Circulating IGF binding protein-1 is inversely associated with leptin in non-obese men and obese postmenopausal women

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Abstract

Objective: Hyperleptinaemia and hyperinsulinaemia interrelate to insulin-like growth factor binding protein 1 (IGFBP-1), and disturbances in the growth hormone–IGF-I axis are linked to obesity and cardiovascular diseases. However, whether the association between leptin and the GH–IGF-I axis is altered with increasing obesity is not known. We therefore examined the relationship between leptin, IGF-I, IGFBP-1, insulin and proinsulin in men and women with or without obesity in a population study.

Design and subjects: Healthy subjects (n = 158; 85 men and 73 pre- and postmenopausal women) from the Northern Sweden MONICA (Monitoring of Trends and Determinants in Cardiovascular Disease) population were studied with a cross-sectional design.

Methods: Anthropometric measurements (body mass index (BMI) and waist circumference) and oral glucose tolerance tests were performed. Radioimmunoassays were used for the analyses of leptin, IGF-I and IGFBP-1, and ELISAs for specific insulin and proinsulin.

Results: Leptin inversely correlated to IGFBP-1 in non-obese men (P < 0.05) and obese postmenopausal women (P < 0.05). In contrast, leptin did not correlate to IGF-I. IGFBP-1 was also significantly associated with proinsulin in non-obese men (P < 0.01) and non-obese premenopausal women (P < 0.05). The association between leptin and IGFBP-1 was lost after adjustment for insulin. In multivariate analyses taking measures of adiposity into account, low proinsulin, and IGF-I in combination with old age, but not leptin, predicted high IGFBP-1 levels.

Conclusions: Leptin was inversely associated with IGFBP-1 in non-obese men and obese postmenopausal women. However, these associations were lost with increasing central obesity in men and premenopausal women and after control for insulin. Therefore, this study suggests (i) that leptin is of minor importance for regulation of IGFBP-1 levels and (ii) that the insulin resistance syndrome is characterised by an altered relationship between leptin, IGFBP-1 and insulin.

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Introduction

The cluster of risk factors named the insulin resistance syndrome, which is associated with (central) obesity (1, 2), is characterised by hyperinsulinaemia, hypertension, dyslipidaemia and abnormal fibrinolysis (3). Increased leptin levels may mediate several of these manifestations through metabolic and hormonal interactions and effects on blood pressure (4–6). In line with this, we have reported that leptin is independently associated with increased risk for cardiovascular disease (7, 8). In addition, the insulin resistance syndrome may include insufficient peripheral effects of insulin-like growth factor-I (IGF-I) (9), possibly due to insulin-mediated inhibition of IGF binding protein-1 (IGFBP-1) production (10). Leptin might also be involved in this regulation, since leptin interacts with the growth hormone (GH)–IGF axis in a bi-directional way. Besides hypothalamic effects (11), it is possible that leptin influences GH effects indirectly in the periphery through interaction with the IGFBPs (12) and, conversely, levels of leptin decrease after GH substitution (13). However, whether the association between leptin and the GH–IGF-I axis is altered in insulin resistance is not known.

In addition, states of insulin resistance are characterised by high circulating levels of proinsulin-like molecules (14), but the association between the GH–IGF-I axis and these molecules has not yet been fully elucidated (15).
The aim of this study was to explore the association between circulating levels of IGF-I and IGFBP-1 on one hand and leptin and proinsulin on the other in healthy men and women representing a wide range of body composition and age, and to explore if these associations differ between men and women in general, and between pre- and postmenopausal women.

Patients and methods

Study population

The study was performed within the framework of the Northern Sweden MONICA Project, which, in turn, is part of the WHO MONICA (Monitoring of Trends and Determinants in Cardiovascular Disease) Project (16). In 1994, a population was screened for cardiovascular risk factors. A total of 2500 individuals in the 25–74 year range were invited by mail from a total population of 367 000 in this age range. Within each age group (25–34, 35–44, 45–54, 55–64 and 65–74 years) 250 men and 250 women were randomly selected from continuously updated population registers in Norrbotten and Västerbotten, the two northernmost provinces of Sweden. In total 1921 subjects participated in the study (76.8%). Subjects were included whose provinces of Sweden. In total 1921 subjects participated in the study (76.8%). Subjects were included whose

Baseline characteristics

Body mass index (BMI) was calculated as total body weight in kilograms divided by the square of height in meters, and waist hip ratio (WHR) was calculated as the ratio of the circumference of the narrowest part of the waist divided by the broadest part of the hip. All measurements were taken with the subject standing upright and breathing lightly. Blood pressure was measured with the subjects in the sitting position after 5 min of rest using the random zero method.

