Leptin levels in non-obese and obese children and young adults with type 1 diabetes mellitus

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Abstract

Objective: To evaluate serum leptin levels in children and young adults with type 1 (insulin-dependent) diabetes mellitus and to investigate whether they are different in prepuberty, puberty and young adulthood.

Design: Three groups of diabetics (prepubertal, pubertal and young adults) subdivided into obese and non-obese were studied. Three groups of healthy subjects matched for sex, age and body mass index served as controls.

Results: Diabetic patients had serum leptin concentrations similar to those of controls in all three groups. A small non-significant increase in leptin from the prepubertal to the young adult age group for both diabetics and controls was found. A significant association of serum leptin level with body mass index ($P < 0.001$), female sex ($P < 0.001$) and age ($P < 0.01$) in both the diabetic and control group was present. Insulin-dependent diabetes was not associated with higher leptin concentration.

Conclusions: Serum leptin concentrations are similar in diabetic patients and healthy controls. The association between obesity and leptin concentration was similar in the diabetic and non-diabetic subjects. Type 1 diabetes mellitus does not modify serum leptin concentration.

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Introduction

Identification of the obese gene and its protein product, leptin, has increased our understanding of the pathophysiology of obesity (1, 2). Recently, it has been demonstrated that changes in blood glucose precede changes in body weight, suggesting that leptin may have a direct effect on insulin action (3). Furthermore, insulin may also have a direct effect on leptin production.

Studies performed in whole rats and in primary rat adipocytes demonstrate that insulin directly regulates *ob* gene expression (4). These data suggest that there are several relevant interactions between insulin and leptin. In fact, serum leptin concentrations in humans are positively associated with body fat percentage, body mass index (BMI) and serum insulin concentration (5–8). Moreover, recently, Tuominen *et al.* (5) demonstrated that leptin levels were higher in subjects with insulin-dependent diabetes mellitus (IDDM) and remained unchanged during hyperinsulinemia in a 240 min euglycemic–hyperinsulinemic clamp.

In spite of many studies (5–8) on serum leptin levels in non-obese and obese subjects, very few studies on adult diabetic patients (5, 9–11) have been reported

and only one on diabetic children (12). In the present study, we measured leptin levels in a group of non-obese and obese diabetic adolescents and young adults; the aims of the study were: (1) to compare the leptin levels of diabetic patients with those of sex-, age- and BMI-matched healthy (non-obese and obese) controls; (2) to determine if leptin levels in diabetics are different in prepuberty, puberty and young adulthood.

Subjects and methods

Three groups of children, adolescents and young adults with type 1 diabetes mellitus (IDDM), who were carefully matched on the basis of BMI, pubertal stage, duration of disease, metabolic control and insulin requirement, participated in the study. Three groups of healthy children, adolescents and young adults without any metabolic and endocrine diseases served as controls. The pertinent data of the three groups of diabetics and controls are shown in Table 1.

All groups (prepubertal, pubertal and young adults) were subdivided into two subgroups: obese and non-obese.

Tanner stages of the pubertal subjects were as follows: diabetics: non-obese, Stage II = 3/3 (M/F),

Table 1 Pertinent data of diabetic patients and controls. Values are mean $\pm$ S.E.

