Human Plasma Leptin in Obese Subjects and Diabetics

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Abstract. Leptin is the protein product of the ob gene, an adipocyte-specific gene, recently discovered in mice. Plasma leptin levels were determined in six normals, twenty-one subjects with impaired glucose tolerance, and forty-nine untreated NIDDM subjects. They increased with the augmentation of obesity (body mass index, BMI kg/m²) and were higher in females than in males: in BMI less than 25 kg/m² the values of plasma leptin were 2.24 ± 0.25 ng/ml (n=29) in males and 3.01 ± 0.39 ng/ml (n=13) in females (P<0.054), respectively, in BMI between 25 kg/m² and 30 kg/m² they were 3.14 ± 0.31 ng/ml (n=10) in males and 10.66 ± 2.86 ng/ml (n=7) in females (P<0.0018) and in BMI higher than 30 kg/m² their levels were 8.98 ± 1.5 ng/ml (n=11) and 11.74 ± 2.2 ng/ml (n=6) (P<0.023), respectively. The severity of diabetes mellitus judged from the fasting plasma glucose level had no influence on the plasma leptin levels during OGTT, but the leptin levels decreased significantly during a tolerance test (P<0.001), and similar results were also seen during a breakfast test. The fasting plasma leptin in the male with FBS less than 140 mg/dl had a significant correlation with the fasting plasma IRI level, but this correlation disappeared after taking obesity into consideration. Thus the plasma leptin was chiefly dependent on the body weight and gender and had no special relation to diabetic severity.

Key words: Plasma leptin, Obesity, Diabetes mellitus, Gender

Materials and Methods

Seventy-six subjects were studied. They were six normal, twenty-one impaired glucose tolerant and forty-nine untreated diabetic subjects. The mean age of the 50 males was 46.5 ± 1.9 yrs (M ±
SEM) and that of 26 females 48.3 ± 3.1 yrs. The body mass index (BMI) of the males was 25.6 ± 0.87 kg/m² and that of the female was 26.5 ± 1.39 kg/m². They all received 75 g or 50 g OGTT for the determination of glucose tolerance. The plasma immunoreactive leptin was determined with an immunoassay kit supplied by Linco Research, Inc. (St. Louis, USA) and the immunoreactive insulin (IRI) was determined by the double-antibody system of Morgan-Lazarow [16]. Both the leptin calibrators (0.5–100 ng/ml) and 125I-labeled leptin were prepared with recombinant human leptin and other details have already been reported [7]. Three amounts of leptin (2.85, 5.7 and 14.3 ng) were added to two plasma samples and the leptin concentrations were determined in two duplicate analyses. Recovery ranged from 95.7 to 105%. Recoveries of leptin in two human plasma specimens (initial concentrations 22.69, and 14.69 ng/ml) serially diluted 2 and 4-fold ranged from 109 to 110%, showing a slightly higher dilution effect. Within- and between-assay variation was assessed by repeated analysis of two plasma samples containing 0.9–2.96 ng/ml leptin. CVs ranged from 1.44 to 2.44% within runs and from 4.0 to 5.1% between runs. The recovery of plasma leptin even after 5 repeated freezings and thawings was 104% in two plasma samples (88.6 and 120%).

Statistical analysis

Data are shown as the mean ± SEM. Statistical analysis was carried out by the non-parametric Mann-Whitney U-test. Paired analysis by Student’s t-test was also performed. A probability of 0.05 was considered to indicate significance.

Results

Plasma leptin concentrations and BMI

Fasting plasma leptin concentrations were measured in normal, impaired and diabetic subjects (Fig. 1). Plasma leptin concentrations measured in samples from fasting subjects correlated well with the subject’s BMI values (r=0.688, P<0.01). The distribution of leptin values vs. BMI for the combined study group clearly represented superimposition of largely distinct distribution for men and women. Regression analysis of leptin values in relation to BMI separated by gender yielded a higher slope for women than for men (slope for females 0.526x – 6.76 (r=0.605, n=24); men, 0.458x – 7.38 (r=0.788, n=48).

Fig. 1. Correlation of plasma leptin concentration with BMI for women (■) and men (●). Women, 0.526x – 6.76 (r=0.605, n=24); men, 0.458x – 7.38 (r=0.788, n=48).

Fig. 2. Comparison of plasma leptin levels in males and females. The number in bracket shows patient’s number. Significant difference was found only in the group with BMI 25–30.
than in men at all levels of BMI, especially high in the BMI 25–30 kg/m² range (P<0.002, males: 3.14 ± 0.31, females: 10.66 ± 2.86 ng/ml). In BMI less than 25 kg/m² they were 2.24 ± 0.25 in males and 3.01 ± 0.39 ng/ml in females (P<0.054, not significant difference) and in BMI higher than 30 kg/m² their levels were 8.98 ± 1.5 in males and 11.47 ± 2.2 ng/ml (P<0.23, not significant difference) in females.

