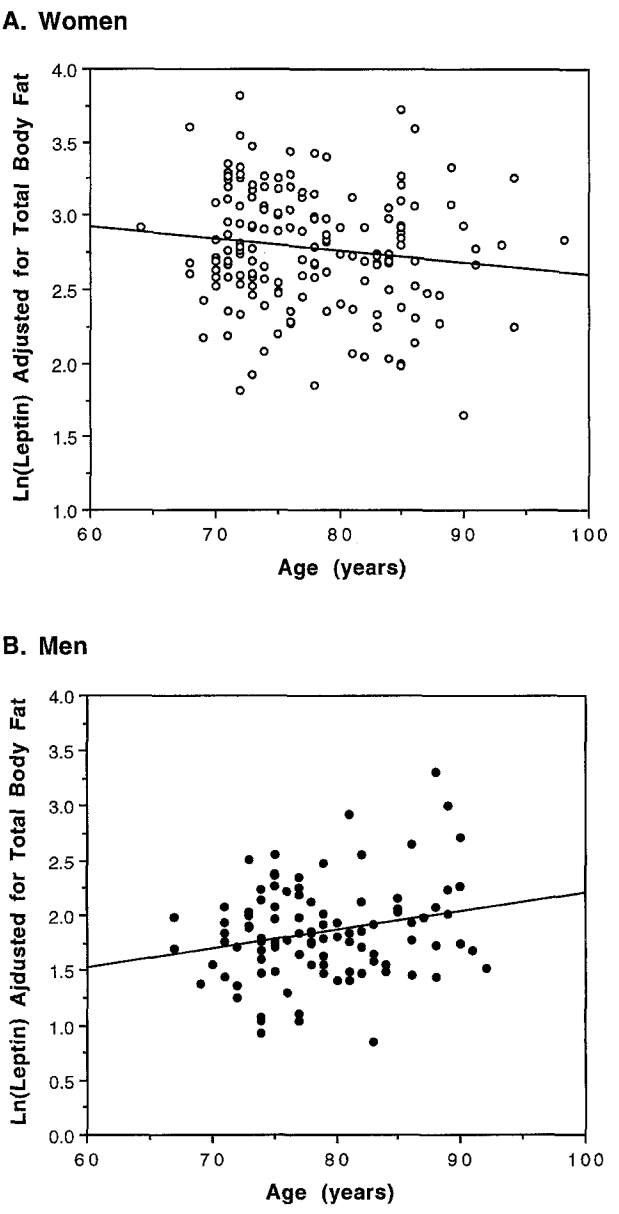

Fig 1. Association of fat-adjusted serum leptin with age in each sex. Fat-adjusted serum leptin was calculated as follows: (1) serum leptin was regressed on total body fat within each sex, (2) the residuals from sex-specific regressions were then added to the mean values for serum leptin in each sex to produce fat-adjusted serum leptin values, and (3) these values were then transformed to natural logarithms to normalize the distribution. Regression of serum leptin on age: (A) fat-adjusted leptin = 3.40 − 0.008 (age), $R^2 = .02$, $P < .10$; (B) fat-adjusted leptin = 0.47 + 0.017 (age), $R^2 = .05$, $P < .02$.

Table 3 shows results for multiple regression analyses to test whether serum sex hormone concentrations are associated with serum leptin after controlling for total body fat, FIRI, and SHBG in each sex. In the model for men, testosterone had a significant negative association with serum leptin (β-coefficient = −0.019 ± 0.008, $P = .03$) independently of total body fat and FIRI, which were strongly independently associated

Table 2. Multiple Regression of Leptin on Age, Sex, and Total Body Fat: Cross-Sectional Data for 1995

Variable	β-Coefficient	SE	P
Intercept	1.881	0.417	<.0001
Total body fat (kg)	0.065	0.003	<.001
Age (yr)	−0.008	0.0045	<.10
Sex (0 = female, 1 = male)	−2.712	0.662	<.001
Age × sex	0.026	0.008	<.003

NOTE. Leptin was transformed to natural logarithms.

