Definition and characterization of relative hypo- and hyperleptinemia in a large Caucasian population

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Abstract

The adipocyte-derived hormone, leptin, has been implicated in the regulation of appetite, weight gain and glucose homeostasis as well as in liver fibrogenesis, hematopoiesis and immune function. No previous reports have clearly defined pathologically elevated or decreased serum leptin levels for Caucasian adults. The aim of this study was to define and characterize subjects with relative hyper- and hypoleptinemia in a large population-based German cohort.

Percentiles of leptin levels by body mass index (BMI) were calculated from 4971 adult Germans, and the participants with leptin levels above the 95th and below the 5th percentile were defined as relatively hyperleptinemic and relatively hypoleptinemic, respectively, for their BMI.

These participants were compared with the intermediate group with respect to anthropometric and clinical data and parameters of glucose and iron metabolism, lipid status, renal, adrenal and reproductive function.

Relatively hyperleptinemic participants (HL) showed higher insulin, c-peptide, and total cholesterol levels than the hypoleptinemic subjects; in males, ferritin levels were higher and testosterone levels lower in the HL group.

In conclusion, we report the first percentile curves for serum leptin by BMI in a large Caucasian population. Relatively low leptin values may be associated with a lower metabolic risk than relatively high serum leptin values.

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Introduction

Obesity research has been immensely stimulated by the cloning of the ob-gene product, the adipocyte-specific proteohormone, leptin. Initially, data from rat and mouse models of leptin (ob/ob mouse) or leptin receptor (db/db mouse, fa/fa rat) deficiency led to the theory that leptin was the hypothetical, long looked-for satiety factor by which adipocytes could signal the amount of fat mass to central appetite centers in the hypothalamus. This was thought to lead to a consecutive downregulation of food intake and an increase in energy expenditure, thereby reducing adipocyte mass. However, later research has shown a more complex role of leptin (Friedman 2000). Up to now, the postulated negative feed-back loop has not been demonstrated convincingly at physiological leptin levels in obese humans. While exogenous hyperleptinemia reduces fat mass in rodents (Halaas et al. 1995, Pelleymentuer et al. 1995, Weigle et al. 1995, Chen et al. 1996), subcutaneous leptin application in humans has not yet been reported to lead to significant weight changes (Westerterp-Plantenga et al. 2001). The Q223R polymorphism of the leptin receptor has been implicated in obesity pathogenesis, but it only seems to account for a small percentage of body weight and body composition variability (Yiannakouris et al. 2001). Looking at dietary interventions in obese patients, some studies have detected a negative correlation between initial leptin levels and total weight lost, hypothesizing that weight loss might, in part, be predicted by leptin levels (Verdich et al. 2001). These conflicting results have been explained by the concept of leptin resistance – an inadequate peripheral or central response to elevated leptin levels. Although this model is intriguing, no exact assay for the quantification of leptin action has been developed yet. Furthermore, reference values for leptin – which would allow the diagnosis of a patient with ‘hyperleptinemia’ and possible leptin resistance – have not been published.
Leptin levels might be important markers of some other aspects of a person’s health. Leptin is involved in the regulation of the body’s insulin and carbohydrate metabolism. In the ob/ob mouse, leptin lowers insulin secretion, whereas insulin upregulates leptin synthesis (Leroy et al. 1996, Kieffer et al. 1997). In perfused rat livers as well as in hepatocyte cultures leptin has been reported to inhibit hepatic glucose output (Rossetti et al. 1997, Cedadia et al. 1999, Nemecz et al. 1999), suggesting it acts as a regulatory hormone for the liver’s function in glucose homeostasis. In the last years, research has identified functional leptin receptors in many other tissues, such as hematopoietic stem cells, hepatocytes, gut epithelia and reproductive tissues. Leptin’s exact role in these organ systems is not yet fully understood, but it is now clear that it is involved in normal sexual maturation and reproduction (Caprio et al. 2001). Besides this, interactions with the hypothalamic–pituitary–adrenal, thyroid and growth hormone (GH) axes and with hematopoiesis and the immune system have also been described in both humans and rodents in vitro and in vivo (Fantuzzi & Faggioni 2000, Glasow & Bornstein 2000, Wauters et al. 2000).

