Clinical Study

Resting metabolic rate, body composition, and serum leptin concentrations in a free-living elderly population

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Abstract

Objective: The present study investigated the relationship between serum leptin concentrations and resting metabolic rate (RMR) in a large study group of elderly individuals with special consideration of body composition and body fat distribution as possible confounders.

Design and methods: The subjects were 122 women (age: 69 ± 6 years, body mass index (BMI): 26.3 ± 3.6 kg/m²) and 82 men (age: 69 ± 5 years, BMI: 26.0 ± 2.6 kg/m²). RMR was measured by indirect calorimetry and body composition by the bioelectrical impedance method. Serum leptin levels were determined by radioimmunoassay.

Results: There was a strong correlation between fat mass (FM) and serum leptin levels in both sexes. An age-related decline in leptin levels adjusted for FM was observed only in the women. After adjustment of RMR for both fat-free mass (FFM) and FM, leptin levels were not associated with RMR. In stepwise multiple regression analysis, FFM was the main predictor of RMR, explaining 35.8% and 47.6% of the variance of RMR in men and women respectively. FM did not explain variance in RMR in men, but accounted for 2.6% of the variance in RMR in women. Waist-hip-ratio and age influenced RMR only in males, explaining 5.7% and 4.0% of the variance in RMR respectively.

Conclusion: Leptin is not a significant predictor of RMR in the elderly, but body composition and distribution of body fat are significantly associated with RMR.

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Introduction

During advanced aging, long-term alterations in body weight (BW) are to be expected. Body weight and body composition are related to health status and life expectancy. While obesity is an important risk factor for mortality in middle age, underweight also becomes a risk factor for mortality in later age (1, 2). Leptin is postulated to be involved in the regulation of BW by inducing a decrease in appetite and an increase in energy expenditure. Therefore, it is of special interest to know whether age-related changes in BW might be associated with leptin-mediated changes in energy expenditure.

While leptin has a clear-cut effect on metabolic rate and body temperature in ob/ob mice, these effects are less obvious in wild-type animals (3). In rat pups, exogenous application of leptin did not increase energy expenditure under thermoneutral conditions. However, upon thermoregulatory activation leptin had an effect on metabolic heat production in these animals. Furthermore, in lean adult mice on food restricted diets leptin attenuated the daily minima of metabolic rate and core temperature (4).

Human studies in a variety of subjects investigating the relationship between serum leptin concentrations and energy expenditure are available with inconsistent results: some of these studies could not detect any association between leptin concentrations and resting metabolic rate (RMR) in adults with stable body weights and a wide range of body mass index (BMI) (5–7), and in patients with lung cancer cachexia (8); positive correlations were found in a group of 18 healthy men (9), in 25 free-living older African-American women (10), and in 18 elderly heart failure patients (11); a negative correlation was observed in a group of 45 obese subjects (12). Some of these inconsistencies may have methodological causes. When investigating the
relationship between RMR and leptin careful consider-
ation has to be given to the body composition of the individuals studied. Fat mass (FM) is not only related to serum leptin levels but, besides fat-free mass (FFM), is also a predictor of RMR (13). Therefore, it is important to adjust RMR appropriately for both body compart-
ments or to eliminate the effects of FFM and FM on RMR by multiple regression analysis before investigating the influence of leptin on RMR. In the afore mentioned studies reporting a positive association of leptin with RMR, some of these aspects were not considered adequately.

Furthermore, the relationship between adiposity and leptin during aging is not clear. As discussed by Mobbs (14), the studies so far indicate that leptin decreases with age mainly after age 60 and that the decrease in leptin with age may be countered by an increase in adiposity with age, which is one of the reasons why the question of whether leptin decreases with age independ-
ently of FM remains to be convincingly demonstrated. In the light of these conflicting results and the few data obtained in elderly subjects, the present study aimed to investigate if serum leptin concentrations are related to RMR in large study groups of elderly males and females with special consideration of body composition as a possible confounder.

Subjects and methods

Study subjects

The study subjects were participants of the longitudinal study in an aging population of Giessen (GISELA), in which free-living citizens of Giessen have been investi-
gated with regard to nutritional and health status at annual intervals since 1994. Inclusion criteria for enrolment in the GISELA study are an age of at least 60 years and physical mobility. 122 female and 82 male subjects who participated in the GISELA study during 1997, who were not suffering from diagnosed diabetes, hypo- or hyperthyroidism, or edema, and who were not taking any thyroid hormones or diuretics were included in the present investigation. The study protocol was approved by the Ethical Committee of the faculty of medicine at the Justus-Liebig-University, Giessen. A written informed consent was obtained from each study participant.