with serum leptin ($P < .001$). SHBG had a significant positive association (β-coefficient = 0.150 ± 0.074, $P = .05$) independently of total body fat, FIRI, and testosterone. Taken together, the results for testosterone and SHBG suggest it is free testosterone that influences serum leptin. Testosterone was not significantly associated with serum leptin when age was included in the model (β-coefficient = −0.015 ± 0.009, $P = .09$). Conversely, age was not significantly associated with serum leptin when testosterone and SHBG were included in the model. This suggests that the effects of age on serum leptin in men are confounded with and possibly the same as those of serum free testosterone. Together, total body fat, FIRI, serum testosterone, and SHBG explained 64% of the variance in serum leptin in men. However, testosterone and SHBG explained only 2% and 1%, respectively, of the variance over and above the 61% explained by total body fat and FIRI. In the model for women, neither serum estrone nor SHBG were significantly associated with serum leptin independently of total body fat and FIRI. Age (not shown) was not significantly associated with serum leptin independently of total fat and FIRI regardless of whether estrone and SHBG were included in the model.

Longitudinal Analyses

Descriptive statistics for the study variables in the longitudinal sample are shown in Table 4. The men and women were approximately 71 years old at baseline. There was no significant change in the mean weight or BMI in men over the 14-year follow-up period. However, changes in the BMI during follow-up study were −4.7 to 2.9 kg/m^2. Serum leptin increased significantly by a mean of 1.3 ± 0.6 ng/mL ($P < .05$) and testosterone decreased by a mean of −7.2 ± 1.4 nmol/L ($P < .0001$) in the men. Changes in serum leptin over time were −13.8 to 11.7 ng/mL in men, and changes in testosterone were −1.39 to −32.0 nmol/L. Although there was a statistically significant loss in mean weight over the follow-up period, among the women (−2.7 ± 1.0 kg), there were no statistically significant changes in the mean BMI, leptin, or serum estrone. Changes in the BMI in women were −9.0 to 5.5 kg/m^2, changes in leptin were −20.0 to 36.0 ng/mL, and changes in serum estrone were −89.7 to 120.7 pmol/L. In sum, although the mean changes in body weight and BMI were, for the most part, small and not statistically significant, there was substantial variability among individuals of both sexes for changes over the follow-up period. There was also substantial variability among subjects for changes in serum leptin and sex hormone concentrations.

Table 3. Multiple Regression of Leptin on Age, Body Fat, FIRI, Serum Sex Hormone, and SHBG Within Each Sex

Variable	β-Coefficient	SE	Partial R^2	P
Men (n = 106)				
Intercept	0.228	0.322		<.479
Total body fat (kg)	0.060	0.006	.57	<.0001
FIRI	0.244	0.074	.04	<.001
Testosterone (nmol/L)	−0.019	0.008	.02	<.027
SHBG (ng/L)	0.150	0.074	.01	<.046
		Total R^2	.64	
Women (n = 166)				
Intercept	1.212	0.243		<.0001
Total body fat (kg)	0.053	0.004	.67	<.0001
FIRI	0.337	0.070	.04	<.0001
Estrone (pmol/L)	0.050	0.045	.00	<.265
SHBG (ng/L)	0.002	0.062	.00	<.975
		Total R^2	.71	

NOTE. Leptin, estrone, and SHBG concentrations and FIRI were transformed to natural logarithms to normalize the distribution.

Table 5 shows results for multiple regression analyses of the change in serum leptin on age, change in BMI, and change in sex hormone concentrations in each sex. In men, the change in serum leptin was not associated with the change in BMI, but it had a statistically significant negative association with the change in serum testosterone (β-coefficient = −0.15 ± 0.005, $P < .009$). The change in testosterone explained 34% of the variance in the change in leptin; the change in BMI and age explained an additional 3%. In the women, the change in serum leptin had a significant negative association with age (β-coefficient = −0.62 ± 2.6, $P < .02$) and a strong positive association with the change in BMI (β-coefficient = 1.84 ± 0.38, $P < .001$), but it was not significantly associated with the change in serum estrone. The change in BMI explained 47% of the variance in the change in leptin in women; age explained an additional 5%.

Because the loss in stature during old age can positively bias the BMI such that changes in the BMI underestimate changes in body fatness, we also analyzed the data using the change in weight and change in stature as independent variables. There was no significant association between the change in BMI and change in stature, but there was a strong linear association between the change in BMI and change in weight in both sexes. In men, the change in weight was not associated with the change in leptin when controlling for the change in stature. However, the change in leptin remained statistically significantly associated with the change in testosterone in men ($P < .01$) after controlling for age and changes in weight and stature. In women, the change in weight was significantly associated with the change in leptin ($P < .001$); there was no association with the change in stature.

