Analysis of the relationship between fasting serum leptin levels and estimates of beta-cell function and insulin sensitivity in a population sample of 380 healthy young Caucasians

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Abstract

Objective: Circulating leptin levels correlate positively with the degree of obesity and prolonged hyperinsulinaemia increases serum leptin levels. Moreover, insulin secreting β -cells express functional leptin receptors indicating a functional relationship between leptin and insulin. The aim of this study was to examine the relationship between fasting serum leptin levels and measures of insulin sensitivity and β -cell function in a population-based sample of 380 young healthy Caucasians.

Design and Methods: Multiple regression analysis was employed to analyse the relationship between fasting serum leptin levels and levels of fasting serum insulin, insulin sensitivity index and acute insulin response (AIR) in a population-based study of 380 young healthy Caucasians who underwent a combined intravenous glucose and tolbutamide tolerance test.

Results and Conclusion: Serum leptin levels were positively correlated to measures of adiposity and were 3.2 times higher in women than in men ($P < 0.00001$). In multiple regression analyses adjusting for age, percentage body fat, waist circumference and maximal aerobic capacity, a significant positive correlation was observed between the fasting serum leptin concentrations and both fasting serum insulin levels ($P < 0.0001$) and AIR ($P = 0.014$) for women. No significant interrelation of these variables was found in men. However, for both genders a significant negative correlation was observed between fasting serum leptin levels and measures of insulin sensitivity index ($P = 0.007$).

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Introduction

Leptin is a secreted protein, expressed almost exclusively in white adipose tissue. The protein has hormonal effects on food intake and energy expenditure mediated through hypothalamic leptin receptors (1). Loss-of-function mutations in the gene encoding leptin are responsible for autosomally recessive inherited obesity associated with hyperinsulinaemia and diabetes in rodents (2) and in rare cases of human obesity (3). In most humans as well as rodents the levels of both circulating leptin and leptin mRNA expression correlate with the degree of obesity (4–6) indicating that the common form of obesity is not caused by absolute leptin deficiency. However, receptors for leptin are expressed in several tissues other than adipocytes and hypothalamic cells which may indicate that the leptin protein has regulatory functions in other tissues (7, 8).

Recent *in vitro* studies have shown that circulating leptin can counteract the effects of insulin in both

muscle (9) and adipocytes (10), thus inducing peripheral insulin resistance, whereas other studies indicate that leptin may stimulate glucose metabolism in muscle (11). These findings indicate that leptin may have direct effects on peripheral insulin action *in vivo*. Studies in humans have shown positive correlations between circulating leptin levels and fasting serum insulin levels (12–15) and negative correlations between fasting serum leptin and insulin sensitivity (12, 16), independently of measures of body fat contents. These findings together with studies showing that prolonged hyperinsulinaemia in humans can increase serum leptin levels indicate that leptin may influence insulin production or sensitivity in humans (17–19).

Moreover, human as well as rodent pancreatic β -cells express mRNA for the long form of the leptin receptor and show binding of recombinant leptin (7, 8). *In vitro* studies have demonstrated that leptin inhibits glucose-stimulated insulin release from isolated rat islets as well as perfused rat pancreas (7, 8, 20), whereas one study

showed no effect of leptin on insulin or glucagon release (21). In relation to these *in vitro* data, one previous study has shown that in women circulating leptin levels correlate positively with measures of acute insulin response (AIR) (22) which could reflect a direct effect of leptin on β -cell function. Thus, although the human studies have comprised relatively small numbers of subjects so far, there is evidence that leptin may influence both insulin sensitivity as well as insulin secretion in man.

The aim of the present study was, therefore, to examine the relationships between fasting serum leptin levels and insulin sensitivity and fasting serum insulin levels and to explore the associations between fasting serum leptin and measures of AIR in an extensively characterised population-based sample of 380 young healthy subjects.