Due to the lack of published reference ranges, the classification of a given person’s ‘leptin status’ is not possible, although it might be of clinical interest. Therefore, we have measured the leptin levels in a large, metabolically well characterized German adult cohort. We here present an analysis of this data, including the definition of leptin percentile curves depending on body mass index. Furthermore, we have compared subjects with relatively high and low leptin levels with regard to their respective metabolic phenotypes.

Materials and Methods

Experimental subjects

Subjects in this study had participated in the Diabetomobile study, an epidemiologic field survey on metabolic disorders in a representative adult German population carried out from 1993 to 1996 (Palitzsch et al. 1999). A total of 6450 persons between 18 and 70 years of age were investigated by a physician from our department with a mobile survey unit in representative German cities and rural communities. Participants were either included in a so-called street setting by randomly choosing streets and house numbers and asking the inhabitants to participate voluntarily in the study (cohort A), or in a market-place setting, where all interested bystanders were included (cohort B). All participants had to be fasting for at least one hour; the time of the last meal was noted. Participants with acute infectious diseases or known major hepatic or renal dysfunction were excluded from this study. All persons were interviewed and responded to a questionnaire on medical history, medication, lifestyle and life-quality. Body height and weight were measured in the survey unit with approved medical care instruments, and body mass index (BMI) was computed as weight (kilograms) divided by squared height (meters squared). Resting blood pressure (BP) was measured with a mercury sphygmomanometer after subjects had been in a sitting position for at least 20 min. Participants with elevated BP (>140/90 mmHg) were re-examined before leaving the mobile survey unit.

All participants remained anonymous throughout the survey and gave their informed consent for further analysis of their blood samples, including all biochemical and genetic studies performed. The study protocol was designed according to the guidelines proposed in The Declaration of Helsinki and was formally approved by the local ethics committee.

Biochemical measurements and definitions

Blood was drawn from all subjects and the time of the last meal was recorded. Serum total and high density lipoprotein (HDL)-cholesterol, creatinine, glucose and HbA1c were determined in the mobile survey unit. All other biochemical measurements were performed on frozen serum samples within 12 months after sample collection. Total and HDL-cholesterol and creatinine were measured by dry chemistry (DT 60, Kodak, Germany). Intra-assay coefficients of variation (CV) were 4·5%, 4·4% and 0·9% respectively, interassay CV were 4·9%, 4·8% and 2·1% respectively. Glucose levels were determined with an Accutrend-Glucose Glucometer (Boehringer Mannheim, Mannheim, Germany). The intra-assay CV was 2·9% at normal range glucose concentrations. HbA1c was measured by an on-site immunoassay (DCA 2000, Bayer AG, Leverkusen, Germany). Intra-assay and interassay CV were 3·1% and 4·4% respectively. Insulin, c-peptide, progesterone, estradiol, cortisol and dehydroepiandrosterone sulfate (DHEAS) levels were measured by immunoassays (Enzymun Insulin, Boehringer Mannheim; Immulite assays, DPC, Bad Nauheim, Germany; cortisol: Immuno-1, Bayer, Leverkusen, Germany; DHEAS-ELISA, IBL, Hamburg, Germany) of frozen plasma samples. Intra-assay CV were 4·9%, 6·2%, 5·9%, 6·0%, 7·5% and 4·3% respectively; interassay CV were 7·0%, 5·9%, 6·3%, 7·5%, 9% and 5·2% respectively. Testosterone and leptin levels were determined by radioimmunoassay (DPC; Linco, St Louis, MO, USA) with 3·8% and 4·2% for the intra-assay CV and 4·2% and 4·5% for the interassay CV respectively. Iron levels were measured using the Guanidine/FerroZine method (Carter 1971), and ferritin was assayed by turbidimetric analysis (Bernard & Lauwerys 1984).