Plasma glucose was analysed by the hexokinase method (Boehringer Mannheim, Germany) on a Hitachi 717 analyser (Tokyo, Japan). Insulin was measured by ELISA without cross-reactivity to human proinsulin (21). Split(32–33)- and des(31,32)-proinsulin do not react, whereas split(65–66)- and des(64,65)-proinsulin cross-react with an efficiency of 30% and 63% respectively, on a molar basis. The detection limit was 5 pmol/l, and the working range was 5–600 pmol/l. Interassay coefficients of variation (CV) were 5–6% at 80–350 pmol/l and 11% at 30 pmol/l. Proinsulin immunoreactivity was measured by ELISA with a detection limit of 0.25 pmol/l and a working range of 0.25–100 pmol/l (22). The four major proinsulin conversion intermediates reacted 65–99% on a molar basis. C-peptide and insulin did not react. Interassay CVs were 6–7% at 5–30 pmol/l. IGF-I was determined by RIA after separation of IGFs from IGFBPs by acid–ethanol extraction and cryoprecipitation. To minimise interference of remaining IGFBPs, des(1–3)-IGF-I was used as radioligand (23). The intra- and interassay CVs were 4% and 11% respectively. IGFBP-1 concentrations in plasma were determined according to the method of Povoëa et al. (24). The sensitivity of the RIA was 3 µg/l, and the intra- and interassay CVs were 3% and 10% respectively. The leptin analysis was performed using a double-antibody RIA with rabbit anti-human leptin antibodies, 125I-labelled human leptin as tracer and human leptin as standard (Linco Res., St Louis, MO, USA). Interassay CV was 1.9% at low levels (<5 ng/ml) and 3.2% at high levels (10–15 ng/ml).

Statistical analysis

All the main study variables were positively skewed and, therefore, (ln) transformed values were used. Means (geometric for transformed values) with 95% confidence intervals (CI) are presented. Bivariate (Pearson’s) and partial correlation coefficients were calculated, adjusted for age and adiposity. One-way ANOVA analyses with adjustments for covariates were used, and significant differences between factor levels were evaluated after Bonferroni correction. Multiple linear regression analysis was performed with a stepwise method. Introducing combination terms in the model tested interaction, and dummy variables the presence of group effects. The influence of potential outliers was tested by analysis of residuals and by excluding subjects. Two-tailed tests were used and a P-value <0.05 was considered significant. All calculations were made with the statistical program SPSS (Chicago, IL, USA) version 6.1 on a Macintosh computer.

Results

Baseline characteristics

Baseline characteristics of the study population are shown in Table 1. Men had higher waist circumference,
WHR and fasting glucose levels in combination with lower leptin concentrations versus both pre- and postmenopausal women. Furthermore, men had higher BMI and higher fasting proinsulin/insulin ratio compared with premenopausal women. IGF-I levels were highest in premenopausal women followed by men who had higher levels than postmenopausal women. Differences in mean IGF-I levels between groups disappeared after adjustment for age whereas differences in proinsulin, glucose, IGFBP-1, and leptin concentrations remained after adjustment for both age and BMI (data not shown).

### Relationship of IGF-I and IGFBP-1 to age, adiposity, insulins and leptin

Levels of IGF-I were decreasing, whilst levels of IGFBP-1 were increasing, with age in men and premenopausal women (data not shown). IGFBP-1 was inversely associated with adiposity indices (BMI, waist circumference and WHR) in men and women irrespective of menstrual status, whereas IGF-I did not correlate to adiposity once adjusted for age (data not shown). IGF-I was positively associated with insulin in non-obese men and in obese premenopausal women, and inversely with the proinsulin/insulin ratio in men and premenopausal women (Table 2).

Leptin inversely correlated to IGFBP-1 in non-obese men and obese postmenopausal women. IGFBP-1 also correlated inversely to insulin and proinsulin in non-obese men, but only to proinsulin in non-obese premenopausal women and not in postmenopausal women. The inverse correlation between proinsulin and IGFBP-1 remained after further adjustment for insulin in premenopausal women ($r = -0.33; P < 0.05$), but not in men, and in all groups the inverse association between leptin and IGFBP-1 was lost after adjustment for insulin (data not shown). Levels of leptin did not correlate to levels of IGF-I, neither in bivariate nor in partial correlation analysis. Finally, the inverse association between IGF-I and IGFBP-1 in men and non-obese postmenopausal women was independent of the effects of insulin ($r = -0.24, P < 0.05$; and $r = -0.64, P < 0.05$ respectively).