Subjects and methods

Phenotype study of a random sample of young healthy Caucasians

Three hundred and eighty subjects, 186 men and 194 women, were randomly recruited from a population of young healthy subjects aged 18–32 years, who in 1979/1980 and again in 1984/1985 had participated as children in an epidemiological blood pressure survey in a representative specified part of Copenhagen. Physiological characteristics of this cohort have been presented previously (23). In short, anthropometric measures (height, weight, waist and hip circumference) as well as measures of insulin sensitivity, β -cell function, physical fitness and serum lipids were recorded (23). Insulin sensitivity index was estimated from an intravenous glucose tolerance test in combination with intravenous injection of tolbutamide as previously described (23). AIR was determined as the area under the insulin curve during the first 8 min after glucose

injection, as reported (23). Body fat content was estimated by a bioimpedance technique as described previously (23, 24).

Measurement of serum leptin

Serum leptin levels were measured using a human leptin RIA kit (Linco, St Charles, MO, USA) (25). Serum samples (100 μ l) were analysed in duplicate for each subject as described in the Leptin RIA kit protocol. The interassay coefficient of variation was less than 0.07.

Statistical analysis

Statistical Package of Social Sciences, version 6.01 was used for statistical analyses. A *P* value <0.05 (two-tailed) was considered significant. For variables that were not normally distributed the natural logarithm transformed values were used. Normality was tested by visually inspecting normal distribution plots and histograms as well as residual plots of variables. From previous studies maximal aerobic capacity (VO_2max) and use of contraceptive pills are known to influence insulin sensitivity (23) and are thus included in the multiple regression analyses. Where gender had a significant effect, a gender stratification was performed.

Results

General anthropometric measures

The clinical and biochemical characteristics of all 380 subjects are described in Table 1, stratified according to gender. Several measures of body fat composition were significantly different between genders, as previously described (23), e.g. the percentage mean body fat was 0.3 times higher in women than in men, whereas body mass index (BMI), total lean mass, total body weight and waist to hip ratio values were all higher in men.

Table 1 Clinical characteristics of a population sample of 380 young healthy Danish Caucasians (mean values $\pm$ s.d.).

	Men (<i>n</i> = 186)	Women (<i>n</i> = 194)	<i>P</i> value*
Age (year)	25.5 $\pm$ 3.4	25.0 $\pm$ 3.5	0.127
Weight (kg)	79.6 $\pm$ 13.1	64.3 $\pm$ 11.2	<0.001
BMI (kg/m ²)	24.1 $\pm$ 3.4	22.9 $\pm$ 3.8	<0.001
Total body fat (kg)	16.3 $\pm$ 7.5	17.7 $\pm$ 7.9	0.067
Body fat percentage (%)	20 $\pm$ 6	26 $\pm$ 7	<0.001
Waist-hip ratio	0.86 $\pm$ 0.05	0.77 $\pm$ 0.06	<0.001
Fasting plasma glucose (mmol/l)	5.2 $\pm$ 0.5	4.8 $\pm$ 0.3	<0.001
Fasting serum insulin (pmol/l)	35 $\pm$ 21	39 $\pm$ 22	0.100
Fasting serum C-peptide (pmol/l)	456 $\pm$ 158	492 $\pm$ 159	0.028
Insulin sensitivity index (Si) ($10^{-5} \times (\text{min} \times \text{pmol/l})^{-1}$)	15.2 $\pm$ 8.8	15.1 $\pm$ 9.6	0.948
Acute serum insulin response AUC _{Insulin} (0–8 min)(min $\times$ pmol/l)	2068 $\pm$ 1372	2430 $\pm$ 1752	0.006
Maximal aerobic capacity (ml O ₂ /(kg $\times$ min))	44 $\pm$ 9	38 $\pm$ 8	<0.001
Fasting serum leptin (ng/ml)	4.6 $\pm$ 3.1	15.0 $\pm$ 10.7	<0.001

* *P* values for the difference between genders are indicated (independent sample *t*-test, equal variances not assumed). AUC, area under curve.

Figure 1 Scatter plot of fasting serum leptin in ng/ml vs body fat percentage measured by a bioimpedance method in 380 young healthy Caucasians. (◆), men; (+), women; dotted line, linear regression for men (R^2 value = 0.523); solid line, linear regression for women (R^2 value = 0.551).