Plasma leptin concentrations and diabetes

Plasma leptin levels were compared in the untreated subjects with different fasting plasma glucose levels including diabetes mellitus. They were all subjected to a 50 g OGTT test. The subjects were divided into three groups according to the fasting plasma glucose level: 140<, 140–200 and >200 mg/dl. All of the BMIs in these subjects were less than 25 kg/m² since the leptin levels varied greatly in cases with a BMI higher than 25 kg/m², as shown in Fig. 2, and each group contained the same number of males and females in order to avoid any effect on the leptin levels. The plasma responses of glucose and IRI are shown in Fig. 3. As already reported in many publications, plasma responses of IRI decreased reciprocally to plasma glucose level. On the other hand, plasma levels of leptin were similar at any time during OGTT in these three groups (Fig. 4). There was no significant change in plasma leptin levels after OGTT test in these number of cases. Plasma leptin concentrations in OGTT and breakfast tests were measured (Fig. 5). The upper figure shows the results of the breakfast test and the lower one those of the 50 g OGTT. In the breakfast test the subjects happened to be obese people, and therefore had a higher leptin level than in OGTT. In both groups the plasma leptin concentration decreased significantly during these loading tests. In 50 g OGTT the plasma leptin level in the fasting state was 2.74 ± 0.31 ng/ml, then the value fell significantly to 2.36 ± 0.24 ng/ml after 2 h (P<0.001). Similarly in the breakfast test the plasma leptin concentration decreased from 5.56 ± 1.15 ng/ml at fasting to 5.09 ± 1.21 ng/ml at 2 h (P<0.05).

The relation between fasting plasma IRI and IRL was investigated. The cases with fasting plasma glucose less than 140 mg/dl were selected since the subjects with fasting plasma glucose more than 140 mg/dl are real diabetics and low insulin responders, and they were all males in this case. As shown in Fig. 6, a significant correlation was found between them (r=0.613, P<0.001), but when the same statistics was done in the subjects with a BMI less than 30 kg/m² in order to avoid the influence of obesity, no significant relation was present (r=−0.616, n=8).
The RIA kit for plasma human leptin provided accurate and precise analysis of the recently discovered hormone. Both the lower detection limit (0.5 ng/ml) and the dynamic range of the assay proved to be optimal. Maffei et al. [5] found plasma leptin concentrations ranging between 1 and 200 ng/ml in a patient population that included obese subjects (BMI up to 62 kg/m²). Leptin concentrations reported by Considine et al. [4] were 7.5 ± 9.3 ng/ml in normal-weight subjects and 31.3 ± 24.1 ng/ml in obese subjects, and those by Zhongmin et al. [7] were 3.84 ± 1.79 ng/ml in males (BMI between 18 and 25 kg/m²) and 7.36 ± 3.73 ng/ml in females, respectively. In our study plasma leptin concentrations measured in samples from fasting subjects correlated well with their BMI and in each of three stages the leptin level was higher in females than in males, especially in BMIs between 25 and 30 kg/m². The reason why the plasma leptin concentrations are higher in females than in males is not known [7, 8, 15, 17]. Rosenbaum et al. [15] mentioned that leptin was significantly higher in pre- and post-menopausal females than in males, even when leptin was corrected for differences in body composition (pre-menopausal females > post-menopausal females > males). Considine et al. [4] reported that gender had no significant effect on the human adipocyte content of mRNA, while Lonnqvist et al. [18] found higher ob mRNA content in adipocytes from obese females.
females, but not in non-obese females compared to obese and non-obese males, respectively. Leptin was reported to be better correlated with absolute fat mass than BMI or percentage body fat at usual body weight [15] and the fat mass is richer in females than in males. More studies from these viewpoints will therefore be necessary to solve this problem.

The relationship between the severity of diabetes and the plasma leptin level was studied. In consideration of the influence of both gender and body weight on the leptin level, the same numbers of males and females with a BMI less than 25 were selected. As shown in this study the severity of diabetes had no influence on the plasma leptin levels. Segal et al. [6] reported that marked alterations in plasma glucose and insulin concentrations induced by glucose and tolbutamide injection did not cause any change in plasma leptin levels. Moreover, the plasma leptin level decreased significantly during the OGTT. This decrease was found also not only during the breakfast test but also during a long fast until lunch (results not shown). Similar findings were reported previously [23]. Such a decrease was thought to be a diurnal variation in plasma leptin. Saladin et al. [20] showed that rat adipose tissue ob mRNA levels were lowest during the light circle, increasing soon after the rats start eating, reached the maximum around 0400 h and thereafter ob mRNA steadily decreased, reaching the minimum in the afternoon. Although insulin increased plasma leptin concentrations in normal subjects and patients with NIDDM [21], the plasma level of insulin caused by infused insulin was higher than that after OGTT or breakfast test (480 fmol/ml vs. 246 fmol/ml in our OGTT) and stimulated insulin by OGTT or acute insulin administration was reported not to affect the plasma leptin level [22]. Sinha et al. [19] found that the plasma leptin level is highest between midnight and the early morning and lowest from noon to mid-afternoon. In this study a significant correlation was found between fasting plasma IRI and leptin levels in male NIDDM patients with fasting plasma glucose less than 140 mg/ml, but it disappeared after adjusting for obesity. In Western Samoans serum leptin concentrations were strongly correlated with serum insulin concentrations even after adjusting for obesity in both sexes [8], and a possible role in insulin resistance or hyperinsulinemia was suggested. These differences might be due to the ethnic differences as seen in the pathogenesis of NIDDM.

Plasma leptin is considered to be regulated chiefly by an adipose tissue-hypothalamus relationship, but another mechanism including sex hormone, corticosteroid [24] or other unknown factors would be associated with it. Further investigation will be necessary for its clarification.

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References