Methods

Blood sampling and all the measurements were carried out in the morning after an overnight fast in the Institute of Nutritional Sciences using standardized methods.

Anthropometry and body composition

Body weight was measured with a calibrated digital scale (SECA, Vogel & Halke, Hamburg, Germany) to the nearest of 0.1 kg after shoes, coats, and sweaters had been removed. From the measured weight, 0.5 to 1.0 kg were subtracted for the remaining clothes. Body height was measured to the nearest 0.5 cm by a height measurement device integrated in the scale. Waist and hip circumferences were measured to the nearest of 1.0 cm using a non-elastic tape. Body composition was determined by a bioelectrical impedance method (BIA 101, RJL Systems, Data-Input, Frankfurt, Germany). FFM and FM were calculated using the equation of Deurenberg et al. (15).

Resting metabolic rate

Resting metabolic rate was measured on an outpatient basis by indirect calorimetry for 25–35 min using a ventilated hood system (Deltatrac MBM-100, Hoyer, Bremen, Germany), with measurements of CO₂ produc-
tion and O₂ consumption performed with 1-min intervals. The subjects were allowed to acclimatize appropriately before the ventilated hood was placed over their head and measurements were started. The initial 10 min of calorimetry were used for further acclimati-
ization. Thereafter, gas exchange of the subjects had reached stable levels and the following 15 to 20 min were taken to calculate RMR using the equation of Weir (16).

Measurement of serum leptin

Serum was obtained from blood drawn by venipuncture and stored at −80°C until analysed. Leptin levels were measured by radioimmunoassay as previously described in detail (17).

Statistical analysis

All statistical analyses were performed with the SPSS/ PC Statistical Package version 6.1.3 (SPSS Inc, Chicago, IL, USA). Data are expressed as means ± S.D. The vari-
ables were checked concerning normal distribution with Kolmogorow-Smirnow’s test. Leptin concentrations were log₁₀ transformed to normalize the distribution. Student’s unpaired t-test was used for comparison between the group means of males and females. Pearson’s product-moment correlations (r) were calculated to measure the strength of associations between RMR or leptin and different variables. Multiple linear regression with a stepwise procedure was applied to define the variables most predictive of RMR. Because FFM and FM are significant determinants of RMR, RMR was statistically adjusted for FFM and FM as delineated by Ravussin and Bogardus (18) to control for variation attributable to differences in body composition. For this purpose the following formula derived from our data was applied: RMR (kJ/24 h)[adjusted for FFM+FM] = RMR (kJ/24 h)[measured] + (82.7 × (45.1 – FFM (kg)[measured])) + (10.4 × (27.4 – FM (kg)[measured])). In a similar way,
adjustment of Ig leptin for FM was achieved by using the equation: Ig leptin_{adjusted for FM} = Ig leptin + 0.04 \times (27.4 - FM (kg)_measured). Descriptive comparisons of physical characteristics, RMR, and leptin data between male and female subjects were performed (Table 1). Pearson’s product-moment correlation coefficients for the associations of leptin, Ig leptin_{adjusted for FM}, RMR, RMR/BW, and RMR/FFM, respectively, with anthropometric characteristics, body composition, and age were also assessed in a descriptive fashion (Tables 2 and 3). Two tests for statistical significance were applied to the correlations between RMR_{adjusted for FFM+FM} and Ig leptin in both males and females; statistical significance is assumed for a \( P \) value less than 0.025 (Bonferroni correction for two independent tests). We assume that the adjustment of RMR for both FFM and FM is the optimal procedure for assessing the independent influence of leptin on RMR. The multiple regression analysis was performed on a descriptive basis.

### Results

Physical characteristics, RMR, and serum leptin data of the subjects are presented in Table 1. Age and BMI did not differ by gender. Waist-hip-ratio (WHR) was higher in males than in females. Females had higher FM and lower FFM both in absolute terms and in percentage of BW compared with male subjects. RMR and RMR per kg BW were lower in females than in males, whereas RMR per kg FFM was higher in the female subjects. However, there was no gender difference in RMR after adjustment for FFM and FM. After adjustment for FM, serum leptin levels in the women were still nearly twice as high as in men.