Fasting serum leptin levels were on average more than three times higher in women than in men, as expected from the higher percentage fat mass in women. Fasting serum leptin levels were positively correlated with percentage body fat in both men and women (Fig. 1).

Fasting serum insulin

Levels of fasting serum insulin showed no differences between genders. A gender stratified multiple regression analysis with $\ln(\text{fasting serum insulin})$ as dependent variable and age, percentage fat, VO_2max , waist

circumference and circulating serum leptin as independent variables showed a strong positive correlation between circulating serum leptin levels and levels of fasting serum insulin which was independent of measures of fat mass. This correlation was, however, only found in women (Table 2).

Insulin sensitivity (Si)

Performing a multiple regression analysis with $\ln(\text{Si})$ as dependent variable and age, percentage fat mass, waist circumference, VO_2max and fasting serum leptin

Table 2 Gender stratified multiple regression analysis using $\ln(\text{fasting insulin})$ as dependent variable.

	Regression coefficient	95% CI		P value
		Lower	Upper	
Men				
(Constant)	2.6	1.6	3.6	<0.001
Age	−0.029	−0.047	−0.011	0.002
Fat percentage	−0.004	−0.023	0.015	0.693
Waist circumference	0.024	0.012	0.037	<0.001
Fasting serum leptin	0.023	−0.008	0.053	0.150
Maximum aerobic capacity	−0.011	−0.019	−0.003	0.010
Women				
(Constant)	3.1	2.2	4.0	<0.001
Age	−0.039	−0.056	−0.021	<0.001
Fat percentage	−0.025	−0.043	−0.007	0.006
Waist circumference	0.026	0.014	0.038	<0.001
Fasting serum leptin	0.020	0.012	0.029	<0.001
Maximum aerobic capacity	−0.005	−0.014	0.004	0.250

CI, confidence interval; Constant, constant in regression equation.

Table 3 Multiple regression analysis using ln(Si) as dependent variable.

	Regression coefficient	95% CI		P value
		Lower	Upper	
(Constant)	3	2.8	5.0	<0.001
Age	0.025	0.009	0.041	0.002
Fat percentage	0.016	−0.001	0.032	0.061
Waist circumference	−0.034	−0.045	−0.024	<0.001
Fasting serum leptin	−0.013	−0.022	−0.003	0.007
Maximum aerobic capacity	0.017	0.009	0.024	<0.001
Gender	−0.199	−0.429	0.031	0.090

CI, confidence interval; Si, insulin sensitivity index; Constant, constant in regression equation.

as independent variables, ln(Si) was negatively associated with fasting serum leptin levels independently of body fat in both genders (Table 3).

Acute insulin response (AIR)

Performing a gender stratified multiple regression analysis with ln(AIR) as dependent variable and age, percentage fat mass, waist circumference, VO₂max and leptin as independent variables, there was a significant positive correlation with fasting serum leptin levels among women (Table 4). The inclusion of fasting serum insulin levels as a dependent variable in the multiple regression analysis eliminated the correlations between fasting serum leptin and AIR (data not shown).

Discussion

In the present study multiple regression analysis showed a significant positive correlation between circulating fasting leptin levels and fasting serum insulin levels for women even when percentage body

fat was included in the analysis (Table 2). This finding is in accordance with previous studies confirming a positive correlation between circulating levels of leptin and insulin (12–15). This relationship may reflect either a direct effect of leptin on pancreatic β -cells increasing insulin secretion or a correlation between leptin and insulin sensitivity, where decreased insulin sensitivity may induce an adaptive increase in fasting insulin levels.

Recent *in vitro* studies show that circulating leptin levels influence energy metabolism in both muscle and adipocyte cells (9–11). Using multiple regression analysis we have demonstrated a significant negative correlation between fasting serum leptin and Si for both genders, indicating a potential impact of leptin on whole body insulin sensitivity. A negative correlation between measures of Si and fasting serum leptin levels has previously been reported (12, 16). However, fasting serum insulin levels correlate well with Si (23) and thus fasting leptin levels could be correlated to Si through fasting insulin levels. As the *in vitro* data are contradictory on this issue, the nature of the correlation between fasting serum leptin and Si is difficult to

Table 4 Gender stratified multiple regression analysis using ln(AIR) as dependent variable in 186 male and 194 female young Danish Caucasians.