Diabetes mellitus was assumed (i) when the HbA1c was elevated (>6% according to the manufacturer’s instructions) or (ii) the proband indicated a known history of diabetes in the interview and the questionnaire. To
differentiate type 1 diabetes mellitus from type 2 diabetes mellitus we used age, diabetes onset, BMI and c-peptide levels. Type 1 diabetes mellitus was defined as diabetes onset before 35 years of age, BMI <25 kg/m² and c-peptide levels <1 ng/ml. All other subjects with diabetes were assigned to the type 2 diabetes mellitus group.

All instruments used for anthropometric and biochemical measurements were calibrated and technically looked after weekly by a technical assistant.

Statistical analysis

The aim of our study was to characterize metabolic parameters in participants with different leptin levels. The anthropometric and biochemical parameters we measured were not normally distributed. Therefore, subjects were compared for differences in these parameters by two-tailed Mann-Whitney or Kruskal-Wallis tests for comparison of two or more independent samples. Data are expressed as means and standard errors of the mean (S.E.M.) for simplicity. Bonferroni correction was used when calculating multiple tests; the significance levels are stated in the text.

To examine associations as exactly as possible, tests were first performed with all three leptin subgroups together. When a significance level <0·1 was achieved, we examined correlations of the possibly associated parameter to the leptin subgroups in every possible combination. The significance level of these results was adjusted by the Bonferroni procedure.

Relative risks were calculated using crosstabs statistics. Correlation coefficients and $r^2$ were determined by the Spearman procedure.

All statistical calculations were performed using the SPSS 9·0 Software package (SPSS, Munich, Germany).

Results

Serum leptin levels were measured in a total of 4971 Diabetomobile study participants. When comparing the population-based cohort A with cohort B no significant differences in leptin levels could be detected after adjustment for gender and BMI (data not shown). We therefore pooled all data for further analysis. The basic anthropometric and biochemical data of the study population are included in Tables 1 and 2 (right columns, Total).

Leptin percentiles

Leptin levels are shown as scattered plots for women and men in Fig. 1. In both sexes, there was a highly significant correlation between BMI and serum leptin levels (female: $r=0·66$, $P<0·001$; male: $r=0·52$, $P<0·001$). Because of the strong correlation between leptin levels and gender and BMI, percentile curves for the 5th, 20th, 50th, 80th and 95th percentile of leptin concentrations by BMI were computed for both sexes separately. The slope of these curves was comparable in both sexes: the lower percentiles showed slightly positive slopes, whereas the higher percentiles rose with steeper slopes, thus leading to divergent curves especially for higher BMIs.

Leptin percentile curves for females In females, the percentile values were fitted to linear graphs for all percentiles (Fig. 2). The graph equations as determined by linear regression analysis are as follows: 5th percentile: $y=0·4284x−5·2922$; 20th percentile: $y=0·8585x−12·409$; 50th percentile: $y=1·6285x−26·362$; 80th percentile: $y=2·2005x−34·071$; 95th percentile: $y=2·7947x−40·181$.

The range between the 5th and 95th percentile increased with BMI. In the lowest BMI class (BMI<18 kg/m²), the value for the 5th percentile of serum leptin was 1·8 ng/ml and the value for the 95th percentile was 12·6 ng/ml, giving a range of 10·8 ng/ml. The corresponding leptin levels in the highest BMI class (≥40 kg/m²) were 8·7 ng/ml and 59·2 ng/ml, the range here being 50·5 ng/ml.

Leptin percentile curves for males In males, the 5th, 20th and 50th percentile values were fitted to linear graphs. The best fit for the 80th percentile was exponential, and the best fit for the 95th percentile was polynomic (Fig. 3). The graph equations as determined by computed best fit analysis are as follows: 5th percentile: $y=0·2197x−3·2838$; 20th percentile: $y=0·3627x−5·7404$; 50th percentile: $y=0·7322x−13·281$; 80th percentile: $y=0·5496e^{0·0996x}$; 95th percentile: $y=0·064x^2−2·3856x+31·186$.

As in females, the range between the 5th and 95th percentile increased with BMI. In the lowest BMI class (BMI<18 kg/m²), the value for the 5th percentile of serum leptin was 1·1 ng/ml and the value for the 95th percentile was 12·9 ng/ml, giving a range of 11·8 ng/ml. The corresponding leptin levels in the highest BMI class (≥40 kg/m²) were 5·3 ng/ml and 33·4 ng/ml, the range here being 28·1 ng/ml.