DISCUSSION

The results of the present study suggest that changes in serum leptin occur with age in elderly men and women that are only partly explained by changes in body fat or the BMI. The sex difference in serum leptin that is well established in younger adults tends to diminish with age in the very old. This convergence of sex-specific serum leptin values appears to be primarily due to a trend for increasing concentrations in men with age and over time that is independent of age-related differences or changes in body fat. Age-related differences or changes over time in serum leptin appear to be more closely

Table 4. Descriptive Statistics (mean ± SE) for Longitudinal Sample

Parameter	Baseline	1994	Mean Difference	P*	Range
Men (n = 22)					
Age (yr)	70.5 ± 0.7	—	—	—	—
Weight (kg)	74.2 ± 1.8	73.3 ± 2.4	−0.9 ± 1.3	.57	−9.8-8.9
BMI (kg/m²)	25.0 ± 0.5	24.8 ± 0.7	−0.2 ± 0.4	.54	−4.7-2.9
Leptin (ng/mL)	6.1 ± 0.7	7.4 ± 0.8	1.3 ± 0.6	.05	−13.8-11.7
Testosterone (nmol/L)	21.5 ± 1.1	14.3 ± 1.3	−7.2 ± 1.4	.0001	−32.0--1.4
Women (n = 52)					
Age (yr)	71.6 ± 0.60	—	—	—	
Weight (kg)	60.7 ± 1.1	58.0 ± 1.3	−2.7 ± 0.96	.01	−24.1-12.4
BMI (kg/m²)	24.2 ± 0.4	23.9 ± 0.5	−0.33 ± 0.39	NS	−9.0-5.5
Leptin (ng/mL)	15.5 ± 1.6	15.6 ± 1.8	0.02 ± 1.29	NS	−20.0-36.0
Estrone (pmol/L)	15.9 ± 2.4	13.3 ± 3.5	−2.9 ± 3.2	NS	−89.7-120.7

*Paired *t* test for difference between 1994 and 1980-1982 values.

related to differences or changes in body fat in elderly women than in men. Our data support the hypothesis that age-related increases in serum leptin in men are associated with age-related decreases in serum testosterone independently of body fat. Our data do not support the hypothesis that changes in serum leptin in elderly women are associated with age or serum estrogens independently of body fat.

We also found a significant positive association between insulin resistance measured by FIRI and serum leptin that was independent of body fatness in both sexes, as reported in previous studies.[9,28-30] In our study, the association was mainly attributable to fasting insulin and not glucose. However, the association between leptin and FIRI was slightly stronger than with fasting insulin, suggesting that controlling for variation in serum glucose in the FIRI improves the association.

Some studies have reported that serum leptin adjusted for body fatness does not differ significantly between premenopausal and postmenopausal women and is not increased in postmenopausal women taking HRT.[16,31,32] However, Rosenbaum et al[8] found that serum leptin adjusted for body fat was significantly higher in premenopausal compared with postmenopausal women. Shimizu et al[21,22] also reported that the mean serum leptin concentration was significantly lower in postmenopausal versus premenopausal women matched for body fat mass, and that leptin concentrations were higher in 13 young premenopausal women during the luteal versus follicular phase of the menstrual cycle. In their cross-sectional study of women, Perry et al[10] reported a significant correlation ($r = .27$) between plasma leptin and estradiol. A possible limitation of the present study is the measurement of estrone, which has less potent effects than estradiol. However, estrone is the primary estrogen in postmenopausal women and is mainly produced by the aromatization of androgens in adipose tissue. Serum estrone is highly correlated with estradiol in women taking HRT. In our cross-sectional data set, serum estrone was substantially higher in women taking HRT, and most of the variation among women was not attributable to differences in body fatness. Thus, an effect of estrogens on serum leptin cannot be entirely ruled out. Nonetheless, it has been suggested that the sex difference in serum leptin is mainly due to inhibition of leptin production by testosterone in men, since the levels in postmenopausal women not taking HRT are still higher than the levels in men.[8]

Table 5. Multiple Regression of the Change in Serum Leptin on Age, Change in BMI and Change in Serum Testosterone Over 14 Years of Follow-up Study

Variable	β-Coefficient	SE	P
Men (n = 22)			
Intercept	−12.026	11.292	<.302
Age at baseline (yr)	0.155	0.163	<.356
Change in BMI	0.075	0.295	<.802
Change in testosterone	−0.015	0.005	<.009
Women (n = 52)			
Intercept	45.158	18.824	<.02
Age at baseline (yr)	−0.621	0.263	<.02
Change in BMI	1.844	0.384	<.001
Change in estrone	0.029	0.045	<.513