### Linear regression analyses with IGFBP-1 as dependent variable

Multiple linear regression analysis with IGFBP-1 concentrations as the dependent variable was performed (Table 3). The model included fasting levels of insulin, proinsulin and glucose, in combination with IGF-I, BMI, waist circumference, age and gender (when applicable) as explanatory variables. Significant predictors for high IGFBP-1 levels were low levels of proinsulin ($P < 0.01$) and IGF-I ($P < 0.05$) in combination with low waist circumference ($P < 0.001$) and
old age ($P < 0.001$) in the entire population, and these variables explained 48% of the IGFBP-1 variation. The addition of menstrual status (dummy variables) to this model revealed that premenopausal women had significantly higher IGFBP-1 levels than men ($P < 0.05$), and that postmenopausal women did not have significantly different IGFBP-1 levels compared with men and premenopausal women. BMI, waist circumference or menstrual status in combination with proinsulin or insulin was not associated with IGFBP-1 levels. The following variables were associated with IGFBP-1 levels after stratification for menstrual status: low levels of proinsulin ($P < 0.01$) and IGF-I ($P < 0.01$) in combination with low BMI ($P < 0.001$) and old age ($P < 0.001$) in men; low proinsulin ($P < 0.01$), low waist circumference ($P < 0.05$) and old age ($P < 0.001$) in premenopausal women; and low IGF-I ($P < 0.05$) and low BMI ($P < 0.01$) in postmenopausal women. Further stratification for waist circumference revealed that low levels of proinsulin ($P < 0.01$) and IGF-I ($P < 0.05$) in combination with high waist circumference ($P < 0.001$) were associated with high IGFBP-1 in non-obese premenopausal women, whilst only low waist circumference ($P < 0.01$) predicted high

Table 2 Partial correlations between determined variables. Stratified for central obesity and adjusted for age, BMI and waist circumference. Median of waist circumference used as cut-off: pre- and postmenopausal women 0.77 and 0.82 m respectively, and men 0.92 m.

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Premenopausal women</th>
<th>Postmenopausal women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All ≤median &gt;median</td>
<td>All ≤median &gt;median</td>
<td>All ≤median &gt;median</td>
</tr>
<tr>
<td>IGF-I versus:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptin</td>
<td>0.19 0.23 0.15</td>
<td>0.23 0.09 0.39</td>
<td>0.21 0.06 0.23</td>
</tr>
<tr>
<td>IGFBP-1</td>
<td>−0.32† −0.28 −0.30</td>
<td>−0.09 −0.39 −0.04</td>
<td>−0.37 −0.60∗ −0.32</td>
</tr>
<tr>
<td>Fasting insulin</td>
<td>0.30‡ 0.39∗ 0.26</td>
<td>0.29 0.27 0.51∗</td>
<td>0.31 0.31 0.29</td>
</tr>
<tr>
<td>Fasting proinsulin</td>
<td>0.09 0.13 0.05</td>
<td>−0.06 −0.12 −0.02</td>
<td>0.15 −0.10 0.30</td>
</tr>
<tr>
<td>Fasting proinsulin/insulin</td>
<td>−0.27∗ −0.30 −0.28</td>
<td>−0.34∗ −0.34 −0.54∗</td>
<td>−0.14 −0.30 0.03</td>
</tr>
<tr>
<td>IGFBP-1 versus:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptin</td>
<td>−0.25∗ −0.33∗ −0.13</td>
<td>−0.11 −0.34 0.18</td>
<td>−0.24 0.26 −0.62∗</td>
</tr>
<tr>
<td>Fasting insulin</td>
<td>−0.35† −0.38∗ −0.33</td>
<td>−0.33∗ −0.39 −0.17</td>
<td>−0.42∗ 0.02 −0.56∗</td>
</tr>
<tr>
<td>Fasting proinsulin</td>
<td>−0.32† −0.42† −0.23</td>
<td>−0.42† −0.50∗ −0.44</td>
<td>−0.22 0.22 −0.38</td>
</tr>
<tr>
<td>Fasting proinsulin/insulin</td>
<td>0.06 −0.03 0.14</td>
<td>−0.02 0.00 −0.23</td>
<td>0.19 0.16 0.19</td>
</tr>
</tbody>
</table>

$P$-values: ∗ < 0.05, † < 0.01, ‡ < 0.001.