Definition of relative hyper- and hypoleptinemia

We now wanted to determine whether relatively high or low leptin levels were correlated with metabolic and endocrine abnormalities. We therefore defined the group of participants with leptin levels beneath the 5th percentile of leptin by BMI as having ‘relative hypoleptinemia’ and termed this group low leptin (LL). A subset with ‘relative hyperleptinemia’ was defined from participants with leptin levels above the 95th percentile of leptin by BMI (high leptin (HL)). These subsets were compared with the main group with ‘normal’ leptin levels between the 5th and 95th percentiles (Fig. 2). The graph equations as determined by computed best fit analysis are as follows: 5th percentile: $y=0·4284x−5·2922$; 20th percentile: $y=0·8585x−12·409$; 50th percentile: $y=1·6285x−26·362$; 80th percentile: $y=2·2005x−34·071$; 95th percentile: $y=2·7947x−40·181$.

The corresponding leptin levels in the highest BMI class (≥40 kg/m²) were 8·7 ng/ml and 59·2 ng/ml, the range here being 50·5 ng/ml.
95th percentile of leptin by BMI (intermediate leptin (IL)). The results are outlined below and shown in detail in Table 1 (females) and Table 2 (males).

**General parameters**

**Females** In females, age, BMI, systolic blood pressure and time since last meal were not significantly different between the three subgroups. The mean diastolic blood pressure was slightly, but significantly higher in IL when compared with LL (86 vs 82 mmHg), but still remained within the normal range. The prevalence of type 1 or type 2 diabetes as we had defined it previously did not differ between the subgroups (not shown).

**Males** The male participants in the subgroup LL were significantly younger than those in subgroups IL and HL (43 vs 50/51 years). When we correlated leptin levels and age in controlling for BMI we found a weak correlation only in the group with a BMI between 25 and 30 kg/m² (correlation coefficient \( r^2 = 0.24; P < 0.001 \)). We corrected for age in the subsequent statistical analysis as detailed below to exclude age-related effects.

Body mass index, diastolic blood pressure and time since last meal did not differ significantly in HL, IL and LL groups. The prevalence of type 1 or type 2 diabetes did not differ between the groups (not shown). Systolic blood pressure was not pathologically elevated in any of the groups, but in the overall comparison of group means it was 6 mmHg higher in IL than in HL (140 vs 134 mmHg). Because the measured parameters were not normally distributed we were not able to conduct a multivariate analysis of the relationship between leptin and systolic
blood pressure. Mann–Whitney tests in different age classes showed a slightly, but not significantly, higher systolic blood pressure in males between 31 and 40 years of age (P<0.07), and partial correlations of leptin and systolic blood pressure controlling for age in different BMI classes showed a weak correlation only in participants between 20 and 30 kg/m² ($r^2=0.18$, $P<0.01$). Taken together, these data argue against a major difference in systolic blood pressure between the three leptin percentile subgroups.

**Endocrine parameters**

**Females** Analysis of serum cortisol, DHEAS, estradiol, testosterone and progesterone was completed in 676 female participants. Mean serum testosterone was not pathologically elevated in any subgroup, but it was about 60% lower in the IL and HL groups than in the LL group (0.44±0.0 and 0.43±0.1 respectively vs 1.09±0.3 µg/l). This was statistically highly significant for the comparison of LL and IL (P<0.001). No significant differences between the three subgroups were seen with regard to the other endocrine parameters we examined.

**Males** We measured the above mentioned parameters in 734 male participants. There was a 60% decline in mean testosterone levels from LL to IL to HL (P<0.01). This finding was not only related to the age difference as partial correlations between serum leptin and testosterone revealed a correlation coefficient of $-0.39$ after controlling for age (P<0.006). The effect was most pronounced in
males between 41 and 50 years of age (LL 5·1 ± 0·6, HL 1·3 ± 0·7 µg/l, \(P<0·05\)). Mean serum cortisol was about 30% higher in LL than in IL. Analysis in different age groups showed a significant effect only in males between 41 and 50 years of age (\(P<0·05\)), partial correlations revealed only a very weak relationship between leptin and cortisol (\(r^2 = -0·12\), \(P<0·027\)). DHEAS, estradiol and progesterone were not different in the three leptin percentile subsets.

