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Published in:
Clinical Endocrinology

DOI:
[10.1046/j.1365-2265.2003.01872.x](https://doi.org/10.1046/j.1365-2265.2003.01872.x)

2003

[Link to publication](#)

Citation for published version (APA):

Bülow, B., Ahrén, B., Fisker, S., Dehlin, O., Hagberg, B., Jensen, E., Svensson, T., Samuelsson, G., & Erfurth, E. M. (2003). The gender differences in growth hormone-binding protein and leptin persist in 80-year-old men and women and is not caused by sex hormones. *Clinical Endocrinology*, 59(4), 482-6.
<https://doi.org/10.1046/j.1365-2265.2003.01872.x>

Total number of authors:
9

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The gender differences in growth hormone-binding protein and leptin persist in 80-year-old men and women and is not caused by sex hormones

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(Received 20 January 2003; returned for revision 7 April 2003; finally revised 17 April 2003; accepted 22 May 2003)

Summary

OBJECTIVE Leptin and growth hormone-binding protein (GHBP) both show gender differences that might be explained by sex hormones. To study the potential relevance of oestradiol and testosterone, we have examined 80-year-old subjects in whom oestradiol is higher in men than in women. The interrelationships between leptin, insulin, GHBP and fat mass in this age group were also investigated.

DESIGN AND SUBJECTS Ninety-four subjects (55 females and 39 males), all 80 years old, were investigated in a community-based study. None of the investigated subjects was being treated for diabetes mellitus and none of the women had oestrogen replacement.

METHODS Levels of testosterone, oestradiol, SHBG, IGF-I, GHBP, glucose, insulin and leptin were analysed. Body composition was measured with bioimpedance analysis (BIA).

RESULTS As in younger age groups, serum leptin, the ratio leptin/kilogram fat mass and serum GHBP were higher in the women (all, $P \leq 0.007$), although serum oestradiol was higher in the men ($P < 0.001$). There were no significant associations between sex hormones and leptin or GHBP either in women or in men (all, $r < 0.13$, $P > 0.1$). Leptin correlated to kilogram fat mass in both women ($r = 0.55$, $P < 0.001$) and men ($r = 0.47$, $P = 0.003$), but in contrast, there were no significant

correlations between GHBP and fat mass and GHBP and IGF-I, either in women or in men (all, $r < 0.24$, $P > 0.2$). Insulin and leptin were significantly associated with GHBP, both in women ($r = 0.48$, $P < 0.001$ and $r = 0.43$, $P = 0.001$, respectively) and in men ($r = 0.40$, $P = 0.01$ and $r = 0.34$, $P = 0.03$, respectively).

CONCLUSIONS Although the 80-year-old men had higher oestradiol levels than the women, the women had higher levels of leptin and GHBP. There were no correlations between sex hormones and leptin and GHBP, which indicates that the gender differences are not caused by sex hormones in old age. In contrast to studies in younger subjects, GHBP did not correlate to fat mass in the investigated 80-year-old men and women. In the older subjects investigated, as in younger subjects, GHBP was significantly correlated with leptin and insulin.

In younger age groups there is a sexual dimorphism in levels of leptin and growth hormone-binding protein (GHBP), with higher concentrations in women than in men (Hattori *et al.*, 1991; Saad *et al.*, 1997). This gender difference might be caused by a regulatory effect of sex hormones. In view of the decline in sex hormones with older age (Simon *et al.*, 1992; Federman, 1993), when men often exhibit higher oestradiol levels than women, it would be of interest to examine if the gender differences in leptin and GHBP still exist.

GHBP corresponds to the extracellular domain of the GH receptor (Leung *et al.*, 1987) and is suggested to be an indirect measure of the GH receptor function. GHBP is known to correlate to fat mass in young and middle-aged adults (Fisker *et al.*, 1997). It is still unknown whether the observed age-associated decline in levels of GHBP (Maheshwari *et al.*, 1996) affects the correlation between fat mass and GHBP in old age.

A relationship has been found between leptin and GHBP after adjustment for body mass index (BMI) in children (Bjarnason *et al.*, 1997) and adults (Llopis *et al.*, 1998). Moreover, insulin secretion has been found to be a predictor of GHBP activity in middle-aged adults (Fernandez-Real *et al.*, 2000). Information about these associations in old age is, however, lacking.