The associations of leptin with anthropometric characteristics, body composition, and age were presented in Table 2. After adjustment for FM only the negative association of leptin with age observed in female subjects, but not in males, persisted. The associations of leptin with BMI, WHR, age, and leptin and RMR are shown. Using the expression of RMR per kg BW or per kg FFM yielded associations with leptin. Our main analysis revealed that after adjustment of RMR for both FFM and FM leptin was not associated with RMR. Thus, the observed

### Table 1 Physical characteristics, RMR, and leptin data of the subjects.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Female ((n = 122))</th>
<th>Mean</th>
<th>S.D.</th>
<th>Male ((n = 82))</th>
<th>Mean</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td></td>
<td>69</td>
<td>6</td>
<td>69</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Anthropometric characteristics</td>
<td></td>
<td></td>
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<tr>
<td>Body weight (kg)</td>
<td>67.0</td>
<td>9.7</td>
<td></td>
<td>77.9(^c)</td>
<td>9.3</td>
<td></td>
</tr>
<tr>
<td>Body height (m)</td>
<td>1.60</td>
<td>5.2</td>
<td></td>
<td>1.73(^c)</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>26.3</td>
<td>3.6</td>
<td></td>
<td>26.0(^c)</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>Waist-hip-ratio</td>
<td>0.83</td>
<td>0.04</td>
<td></td>
<td>0.95(^c)</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Body composition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FM (kg)</td>
<td>29.9</td>
<td>5.8</td>
<td></td>
<td>24.9(^c)</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>FM (%)</td>
<td>44.3</td>
<td>3.4</td>
<td></td>
<td>31.8(^c)</td>
<td>3.0</td>
<td></td>
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<tr>
<td>FFM (kg)</td>
<td>37.2</td>
<td>4.6</td>
<td></td>
<td>53.0(^c)</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>FFM (%)</td>
<td>55.7</td>
<td>3.4</td>
<td></td>
<td>68.2(^c)</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>RMR</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>RMR (kJ/24 h)</td>
<td>5421</td>
<td>588</td>
<td></td>
<td>6745(^c)</td>
<td>704</td>
<td></td>
</tr>
<tr>
<td>RMR/BW (kJ/kg/24 h)</td>
<td>81.7</td>
<td>8.7</td>
<td></td>
<td>87.2(^c)</td>
<td>8.9</td>
<td></td>
</tr>
<tr>
<td>RMR/FFM (kJ/kg/24 h)</td>
<td>146.8</td>
<td>14.0</td>
<td></td>
<td>127.9(^c)</td>
<td>12.6</td>
<td></td>
</tr>
<tr>
<td>RMR_{adjusted for FFM+FM} (kJ/24 h)</td>
<td>5927</td>
<td>418</td>
<td></td>
<td>5993(^c)</td>
<td>482</td>
<td></td>
</tr>
<tr>
<td>Leptin</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Serum leptin (ng/ml)</td>
<td>18.7</td>
<td>12.3</td>
<td></td>
<td>4.7(^c)</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>Serum leptin/FM (ng/ml/kg)</td>
<td>0.59</td>
<td>0.31</td>
<td></td>
<td>0.18(^c)</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>Ig Leptin (ng/ml)</td>
<td>1.17</td>
<td>0.31</td>
<td></td>
<td>0.56(^c)</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>Ig Leptin_{adjusted for FM} (ng/ml)</td>
<td>1.08</td>
<td>0.21</td>
<td></td>
<td>0.65(^c)</td>
<td>0.27</td>
<td></td>
</tr>
</tbody>
</table>

\( a \) \( P < 0.05 \); \( b \) \( P < 0.01 \); \( c \) \( P < 0.001 \) (nominal \( P \) values).

### Table 2 Pearson’s product-moment correlation coefficients for the associations of leptin and Ig leptin_{adjusted for FM} with anthropometric characteristics, body composition, and age.

<table>
<thead>
<tr>
<th></th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BMI (kg/m(^2))</strong></td>
<td>0.72(^c)</td>
<td>0.47(^c)</td>
</tr>
<tr>
<td><strong>WHR</strong></td>
<td>-0.08</td>
<td>0.29(^c)</td>
</tr>
<tr>
<td><strong>FM (kg)</strong></td>
<td>0.74(^c)</td>
<td>0.55(^c)</td>
</tr>
<tr>
<td><strong>FM (%)</strong></td>
<td>0.58(^c)</td>
<td>0.49(^c)</td>
</tr>
<tr>
<td><strong>FFM (kg)</strong></td>
<td>0.52(^c)</td>
<td>0.30(^b)</td>
</tr>
<tr>
<td><strong>FFM (%)</strong></td>
<td>-0.58(^c)</td>
<td>-0.49(^c)</td>
</tr>
<tr>
<td><strong>Age (year)</strong></td>
<td>-0.28(^b)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