**Serum lipids**

**Females** In females, total serum cholesterol was slightly elevated in IL and HL. There was a continuous increase in the three leptin percentile subgroups with cholesterol levels being 8% and 14% higher than LL levels in IL and HL respectively. HDL-cholesterol and lipoprotein(a) levels were not significantly different between the subgroups.

**Males** Similarly, male participants showed lower total cholesterol levels in LL with an 11% rise to IL and HL. The effect was significant in the age range between 18 and 40 years; older participants showed no differences in cholesterol levels. Partial correlations controlling for age revealed a low overall correlation coefficient of 0·08; analysis of BMI subgroups showed significant relationships with correlation coefficients between 0·1 and 0·5 for participants with a BMI between 20 and 30 mg/kg\(^2\). No significant correlations were found in obese participants. HDL-cholesterol and lipoprotein(a) were not significantly different between the three leptin percentile subgroups.

**Parameters of iron metabolism**

**Females** Serum iron values were higher in the LL and the IL groups when compared with the HL group (149 ± 5 and 142 ± 1·1 vs 129 ± 5·3 µg/dl, \(P<0·01\) and \(P<0·001\)). Serum ferritin and transferrin levels were not significantly different.

**Males** In males, serum iron was also higher in LL and IL compared with HL (156 ± 5·5 and 152 ± 1·1 vs 138 ± 4·2 µg/dl, \(P<0·01\); this difference was also noted after correction for age by analyzing iron levels in different age classes. Serum ferritin was lower in LL when compared with IL and HL (116 ± 9·7 vs 175 ± 3·8 and 168 ± 17 ng/ml respectively, \(P<0·01\) and \(P<0·001\)). Looking at different age classes, we found this effect to be significant only in participants older than 50 years of age (\(P<0·05\)). Transferrin values did not differ between the three groups.

**Parameters of renal function**

**Females** Serum uric acid was significantly lower in LL than in HL (5·1 ± 0·2 vs 5·7 ± 0·2 mg/dl, \(P<0·02\)). Serum urea and creatinine levels showed no significant differences between the three groups.

**Males** All three examined parameters, serum uric acid, urea and creatinine did not differ between the three leptin percentile subsets.

**Parameters of glucose metabolism**

**Females** In females, there was a 42% rise of insulin levels from LL to IL and a 29% rise from IL to HL (\(P<0·001\)) to a maximum of 25·5 ± 1·7 mU/l. The continuous increase in serum insulin remained significant after correction for time since last meal. This effect was also reflected by a corresponding 50% rise in c-peptide levels from LL to IL to HL (\(P<0·001\)). The HbA1c was not significantly different in these groups.
Males In males, HbA1c values were the lowest in LL when compared with IL and HL (5.2 ± 0.1 vs 5.5 ± 0.1% in both IL and HL, $P<0.01$ and $P<0.001$ respectively). Analysis in different age classes showed this effect to be significant only in participants older than 40 years ($P<0.05$). Similar to the female participants, both insulin and c-peptide levels rose from LL to IL to HL (insulin: 17 ± 1.0 vs 21.2 ± 0.4 vs 27.9 ± 2.7 mU/l, $P<0.001$; c-peptide: 1.6 ± 0.1 vs 2.0 ± 0.0 vs 3.0 ± 0.3 µg/l, $P<0.001$). Analysis in different age classes confirmed these results, thereby ruling out an age bias as the cause for the observed effects.

To definitely rule out BMI or time after the last meal as an underlying cause for the observed relationships, we analyzed insulin and c-peptide levels in normal weight, overweight and obese subjects depending on leptin percentile subsets for both sexes. No difference between time after last meal was observed between LL, IL, and HL groups. The rise of c-peptide and insulin with higher leptin levels was confirmed in all BMI groups. The relationship was statistically strongest in overweight males and females (Tables 3 and 4). In this subgroup, variability in insulin accounted for up to 24% of variability in leptin levels, whereas in subjects with normal weight only 2–8%
Assessment of relative risks for metabolic syndrome

Given the tendency of HL participants to have more factors of the metabolic syndrome such as hyperinsulinemia, hypercholesterolemia or hypertension we calculated the relative risk of these conditions at different quintiles of leptin by BMI. Generally, the relative risk of the examined parameters of the metabolic syndrome was significantly lower in the lowest quintile of leptin by BMI and significantly higher in the highest quintile of leptin by BMI when compared with the middle quintile for both sexes (Table 5).