	Regression coefficient	95% CI		<i>P</i> value
		Lower	Upper	
Men				
(Constant)	7.1	5.4	8.7	<0.001
Age	−0.037	−0.072	−0.003	0.034
Fat percentage	0.009	−0.027	0.044	0.627
Waist circumference	0.012	−0.012	0.036	0.338
Fasting serum leptin	0.023	−0.033	0.080	0.414
Women				
(Constant)	6.5	5.5	7.5	<0.001
Age	−0.011	−0.036	0.014	0.370
Fat percentage	−0.023	−0.048	0.002	0.077
Waist circumference	0.024	0.007	0.041	0.007
Fasting serum leptin	0.015	0.003	0.027	0.014

CI, confidence interval; AIR, acute insulin response; Constant, constant in regression equation.

evaluate. The finding from several studies that prolonged hyperinsulinaemia (>4 h) can increase circulating leptin levels in humans (17–19) and also that insulin has been shown to be a potent stimulator of leptin secretion *in vitro* (26) underscore the possibility that a regulatory mechanism may exist between serum leptin and serum insulin levels and further points to insulin as the primary determining factor. However, as the ob mice lacking leptin are both obese and insulin resistant (2), leptin does not seem to constitute an essential part of the insulin resistance conveyed by obesity.

On the other hand, the presence of leptin receptors on β -cells (7, 27) and their sensitivity to leptin in *in vitro* studies (7, 8, 20) may also indicate that leptin affects β -cell function directly. We therefore analysed the relation between circulating leptin levels and measures of AIRs using multiple regression analysis. Including, among many other factors, fat mass as a dependent factor in the analysis, AIR in women showed a positive correlation to fasting serum leptin levels. As the positive correlation between fasting serum insulin levels and leptin seems to be stronger in women compared with men (28) and as a positive correlation has previously been found between circulating leptin levels and AIR to arginine and glucose in a study of 36 postmenopausal women (22), it is possible that these findings reflect a direct effect of leptin on AIRs, which appears only to be detectable in women. However, since a very strong positive correlation exists between fasting serum insulin and AIR, the correlation between fasting serum leptin and AIR may be secondary to the latter relationship (23). When including fasting serum insulin levels as an independent variable in the multiple regression analysis, the correlation between leptin and AIR disappears. Still, acute hyperglycaemia or hyperinsulinaemia do not increase circulating leptin levels in man (29, 30), and one could hypothesise that although long-term hyperinsulinaemia increases circulating leptin levels, a short-term effect of leptin on pancreatic β -cells and AIR might exist. Determining a primary effect of leptin on insulin secretion will, however, require further investigation. In Pima Indians, a genetic linkage has been reported between measures of AIR to an intravenous glucose challenge and a locus which encompasses the leptin receptor indicating a potential role of leptin action in controlling AIR in this ethnic group (31).

Fasting serum insulin and AIR show positive correlations to leptin levels only in women in this and other studies (22, 28). A possible reason for this finding could be that a higher variance is seen for leptin levels among women. However, leptin levels are also higher in women, and thus the relative variation is similar among men and women. Rather, the correlations seen in women could be due just to the higher serum leptin levels, since a potential effect of leptin would be stronger.

In summary, the fasting serum leptin level is positively correlated to both fasting serum insulin

levels and AIR in women in this population sample of young healthy subjects. Moreover, Si is negatively correlated to fasting serum leptin levels in both genders. As described by Clausen *et al.* (23), AIR, fasting serum insulin and Si are highly correlated to measures of body fat as is circulating leptin levels, making body fat content a possible confounder in the interpretation of the results obtained in the correlation analysis. However, the multiple regression analysis indicates that fasting serum leptin may be independently correlated to measures of fasting serum insulin, insulin responses and insulin sensitivity. Whether these results point to a direct effect of leptin on β -cell function and insulin sensitivity or whether circulating insulin levels influence circulating leptin levels remain to be determined.

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