\( a \) \( P < 0.05 \); \( b \) \( P < 0.01 \); \( c \) \( P < 0.001 \) (nominal \( P \) values).
negative correlation between leptin and RMR per kg BW or per kg FFM are due to inadequate consideration of the effects of body composition on RMR. Additional descriptive analyses revealed that after adjustment of RMR for both FFM and FM age was not associated with RMR. A positive correlation of WHR with RMR adjusted for FM and FFM was found only in males.

In order to descriptively assess the magnitude of the explained variance of RMR we performed multiple stepwise regression analysis of RMR upon FFM, FM, leptin, WHR, and age as independent variables. The main predictor of RMR was FFM, explaining 35.8% and 47.6% of the variance in RMR of men and women respectively (nominal $P < 0.001$). In accordance with the afore mentioned lack of correlation between RMR adjusted for both FFM and FM, serum leptin did not explain variance in either gender. FM did not influence RMR in men, but explained 2.6% of the variance in RMR in women (nominal $P < 0.001$). WHR and age had no influence on RMR in females. However, in males WHR and age explained 5.7% (nominal $P < 0.01$) and 4.0% (nominal $P < 0.05$), respectively, of the variance in RMR.

Discussion

The results of this study in a large group of elderly subjects within an age range of 60 to 88 years clearly show that both in men and women, RMR adjusted for both FFM and FM is not associated with circulating leptin levels. This seems to be in contradiction to the results from Nicklas et al. (10), who observed that plasma leptin concentrations were significantly and positively related to RMR, even after controlling for the effects of FFM on energy expenditure in a group of 25 free-living older African-American women, but not in men. Besides FFM, FM can also have significant effects on RMR, especially when located intra-abdominally. Oxygen consumption of visceral adipose tissue was shown to be significantly higher than that of subcutaneous adipose tissue (19), and several authors have reported a positive relationship between WHR or waist circumference and RMR, especially in overweight women (20–22). In the above mentioned study from Nicklas et al. (10), the women had a considerably higher BMI compared with our female subjects (32 vs 26 kg/m$^2$) but no information was given on the fat distribution of their subjects. It would have been of interest to know whether the observed association between leptin and energy expenditure would persist when correcting RMR not only for FFM but also for FM and for fat distribution. With regard to this question, Nagy et al. (23) reported that the significant relationship between leptin and RMR in the postmenopausal African-American women investigated by Nicklas et al. (10) no longer persisted after adjustment of RMR for both FFM and FM.

In our study, there was no difference in BMI between males and females, whereas females had significantly more FM but a lower WHR than the male subjects. Our descriptive regression analysis revealed that WHR, independently of FM, accounted for 5.7% of the variance in RMR of the men. The mechanism by which a higher waist circumference influences RMR is not clear. Several studies support the hypothesis that a higher sympathetic and metabolic activity in the visceral tissues might explain the differences seen in the RMR (24, 25). Thus, body fat distribution should be considered as a confounding factor when evaluating the influence of leptin on RMR.

In another study not in accordance with our results, Jørgensen et al. (9) reported a significant association between circulating leptin and energy metabolism, inasmuch as leptin was positively correlated with RMR and the quotient of RMR/FFM in men but not in women. However, in their study only small groups, each of 18 males and females, were investigated and no adequate adjustment of RMR was made for either FFM or FM. In studies on energy expenditure, data for RMR are often related to BW or FFM, as these metabolic measures are important determinants of RMR in that an increase in BW or FFM is associated with an elevation in RMR. However, when expressing RMR per kg BW or FFM it is mostly neglected that these quotients usually decrease with increasing BW or FFM. This is evident across a wide range of body compositions.
from our results when looking at the negative associations between FFM and RMR/BW and RMR/FFM respectively, listed in Table 3. The reason for this phenomenon is that usually an increase in BW is associated with a disproportionate increase in FM in comparison with FFM and that, similarly, an increase in FFM is associated with a disproportionate increase in metabolically less active tissue mass (like muscle mass) in comparison with tissue mass of high metabolic activity (like heart, brain, and visceral organs). Thus, the use of these quotients may result in spurious correlations, which we also observed between lg leptin and RMR and potentially influencing factors it is necessary to adjust RMR appropriately for body composition.