Several cross-sectional studies have reported that testosterone is associated negatively with serum leptin independently of body fatness.[13,33,34] However, Haffner et al[35] did not find a significant association after adjusting for BMI in a study of 87 middle-aged men. It is important to note that in the present study, the size of the effect of testosterone on serum leptin was small in both cross-sectional and longitudinal analyses, indicating that it may only be detectable in a larger sample or over a long follow-up period in men who are not under any form of experimental treatment. In addition, there may be a threshold level for serum testosterone that is necessary for the suppression of leptin, and few men have been included in previous cross-sectional studies with serum testosterone concentrations below this threshold. Serum leptin levels are reported to decrease in boys during adolescence and are significantly lower than the levels in adolescent girls after adjusting for body fatness.[14,23,36] In clinical trials, testosterone treatment has been reported to decrease leptin concentrations in hypogonadal men, boys with delayed puberty, and female transsexuals independently of changes in body fatness.[12,15,17,20] Ours is the first study that we are aware of to suggest that natural, age-related decreases in testosterone levels are associated with increases in serum leptin independently of changes in body fatness. Taken together, the results of these various cross-sectional, longitudinal, and experimental studies strongly indicate that testosterone is involved in the physiological regulation of leptin in humans.

The exact mechanism by which testosterone is associated

with leptin remains to be established. Barash et al[37] reported that leptin treatment of male ob/ob mice increased serum follicle-stimulating hormone, testicular and seminal vesicle weight, and the sperm count, which suggests that leptin may stimulate testosterone production. However, if this were the case in humans, one would expect a positive association between serum leptin and testosterone, or the reverse of what has been reported as reviewed herein. Lonnqvist et al[38] reported that leptin gene expression was lower in abdominal adipose tissue of men compared with women. Wabitsch et al[23] recently reported that dihydrotestosterone suppresses leptin mRNA in adipocytes and secretion of leptin in vitro. These data suggest a direct effect of testosterone on leptin production. To our knowledge, alternative effects of testosterone on leptin binding proteins, renal clearance, or tissue sensitivity have not been studied.

The results of our study appear to contrast with those reported by Nicklas et al[39] for changes in leptin with diet-induced weight loss in older obese men and women. They found that although men and women lost a similar amount of weight in response to a 6-month hypocaloric diet, changes in serum leptin were correlated significantly with the change in fat mass in men but not in women. There are some important differences between our studies that affect any comparison of the results. First, we studied the association of leptin with long-term naturally occurring changes in weight in an older group of non-obese men and women: no experimental treatment to produce a weight change was administered. We did not control for the possible effects of incident morbidity during follow-up study or discriminate voluntary from involuntary weight change in our longitudinal analyses. Given the general good health and nutritional status of our participants, serious confounding by these factors, although possible, seems unlikely. Our focus was the possible association of age-related differences and changes over time in sex hormones with leptin concentrations when controlling for changes in body weight or composition. The study by Nicklas et al[39] did not measure or control for changes in sex hormone levels with weight loss. Nonetheless, our results could be interpreted as suggesting that changes in leptin are less closely linked to changes in body composition in elderly men versus women, which is the reverse of the finding reported by Nicklas et al.[39] It is possible that acute changes in sex hormones or other regulatory factors result in a dissociation between short-term changes in body fat and serum leptin more in women versus men, but that long-term age-related changes in sex hormones, specifically testosterone, result in a more gradual dysregulation and subsequent dissociation between body composition and serum leptin in elderly men but not in women.

In summary, our results confirm that although the body fat mass is the primary determinant of differences in serum leptin concentrations among elderly men and women, decreasing serum testosterone with age may be additionally associated with an age-related increase in serum leptin in elderly men that is independent of changes in body fat. This may explain the gradual trend for convergence of the sex difference in fat-adjusted serum leptin concentrations in the present study. Although the magnitude of the association of testosterone with leptin is small in our cross-sectional analyses for men, results from our longitudinal analyses are supportive in that they suggest long-term changes in serum leptin in elderly men are more closely associated with changes in serum testosterone than changes in body composition. In conclusion, testosterone appears to play a role in the regulation of leptin metabolism. The mechanism by which testosterone affects leptin remains to be determined.

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