The aims of the present study were to examine whether there are gender differences in leptin and GHBP in 80-year-old subjects,

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and also to study the interrelationships between leptin, insulin, GHBP and fat mass in this age group.

Materials and methods

Subjects

The study is based on a subsample from a previously published study of 80-year-old patients (Svensson *et al.*, 1993). At the time of the present investigation the total population of 80-year-old subjects living in the city of Lund was 303. This cohort was invited to take part in the initial study and 147 accepted (Svensson *et al.*, 1993). Of these, 100 accepted a second investigation. Six of these subjects, who were being treated for diabetes mellitus, were excluded. The remaining 94 subjects (55 females and 39 males) were investigated with fasting blood samples and bioimpedance analysis. None of the women was treated with oestrogen replacement, and liver enzymes were in the normal reference range, except in two patients who had slightly elevated levels. Only two of the patients had a BMI of 30 kg/m² or more. The study protocol was approved by the Ethics Committee of Lund University.

Biochemical analyses

With the subjects fasting since midnight, serum samples for analysis were collected between 0800 and 0900 h and then stored at -70°C until analysed. Samples were analysed in a maximum of two assays. Serum oestradiol was measured with a time-resolved solid-phase fluoroimmunoassay (AutoDELFIA™ Oestradiol kit; Wallac Oy, Turku, Finland). The intra- and interassay coefficients of variance (CVs) at 50 pmol/l were 9% and 13%, respectively, and at > 200 pmol/l, 2% and 4%, respectively. Serum SHBG was measured by radioimmunoassay (RIA) (Fernlund *et al.*, 1985) with intra- and interassay CVs of 4.1 and 7.2%, respectively. Serum testosterone was also measured by RIA (Thorell & Larsson, 1978) with an intra-assay CV of 4%. Serum IGF-I was analysed by RIA after formic acid-ethanol extraction (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). The inter-assay CV was 7.9%. Venous blood glucose was measured with a HemoCue Blood Glucose Analyser (HemoCue, Ängelholm, Sweden) (Ashworth *et al.*, 1992). The instrument was controlled daily using a standard microcuvette and weekly using a haemolysate (Eutrol, Wageningen, the Netherlands) with known glucose concentration standards. Serum insulin was measured with a competitive RIA (Thorell & Larsson, 1978) with intra- and interassay CVs of 7.1% or less. Serum leptin was analysed with a double-antibody RIA using rabbit anti-human leptin antibodies, ¹²⁵I-labelled human leptin as tracer and human leptin as standard (Linco Res., St Charles, MO, USA) as described previously (Ma *et al.*, 1996). The intra-assay CV was 5% and the interassay

CV 7%. Serum GHBP was analysed in an in-house immunofunctional assay for GHBP as described previously (Fisker *et al.*, 1996).

Body composition

Bodyweight was measured after a 12-h fast and body height was measured barefoot. BMI was calculated as kg/m². Body composition was measured in the supine position by bioelectrical impedance analysis (BIA) using the BIA 101-S (RJL-Systems, Detroit, MI, USA), according to the manufacturer's instructions. A 50-kHz, 800-μA current was applied.

Statistical methods

Data are presented as the median and range. The Mann-Whitney *U*-test was used for comparison of results between men and women. Univariate correlation was assessed using Spearman's rank order correlation test. The level of significance was set to $P < 0.05$.

Results

Anthropometrical and biochemical characteristics of the 80-year-old men and women are shown in Table 1. Levels of glucose and insulin were similar in women and men (both, $P > 0.5$). Both serum leptin and the ratio leptin/kilogram fat mass were significantly higher in the women than in the men (both, $P < 0.001$). Similarly, serum GHBP was higher in the women than in the men ($P = 0.007$). Serum oestradiol, serum testosterone and the ratio testosterone/SHBG were higher in the men (all, $P \leq 0.02$), but there was no significant difference in serum IGF-I ($P > 0.5$).