Table 3 Parameters of glucose metabolism depending on obesity and leptin percentiles – females. Results are means ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>LL (serum leptin &lt;5th percentile)</th>
<th>IL (serum leptin 5th–95th percentile)</th>
<th>HL (serum leptin &gt;95th percentile)</th>
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<tr>
<td>Normal weight (BMI 20–24·9 kg/m²)</td>
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<tr>
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<tr>
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<td>2·9 ± 2·2</td>
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<tr>
<td>Insulin (mU/l)</td>
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<td>C-peptide (µg/l)</td>
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<tr>
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<tr>
<td>C-peptide (µg/l)</td>
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*P < 0·05/number of tests, †P < 0·001: LL (relative hypoleptinemia) compared with HL (relative hyperleptinemia);
†P < 0·05/number of tests, ††P < 0·001: LL compared with IL (normal leptin levels); ‡P < 0·05/number of tests, ‡‡P < 0·001: IL compared with HL.

Table 4 Parameters of glucose metabolism depending on obesity and leptin percentiles – males. Results are means ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>LL (serum leptin &lt;5th percentile)</th>
<th>IL (serum leptin 5th–95th percentile)</th>
<th>HL (serum leptin &gt;95th percentile)</th>
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*P < 0·05/number of tests, †P < 0·001: LL (relative hypoleptinemia) compared with HL (relative hyperleptinemia);
‡P < 0·05/number of tests, ‡‡P < 0·001: LL compared with IL (normal leptin levels); †P < 0·05/number of tests, ‡‡P < 0·001: IL compared with HL.
Table 5 Leptin quintiles and relative risks for features of the metabolic syndrome. Stated are the relative risks (with confidence intervals) for hyperinsulinemia (defined as serum insulin >24 μU/ml), elevated c-peptide (defined as serum c-peptide >4 μg/l), hypertension (defined as systolic (syst.) blood pressure >140 mmHg and diastolic (diast.) blood pressure >90 mmHg), and hypercholesterolemia (defined as total cholesterol >200 mg/dl) when comparing the lower (1 and 2) and upper (4 and 5) quintiles of leptin by BMI with the middle quintile (3).

## Quintiles of serum leptin by BMI

<table>
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<th>Relative risks – females</th>
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<td>Elevated diast. blood pressure</td>
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<td>0·77</td>
<td>0·87</td>
<td>n/a</td>
<td>0·97</td>
</tr>
<tr>
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<td>0·86</td>
<td>0·97</td>
<td>n/a</td>
<td>1·03</td>
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</tbody>
</table>

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<th>3</th>
<th>4</th>
<th>5</th>
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<tr>
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<td>0·82</td>
<td>0·82</td>
<td>n/a</td>
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</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>0·68</td>
<td>0·68</td>
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<td>n/a</td>
<td>0·94</td>
</tr>
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</table>

n/a. not applicable.

## Discussion

In this paper, we present an analysis of the serum leptin levels in a large, well-characterized, population-based German cohort. The known positive correlations of leptin to BMI and female gender (Maffeï et al. 1995, Considine et al. 1996, Dua et al. 1996, Segal et al. 1996) and the negative correlation to testosterone in males (Behre et al. 1997, Luukkaa et al. 1998) were confirmed. The range of the leptin levels we measured correlates very well with previously published studies examining serum leptin in lean and obese populations (Sinha et al. 1996, Weigle et al. 1997, Langendonk et al. 1998).