	Prepubertals						Pubertals						Young adults					
	Diabetics			Controls			Diabetics			Controls			Diabetics			Controls		
	Non-obese	Obese	10/10	Non-obese	Obese	10/10	Non-obese	Obese	9/11	Non-obese	Obese	9/11	Non-obese	Obese	17/8	Non-obese	Obese	17/8
Sex (M/F)	10/10	10/10	10/10	10/10	10/10	10/10	10/10	9/11	9/11	14.7 $\pm$ 1.9	14.6 $\pm$ 2.1	14.9 $\pm$ 2.2	14.9 $\pm$ 2.3	18.1 $\pm$ 3.1	18.9 $\pm$ 3.7	18.7 $\pm$ 3.5	18.9 $\pm$ 3.9	17/8
Age (years)	8.9 $\pm$ 1.6	9.6 $\pm$ 1.7	9.4 $\pm$ 1.9	9.0 $\pm$ 1.9	9.0 $\pm$ 1.9	9.0 $\pm$ 1.9	9.0 $\pm$ 1.9	9.0 $\pm$ 1.9	9.0 $\pm$ 1.9	22.1 $\pm$ 0.9	33.7 $\pm$ 4.1*	22.7 $\pm$ 0.9	34.9 $\pm$ 4.0*	22.7 $\pm$ 0.8	35.9 $\pm$ 1.1*	22.9 $\pm$ 0.9	35.1 $\pm$ 1.2*	17/8
BMI (kg/m ²)	17.1 $\pm$ 0.9	31.1 $\pm$ 1.4*	18.1 $\pm$ 0.8	33.1 $\pm$ 1.4*	33.1 $\pm$ 1.4*	33.1 $\pm$ 1.4*	33.1 $\pm$ 1.4*	33.1 $\pm$ 1.4*	33.1 $\pm$ 1.4*	10.9 $\pm$ 2.1	11.7 $\pm$ 2.4	10.9 $\pm$ 2.1	11.7 $\pm$ 2.4	10.7 $\pm$ 2.9	10.9 $\pm$ 3.0	10.7 $\pm$ 2.9	10.9 $\pm$ 3.0	17/8
Duration of disease (years)	5.4 $\pm$ 1.8	5.6 $\pm$ 1.9	5.6 $\pm$ 1.9	5.6 $\pm$ 1.9	5.6 $\pm$ 1.9	5.6 $\pm$ 1.9	5.6 $\pm$ 1.9	5.6 $\pm$ 1.9	5.6 $\pm$ 1.9	8.4 $\pm$ 1.7	8.9 $\pm$ 2.0	8.4 $\pm$ 1.7	8.9 $\pm$ 2.0	8.1 $\pm$ 1.5	8.4 $\pm$ 1.7	8.1 $\pm$ 1.5	8.4 $\pm$ 1.7	17/8
HbA _{1c} (%)	7.2 $\pm$ 1.0	7.0 $\pm$ 1.4	7.0 $\pm$ 1.4	7.0 $\pm$ 1.4	7.0 $\pm$ 1.4	7.0 $\pm$ 1.4	7.0 $\pm$ 1.4	7.0 $\pm$ 1.4	7.0 $\pm$ 1.4	1.4 $\pm$ 0.7	1.3 $\pm$ 0.6	1.4 $\pm$ 0.7	1.3 $\pm$ 0.6	1.0 $\pm$ 0.5	1.1 $\pm$ 0.7	1.0 $\pm$ 0.5	1.1 $\pm$ 0.7	17/8
Insulin requirement (U/kg)	0.8 $\pm$ 0.2	0.8 $\pm$ 0.3	0.8 $\pm$ 0.3	0.8 $\pm$ 0.3	0.8 $\pm$ 0.3	0.8 $\pm$ 0.3	0.8 $\pm$ 0.3	0.8 $\pm$ 0.3	0.8 $\pm$ 0.3	8.7 $\pm$ 1.5	36.1 $\pm$ 3.9*	8.7 $\pm$ 1.5	36.1 $\pm$ 3.9*	9.9 $\pm$ 1.7	37.8 $\pm$ 9.1*	9.7 $\pm$ 1.9	38.1 $\pm$ 10.1*	17/8
Leptin (ng/ml)	6.5 $\pm$ 1.1	28.7 $\pm$ 2.9*	6.1 $\pm$ 1.2	27.1 $\pm$ 2.0*	27.1 $\pm$ 2.0*	27.1 $\pm$ 2.0*	27.1 $\pm$ 2.0*	27.1 $\pm$ 2.0*	27.1 $\pm$ 2.0*	8.1 $\pm$ 1.2	37.5 $\pm$ 3.5*	8.1 $\pm$ 1.2	37.5 $\pm$ 3.5*	9.9 $\pm$ 1.7	37.8 $\pm$ 9.1*	9.7 $\pm$ 1.9	38.1 $\pm$ 10.1*	17/8

* $P < 0.0001$ obese vs non-obese (Student's t -test).

Stage III = 3/4; Stage IV = 3/4; obese, Stage II = 3/3, Stage III = 3/4; Stage IV = 3/4. The controls (non-obese and obese) were strictly matched for Tanner stage.