Sex hormones (oestradiol, testosterone, testosterone/SHBG ratio) were not significantly correlated to leptin or GHBP, either in women or in men (all, $r < 0.13$, $P > 0.1$). Leptin correlated to kilogram fat mass and BMI, both in women ($r = 0.55$, $P < 0.001$ and $r = 0.5$, $P < 0.001$, respectively) and in men ($r = 0.47$, $P = 0.003$ and $r = 0.50$, $P = 0.001$, respectively). There was a significant association between GHBP and BMI in men ($r = 0.35$, $P = 0.03$), but not in women ($r = 0.18$, $P = 0.2$). GHBP and kilogram fat mass were not significantly correlated either in women or in men (both, $r < 0.24$, $P > 0.2$).

Insulin and leptin were significantly associated with GHBP, both in women ($r = 0.48$, $P < 0.001$ and $r = 0.43$, $P = 0.001$, respectively) and in men ($r = 0.40$, $P = 0.01$ and $r = 0.34$, $P = 0.03$, respectively). The association between GHBP and leptin is shown in Fig. 1.

In both men and women there were no significant relationships between GHBP and glucose (both, $r < 0.17$, $P > 0.3$) and GHBP and IGF-I (both, $r < 0.003$, $P > 0.5$).

Table 1 Anthropometrical and biochemical characteristics of the 80-year-old men and women included in the study

	Females (<i>n</i> = 55)	Males (<i>n</i> = 39)	<i>P</i>
Weight (kg)	55 (44–88)	76 (56–95)	< 0.001
BMI (kg/m ²)	24.0 (17.9–31.9)	24.5 (19.4–29.3)	0.2
Fat mass (kg)	20.4 (9.7–36.9)	19.4 (10.5–49.5)	0.5
Blood glucose (mmol/l)	4.4 (3.2–5.4)	4.4 (3.5–6.9)	> 0.5
Serum insulin (pmol/l)	86.0 (45.5–449.3)	80.0 (42.8–231.5)	> 0.5
Serum leptin (ng/ml)	11.9 (1.5–100)	7.8 (1.9–22.0)	< 0.001
Serum leptin/kg fat mass (ng/ml/kg)	0.6 (0.08–3.0)	0.4 (0.1–1.0)	< 0.001
Serum GHBP (nmol/l)	1.4 (0.6–3.0)	1.3 (0.5–2.6)	0.007
Serum oestradiol (pmol/l)	43.3 (16.2–79.2)	70.6 (32.8–123.0)	< 0.001
Serum testosterone (nmol/l)	2.3 (0.9–30.3)	10.1 (1.2–36.4)	0.02
Serum testosterone/S-SHBG (nmol/mg)	0.7 (0.2–9.9)	5.4 (0.2–32.0)	0.001
Serum IGF-I (µg/l)	93 (52–266)	93 (10–183)	> 0.5

Values are median (range).

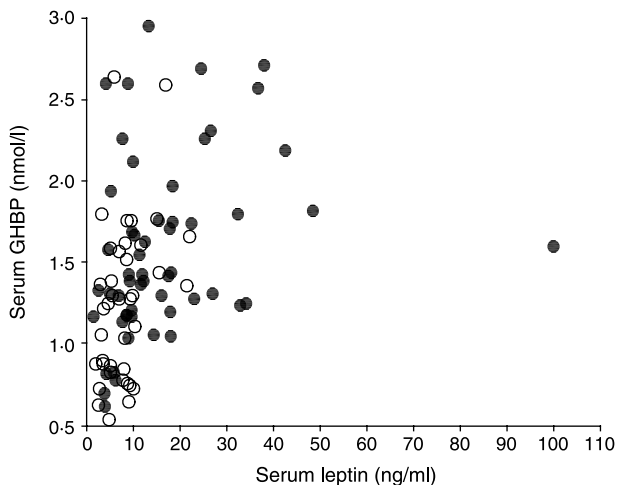


Fig. 1 Relationship between serum leptin and serum GHBP in women (●) ($r = 0.43$, $P = 0.001$) and in men (○) ($r = 0.34$, $P = 0.03$).