### Definition of relative hypo- and hyperleptinemia

Leptin is known to correlate with central obesity, but previous studies have not conclusively analyzed the specific effects of leptin on metabolic and endocrine parameters independently of body mass index. To correct for BMI-dependent effects, we defined states of ‘relative hypo- and hyperleptinemia’ (LL and HL respectively) by analyzing the percentiles of serum leptin levels by BMI. The 5th and 95th percentile as lower and upper limits of ‘relative normoleptinemia’ (IL) were arbitrarily chosen. We did not use the usual definition of reference ranges, e.g. the range between the 2.5th-97.5th percentile, because the resulting HL and LL group sizes would then have been too small to make reasonable statistical comparisons.

It is important to note that the leptin percentiles we report may not be attributed as ‘leptin reference ranges’ for some other reasons. First, leptin is secreted from white adipose tissue in lean and obese subjects following a circadian rhythm, with peak levels in the early night and a nadir around noon (Sinha et al. 1996, Simon et al. 1998). From these published observations the variation of serum leptin during the day can be estimated to be less than 20% of the 0800 h levels. Secondly, the study population was not homogeneous with respect to age, lifestyle and underlying diseases. Several chronic illnesses are known to influence leptin levels, e.g. liver cirrhosis, anorexia nervosa, renal dysfunction and more (Grinspoon et al. 1996, Henriksen et al. 1999, Widjaja et al. 2000). Lifestyle factors such as physical activity also influence leptin levels. Although we excluded participants with known liver cirrhosis or nutrition disorders from the analysis, factors of this kind may have confounded the data in some cases. We assume that the large number of participants prevents major misinterpretations coming from these potential bias sources. Taken together, to obtain correct reference ranges for leptin it would be necessary to analyze a homogeneous group of healthy males and females at a defined point of time, maybe also differentiating between bound and free leptin levels (Brabant et al. 2000).

### Relative hypo- and hyperleptinemia and the metabolic syndrome

The comparison of the relatively hypo- and hyperleptinemic groups with each other and the intermediate group showed a variety of effects which cannot be explained by changes in body mass index, as a confounding effect of obesity was ruled out by defining the leptin percentiles by BMI. In both sexes, insulin and c-peptide levels clearly increased with the leptin percentiles. The relationship between leptin and insulin/c-peptide has been described extensively elsewhere. Briefly, in in vitro settings, leptin had a negative effect on insulin secretion (Kieffer et al. 2002).

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et al. 1997), and insulin upregulated leptin secretion (Leroy et al. 1996, Rentsch & Chiesi 1996). Previous studies in humans have shown positive correlations between leptin and insulin, agreeing with our results (Mohamed-Ali et al. 1997, Asakawa et al. 2001). These results might indicate that in vivo the stimulatory effect of insulin on leptin secretion predominates over the inhibitory effect of leptin on insulin levels, although this issue remains controversial. Previous studies with smaller patient numbers have concluded that leptin levels are associated with the insulin resistance syndrome (Haffner et al. 1999). We propose that relative (i.e. BMI-corrected) hyperleptinemia may be a new component of this syndrome. This is confirmed by the 40% elevation of the relative risk for hyperinsulinemia in the upper quintile of leptin by BMI when compared with the middle quintile. Decreased leptin levels may indicate a low relative risk for the metabolic syndrome, as shown by the respective calculations for the lower leptin quintile (Table 5). This might reflect a higher proportion of athletic individuals in the lower quintiles with similar body mass index but different fat distribution than participants in the higher quintiles, as leptin secretion differs between subcutaneous and visceral fat. As no data concerning body fat distribution were available from the study participants this possible confounding factor should be carefully monitored in future studies.

Obviously, the hyperuricemia and hypercholesteremia in the HL group might also be interpreted as part of the metabolic syndrome cluster that the participants with relatively high leptin levels tended to show. Previous studies have not conclusively shown a BMI-independent correlation between hypercholesterolemia and leptin levels (Ruige et al. 1999, Chu et al. 2001, Martini et al. 2001). Experimental results indicated the capability of leptin to reduce lipid release and lower lipoprotein levels in Caco-2 cells (Stan et al. 2001) and to promote hepatic cholesterol clearance in obese Zucker rats (VanPatten et al. 2001), thus arguing for a direct effect of leptin on lipoprotein metabolism. Taken together, there is a line of evidence connecting leptin and cholesterol, although the clinical significance of this issue must remain controversial.