In accordance with our results, in those studies in which RMR was adjusted for FFM and FM as outlined elsewhere (18) or in which the effects of FFM and FM were statistically eliminated by stepwise multiple regression analysis (5–7), no correlations between leptin and RMR were observed. The different statistical handling of the data may explain inconsistent results with regard to energy expenditure and leptin concentrations in adult humans. However, it has to be considered that most of the subjects investigated in these studies were aged between 20 and 50 years and only one study (5) included 20 elderly males and 9 elderly females. Therefore, the present study meets the need for investigations on the relationship between leptin and RMR in the elderly.

There are more studies in the elderly on serum leptin levels in relation to body composition. The results obtained for leptin in serum of elderly subjects in this study are comparable to those found by other authors for subjects of this age group and BMI (26, 27). In this comparatively large group of elderly people investigated by our group, FM is a strong determinant of serum leptin in both sexes. This is in accordance with the results of Nicklas et al. (10) but in contradiction to the study of Möller et al. (26) who could not find a significant correlation of leptin with body fat in a group of 12 women and 12 men of comparable age, BMI, and leptin levels; however this might be due to the small sample size.

Our results clearly demonstrate that in this large group of elderly postmenopausal females serum leptin levels adjusted for FM were nearly twice as high when compared with male subjects of similar age. In other studies, adjustments for differences in adiposity, as assessed by skinfolds (28) or hydrodensitometry (29), also did not reduce the differences in leptin concentrations between women and men, suggesting that sex-related differences in hormonal milieu could also influence leptin synthesis and release. Rosenbaum et al. (30) reported that post-menopausal females had significantly lower leptin concentrations but these were still greater than in males, and they concluded that it is possible that oestrogen and/or progesterone are not the sole influencing factors, and that androgens may also have a suppressive effect on leptin. A clear contribution of androgen to the gender difference in leptin production has recently been reported (31), with testosterone having an inhibitory effect on leptin (32). On the other hand, after adjustment for body fat content, higher leptin concentrations were seen in nonobese preadolescent girls compared with nonobese preadolescent boys. In this study from Caprio et al. (33) in which obese children and adults were also investigated, the amount of subcutaneous fat appeared to have the greatest impact on circulating leptin concentrations, although the authors imply that further determinants other than sex hormones and fat distribution contribute to the gender difference in leptin levels.

The influence of age on the relationship between leptin and adiposity is not clear. Leptin levels in relation to age were reported to decrease even after statistical control for the effects of adiposity by some authors (34, 35) but not by others (5, 36). In the study of Roberts et al. (5), age was not a significant independent predictor of circulating leptin when body fat content was taken into account. However, in their study only 9 elderly females (74 ± 1.3 years, BMI 23.8 ± 1.0 kg/m²) and 20 males (68 ± 1.3 years, BMI 25.3 ± 0.8 kg/m²) were included, and they were relatively lean subjects. Our results in a group of 122 elderly women and 82 elderly men within the age range of 60 to 88 years and with a comparably higher BMI, show an age-related decline in leptin levels when corrected for FM only in the women. The female subjects had a higher FM primarily distributed in the gluteal femoral region, while the male subjects exhibited a higher abdominal deposition of their body fat (Table 1). Montague et al. (37) reported that leptin mRNA levels were higher in subcutaneous than in omental adipocytes and that the subcutaneous to omental ratio of leptin mRNA expression was markedly higher in women (5.5 ± 1.1-fold) than in men (1.9 ± 0.2-fold). These findings are consistent with the above mentioned results from Caprio et al. (33) who reported that the amount of subcutaneous fat in preadolescents, adolescents, and young adults appeared to have the greatest impact on circulating leptin concentrations. If the age-related decline in leptin levels is due mainly to a decrease in leptin mRNA expression in the subcutaneous adipocytes, then differences in the amounts of intra-abdominally and subcutaneously located FM could explain why in our study an age-related decline in leptin levels corrected for FM is seen only in the women. This hypothesis should be taken into consideration in future studies on leptin levels, RMR, and aging.

In conclusion, the results of this study show that leptin is not a significant predictor of RMR when considering both FFM and FM in the elderly. Furthermore, our results indicate that leptin may decline with age. The present study demonstrates that body
composition can act as a confounder when investigating the relationship between circulating leptin levels and RMR. FM and its distribution is not only associated with leptin secretion but also, and independently from RMR. FM and its distribution is not only associated with the relationship between circulating leptin levels and composition can act as a confounder when investigating results from studies related to this topic.

References