Table 3 Linear regression model for variables associated with circulating (ln) IGFBP-1 levels. The model included (fasting) insulin, (fasting) proinsulin, IGF-I, (fasting) glucose, BMI, waist circumference, age and gender (when applicable) as explanatory variables. Analyses repeated after stratification for central obesity.

<table>
<thead>
<tr>
<th>Group</th>
<th>Factors</th>
<th>Adjusted $R^2$</th>
<th>$B$</th>
<th>SE($B$)</th>
<th>$\beta$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td>Age</td>
<td>48.2</td>
<td>0.020</td>
<td>0.004</td>
<td>0.421</td>
<td>†</td>
</tr>
<tr>
<td></td>
<td>Waist circumference</td>
<td>−0.020</td>
<td>0.004</td>
<td>−0.336</td>
<td>0.05</td>
<td>‡</td>
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<tr>
<td></td>
<td>Fasting insulin</td>
<td>−0.188</td>
<td>0.097</td>
<td>−0.155</td>
<td>0.05</td>
<td>‡</td>
</tr>
<tr>
<td></td>
<td>Fasting proinsulin</td>
<td>−0.290</td>
<td>0.099</td>
<td>−0.241</td>
<td>†</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>IGF-I</td>
<td>−0.390</td>
<td>0.158</td>
<td>−0.185</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>Age</td>
<td>47.9</td>
<td>0.018</td>
<td>0.005</td>
<td>0.362</td>
<td>†</td>
</tr>
<tr>
<td></td>
<td>BMI</td>
<td>−0.094</td>
<td>0.021</td>
<td>−0.403</td>
<td>†</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Fasting proinsulin</td>
<td>−0.295</td>
<td>0.107</td>
<td>−0.243</td>
<td>†</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>IGF-I</td>
<td>−0.708</td>
<td>0.237</td>
<td>−0.283</td>
<td>†</td>
<td></td>
</tr>
<tr>
<td>Premenopausal women</td>
<td>Age</td>
<td>39.7</td>
<td>0.033</td>
<td>0.009</td>
<td>0.468</td>
<td>†</td>
</tr>
<tr>
<td></td>
<td>Waist circumference</td>
<td>−0.015</td>
<td>0.007</td>
<td>−0.294</td>
<td>†</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fasting proinsulin</td>
<td>−0.529</td>
<td>0.182</td>
<td>−0.395</td>
<td>†</td>
<td></td>
</tr>
<tr>
<td>≤median of waist</td>
<td>Waist circumference</td>
<td>67.3</td>
<td>0.069</td>
<td>0.014</td>
<td>0.614</td>
<td>†</td>
</tr>
<tr>
<td></td>
<td>Fasting proinsulin</td>
<td>−0.510</td>
<td>0.156</td>
<td>−0.401</td>
<td>†</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IGF-I</td>
<td>−0.476</td>
<td>0.171</td>
<td>−0.347</td>
<td>*</td>
<td>†</td>
</tr>
<tr>
<td>&gt;median of waist</td>
<td>Waist circumference</td>
<td>32.4</td>
<td>−0.042</td>
<td>0.013</td>
<td>−0.598</td>
<td>†</td>
</tr>
<tr>
<td>Postmenopausal women</td>
<td>BMI</td>
<td>43.8</td>
<td>−0.069</td>
<td>0.019</td>
<td>−0.536</td>
<td>†</td>
</tr>
<tr>
<td></td>
<td>IGF-I</td>
<td>−0.577</td>
<td>0.260</td>
<td>−0.330</td>
<td>*</td>
<td></td>
</tr>
</tbody>
</table>

$B =$ regression coefficient, SE($B$) = standard error of $B$, and $\beta =$ standardised regression coefficient. $P$-values: ∗ < 0.05, † < 0.01, ‡ < 0.001.

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Figure 1 The relation between levels of proinsulin and IGFBP-1 in men (●) and women (□).

Figure 2 The relation between levels of insulin and IGFBP-1 in men (●) and women (□).
IGFBP-1 levels in obese premenopausal women. The stratified analyses did not bring additional information to the analyses of men and postmenopausal women. Finally, leptin was introduced in the model. Leptin replaced BMI and IGF-I as a predictor of circulating IGFBP-1 levels ($P < 0.001$) in postmenopausal women. Leptin did not associate to IGFBP-1 in men and premenopausal women.