Elevated blood pressure is another well known part of the metabolic syndrome. In our study, in male participants systolic blood pressure and in females diastolic blood pressure was lower in the group with relative hypo-leptinemia. There are some previous reports linking leptin levels with human hypertension (Kazumi et al. 1999, Haynes 2000, Matsubara et al. 2000); this is presumed to result from activation of the sympathetic nervous system (Esler et al. 2001). Studies that have administered leptin directly to healthy humans have not reported changes in blood pressure (Mackintosh & Hirsch 2001, Narkiewicz et al. 2001). Considering these results and the small size of the effect we saw it does not seem justified to attribute major effects on blood pressure to relative hyperleptinemia.

Where might the assessment of the leptin status with percentile curves be useful?

Summarizing the phenotype of the subjects with relative hyperleptinemia, it appears that it resembles an early stage metabolic syndrome with elevated insulin and c-peptide levels, elevated cholesterol, to some extent higher blood pressure etc. These changes were marked especially when compared with the group with low leptin levels. The use of BMI-corrected leptin levels as we propose might help to predict a person’s risk of acquiring a metabolic syndrome with consecutively elevated susceptibility to atherosclerosis. Recently, leptin has been identified as a predictor of first-time ever hemorrhagic stroke (Soderberg et al. 1999), and several groups have shown a proatherogenic action of leptin on endothelial cells (Parhami et al. 2001, Yamagishi et al. 2001). Leptin is positively associated with the intima thickness of the common carotid artery (Ciccone et al. 2001). These associations do not prove a causal role of hyperleptinemia for the pathogenesis of atherosclerosis, but they point at serum leptin as a possible cardiovascular risk marker. Persons with relatively high serum leptin should be further examined to find out whether the putative increased risk for the development of glucose intolerance as well as cardiovascular disease and body weight gain (Chu et al. 2001) can be shown prospectively.

Leptin is a polyfunctional hormone. The evaluation of BMI-corrected leptin levels may contribute not only to metabolic clinical problems, but also to questions in the field of liver disease, oncology and others. For example, many studies have shown elevated leptin levels in patients with alcoholic and infectious liver disease (Henriksen et al. 1999, Greco et al. 2000, Campillo et al. 2001, Nicolas et al. 2001). Recently, Nakamuta et al. (2001) demonstrated normal leptin levels in patients with nonalcoholic liver disease; Ben-Ari et al. (2002) found low leptin levels in patients with primary biliary cirrhosis. Both leptin and iron load have been implicated in the pathogenesis of human liver fibrosis (Pietrangelo 1998, Casaril et al. 2000, Ikejima et al. 2001), and iron overload has been associated with steatohepatitis and insulin resistance (Mendler et al. 1999, Moirand et al. 2000). We observed markedly lower iron values in the group with relatively high leptin levels in both sexes, and higher ferritin levels in male subjects older than 50 years with relatively high leptin levels. Alcohol consumption which could explain differences in iron load did not differ in the three subgroups. These data imply that leptin levels might be helpful when differentiating the etiology of liver damage and that they might provide a link between the metabolic syndrome and the associated fatty liver syndrome with putative iron overload. New interesting experimental data have revealed leptin to be a growth factor for colonic epithelial cells (Hardwick et al. 2001); one study suggests a causal role for leptin in the enhancement of colonic carcinogenesis under a high fat diet.
(Liu et al. 2001). Associations to prostate and breast cancer have been presumed (Tessitore et al. 2000, Hsing et al. 2001, Stattin et al. 2001), although no causal relationship has been detected yet.

Clearly, more prospective studies are needed to fully understand the implications of BMI-corrected basal leptin levels on weight gain and weight reduction, glucose intolerance, hypertension and cardiovascular disease as well as the relationship of leptin to (colorectal) cancer risk, liver disease and other chronic illnesses.

References


Hardwick JC, Van Den Brink GR, Offerhaus GJ, Van Deventer SJ & Peppelensboch MP 2001 Leptin is a growth factor for colonic epithelial cells. *Gastroenterology* 121 79–90.


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