Discussion

In the present study, we found a clear gender difference in leptin levels in the 80-year-old subjects despite higher oestradiol levels in the men than the women. It has previously been suggested that oestradiol stimulates (Shimizu *et al.*, 1997) and testosterone inhibits (Luukkaa *et al.*, 1998; Söderberg *et al.*, 2001) leptin production and therefore that the gender difference in leptin might be explained by differences in sex hormones. The findings in the present study suggest that sex hormones are not responsible for the difference in leptin levels between men and women in old age, as there were no correlations between the sex hormones and leptin among the 80-year-old subjects investigated.

In the present study, the gender difference in leptin could not be explained by differences in the amount of fat mass, as the ratio

leptin/kilogram fat, used as a measure of adipocyte secretory function, was also significantly higher in the women. Instead, differences in fat distribution might be the explanation for the gender difference in circulating leptin, because the subcutaneous and not the visceral fat depot is the major source of leptin (Van Harmelen *et al.*, 1998), and women have a higher subcutaneous fat mass than men.

We also found, in accordance with studies in younger subjects (Hattori *et al.*, 1991), a gender difference in serum GHBP levels, with higher levels in women. This difference between the men and women also could not be explained by the differences in sex hormones, as these were not significantly associated with GHBP. Hence the mechanism of the maintenance of the statistically significant gender difference found both in leptin and in GHBP levels in old age remains to be established.

Although there was a significant correlation between BMI and GHBP in the 80-year-old men, the correlation coefficient was not high ($r = 0.35$). Moreover, BMI and GHBP were not correlated in the women and there was no significant association between fat mass and GHBP either in the women or in the men. This is different from the clear association between fat mass and GHBP in children (Juul *et al.*, 2000) and young and middle-aged subjects (Fisker *et al.*, 1997). GHBP is derived from the GH membrane receptor and is identical to the extracellular binding domain of this receptor (Leung *et al.*, 1987). It has been suggested that levels of GHBP reflect the tissue level of GH receptors and thus the GH receptor function. Previously, it has been shown that GHBP gradually declines in older age (Maheshwari *et al.*, 1996). The lack of correlation between GHBP and fat mass in the presently investigated older subjects might indicate that there is an insensitivity to GH in this age group, which could be of importance for the increase in fat observed with older age (Rudman, 1985). However, it still has to be shown convincingly that GHBP mirrors GH receptor activity in the adipose tissue. Another

possibility for altered GHBP in the 80-year-old subjects could be altered fat distribution or muscle atrophy, because GHBP is mainly expressed in abdominal fat tissue and in muscle tissue.

In the present study BIA was used to estimate body composition, a method considered less accurate than dual-energy X-ray absorptiometry (DEXA). However, we found with BIA, in agreement with previous studies on older subjects (Van der Saffele *et al.*, 1999; Isidori *et al.*, 2000; Thomas *et al.*, 2000), a significant association between kilogram fat mass and leptin in the 80-year-old men and women, indicating that the associations between fat mass and leptin and fat mass and GHBP do in fact differ.

In contrast to previous studies on younger subjects (Counts *et al.*, 1992), no significant correlation between IGF-I and GHBP was found, suggesting that factors other than GHBP are involved in regulating the levels of IGF-I in the elderly subjects investigated. This finding may also support the presence of GH resistance in the elderly subjects investigated.

In younger subjects, both leptin and insulin secretion correlate to GHBP activity (Bjarnason *et al.*, 1997; Llopis *et al.*, 1998; Fernandez-Real *et al.*, 2000). These associations were also seen in the 80-year-old men and women currently being investigated, supporting the suggestion that leptin and insulin could be important factors in the control of GHBP activity also in this age group.

In summary, this study of 80-year-old subjects shows that the gender differences in levels of leptin and GHBP persist in older age. There was no correlation between sex hormones and leptin or GHBP, suggesting that the gender differences observed in leptin and GHBP cannot be explained by sex hormones in old age. In contrast to studies in younger subjects, GHBP did not correlate with fat mass at 80 years of age. Finally, we found that both leptin and insulin were significantly correlated with GHBP also in this age group.

Acknowledgements

We thank Lilian Bengtsson and Ann-Sofie Nilsson for expert technical assistance. This study was supported by the Swedish Research Council (grant numbers 6834, 14257-01 and K1999-27X013074-01A), Albert Pahlssons and Novo Nordisk Foundations, Swedish Diabetes Association and the Faculty of Medicine, Lund University.

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