The relationships between levels of proinsulin and insulin on one hand and IGFBP-1 levels on the other are further illustrated in Figs 1 and 2. In Fig. 2, it can be seen that there are three men with high IGFBP-1 despite high insulin, and a premenopausal woman with very low IGFBP-1 and high insulin levels. Exclusion of these subjects (all with waist above median) did not affect the association between leptin on the one hand and IGF-I and IGFBP-1 on the other. In contrast, insulin became inversely associated with IGFBP-1 in obese men ($r = -0.42, P < 0.05$). Furthermore, the following factors were significant predictors for IGFBP-1 in the regression analysis: insulin ($P < 0.01$) (replaced proinsulin) together with age, BMI and IGF-I in men; and IGF-I ($P < 0.01$) (replaced age and waist) together with proinsulin in premenopausal women.

Discussion

Earlier reports have shown that levels of IGFBP-1 are associated with various expressions of the insulin resistance syndrome (25). It was thus of interest to further clarify the role of IGFBP-1 in conjunction with leptin and proinsulin levels. Specifically, we found that leptin was associated with low IGFBP-1 levels in non-obese men after adjustment for adiposity. This association was lost in subjects with increasing central obesity and after adjusting for fasting insulin. This suggests an altered relationship between leptin and IGFBP-1 in subjects with obesity due to the influence of insulin. In line with this, it was recently suggested that the reduction in leptin during fasting is associated with increased IGFBP-1 levels, and that this change is dependent on the concomitant reduction in insulin (26). Furthermore, we found a strong inverse association between circulating levels of proinsulin and IGFBP-1 in non-obese men and premenopausal women. It may be speculated that proinsulin regulates hepatic IGFBP-1 production under conditions of low insulin, to replace the influence of insulin, as in non-obese subjects. If so, this would suggest that the relation between IGFBP-1 and proinsulin is not primarily related to the insulin resistance syndrome, which is in contrast with earlier studies (15).

Interestingly, we have recently shown that circulating levels of leptin and proinsulin are strongly associated in non-obese men and premenopausal women, whereas the association was lost with increasing central obesity (unpublished data). This association between proinsulin and leptin resembles therefore that between IGFBP-1 and leptin. It is possible that these associations reflect normal regulation by proinsulin of both IGFBP-1 and leptin in non-insulin resistant subjects, whereas in obese subjects the influence by insulin predominates. A problem with the interpretation of the data in the present study is that the cohort was divided in subgroups with a limited number of subjects in each group. Although this may limit the generality of the findings, the results clearly indicate that the relation between IGFBP-1 and leptin is stronger in non-obese men and in obese postmenopausal women than in other subgroups. It is not clear why the association between IGFBP-1 and leptin is restricted to non-obese men and obese postmenopausal women. Therefore, the physiological relevance of these findings remains to be established.

The regulation of the IGFBPs will affect circulating levels of IGFs, since IGFBPs maintain adequate levels of IGFs through protection from degradation, and also function as regulators of IGF function at the cellular level (10). IGFBP-1, which is mainly produced in the liver, is thought to be a direct regulator of free IGF levels, and thus reflects the biologically active fraction of IGFs (27). Insulin regulates the hepatic production of IGFBP-1 through insulin responsive elements in the promoter region (28).

We found that IGF-I did not correlate with leptin after adjustments in this healthy and probably weight-stable population, results consistent with earlier studies in both normal and GH-deficient subjects (13, 29). Neither did IGF-I correlate to measures of adiposity once adjusted for age, suggesting that IGF-I production is related more to lean body mass than to fat mass (30). In contrast, there are studies in both humans and rats reporting a sustained decrease in leptin levels after treatment with IGF-I, probably due to decreased circulating levels of insulin (31, 32). Leptin can affect the homeostasis of IGF-I through an interaction with the GH axis, an interaction that could be both direct and indirect. For example, intracerebroventricular administration of leptin antisera reduced GH secretion in rats (11) and GH administration to GH-deficient humans has been found to reduce leptin levels, probably through reduced amount of body fat mass (13). Leptin could also influence GH effects in the periphery through association with the GH-binding protein and the IGFBPs (12, 33).

In conclusion, circulating leptin was inversely associated with IGFBP-1 levels in non-obese men and obese postmenopausal women, whereas leptin did not associate to IGF-I levels. Similarly, circulating levels of proinsulin was inversely associated with IGFBP-1 in non-obese men and premenopausal women. These associations were lost with increasing central obesity in men and premenopausal women and after control for insulin. Therefore, this study suggests (i) that leptin is of minor importance for IGFBP-1 regulation and (ii) that the insulin resistance syndrome is
characterised by an altered relationship between leptin, IGFBP-1 and insulin.

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