We studied only diabetic children who were receiving human insulin and followed a multiple daily injection regimen (three or four injections per day). Patients treated by three injections were instructed in the use of two injections of regular insulin via a pen injector 15 min before breakfast and lunch, plus one injection of a mixture of regular and long-acting insulin via a syringe 15 min before dinner (at 2000 h). The insulin dose distribution was 25% before breakfast, 20% before lunch and 55% before dinner (25% regular and 30% long-acting insulin). Patients treated by four injections received three injections of regular insulin plus the fourth of long-acting insulin at 2200 h. All patients followed an intensive insulin regimen, as suggested by the DCCT (13).

A detailed medical and nutritional history was obtained from each subject, and the food intake of all children studied was analysed by the recall method.

Height and weight were measured while the subjects were fasting and wearing only their undergarments. BMI or the Quetelet index was then calculated as weight (kg) divided by height² (m²), and this was used as an indirect measure of adiposity.

All obese children had a BMI 95th percentile specific for age and sex. Data on the onset of excessive weight gain were obtained from growth curve charts provided by their pediatricians. All children were in good health, taking no medications, and the obese children were not following a weight control program. The Tanner stage of pubic hair, breast or genital development was assessed by physical examination before participation in the study, according to the Tanner classification (14).

Informed consent was obtained from the parents of all children studied, and the study was approved by the ethics committee of the University of Chieti.

Serum leptin levels were measured after an overnight fast, between 07.30 and 08.00 h, before the first insulin administration of the morning; when blood samples were taken, plasma glucose was determined by a glucose oxidase method (Beckman Instruments, Fullerton, CA, USA): serum glucose levels ranged from 4.9 to 6.5 mmol/l. Serum samples for leptin determination were stored at -70°C for an average duration of 5 months and then evaluated in duplicate using a double-antibody RIA (Human Leptin RIA Kit, Linko Research, St Charles, MO, USA), using a polyclonal antibody raised in rabbits against highly purified recombinant human leptin. The limit of sensitivity for the human leptin assay was 0.5 ng/ml, the intra-assay standard coefficient of variation was 10.6% and the interassay coefficient of variation was 6%.

Glycosylated hemoglobin (HbA_{1c}) was measured by HPLC (Bio-Rad Laboratories, Richmond, CA, USA).

All data are presented as means $\pm$ S.E. To compare results of different groups, we used ANOVA (Prophet

Table 2 Correlation analysis between serum leptin levels and the main parameters of the diabetics and controls as a whole.

	Controls		Diabetics	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Male sex	0.07	>0.05	0.11	>0.05
Female sex	0.40	<0.001	0.43	<0.001
Age (years)	0.31	<0.01	0.32	<0.01
BMI (kg/m ²)	0.39	<0.001	0.43	<0.001
Serum glucose (mmol/l)	0.10	>0.05	0.12	>0.05
Insulin dose (U/kg per day)	–	–	0.09	>0.05
Duration of diabetes (years)	–	–	0.08	>0.05
HbA _{1c} (%)	–	–	0.17	>0.05

Statistical Package, BBN Software). Single within-group comparisons were made by a two-tailed unpaired Student's *t*-test. Multiple stepwise regression analysis was used to identify factors exerting independent influences on serum leptin concentrations. Tests were considered significant if a *P* value of 0.05 or less was found.

Results

In the three age groups of subjects studied, diabetic patients showed no significant differences in leptin concentration from the non-diabetic control group matched for age, sex and BMI (Table 1). A non-significant increase in leptin concentration from pre-puberty to young adulthood was observed in both diabetics and controls (obese and normal weight); this increase was more evident in girls.

When we evaluated serum leptin levels in diabetics and controls as a whole and compared male and female subjects (non-obese and obese), females had significantly (*P* < 0.001) higher leptin concentrations than males, in both groups. The correlation between BMI and leptin concentration was similar in each group (*P* < 0.001).

In both diabetic and non-diabetic subjects a multiple regression analysis of the total study sample with serum leptin as a dependent variable demonstrated significant associations with female sex, BMI and age, while no correlation was found for the insulin dose per kg body weight, duration of diabetes or HbA_{1c} (in diabetics). However, the association of leptin concentration with age was due to the age dependency of BMI. The correlations between serum leptin level and the main parameters of the diabetics and controls are shown in Table 2.

Discussion

This is the first study to analyse plasma leptin levels in diabetic children and young adults (non-obese and obese). It shows that diabetic children with good

metabolic control have similar plasma leptin levels to those of the control population, and there are no differences from the controls in both the prepubertal and pubertal periods. We found no significant correlation between blood glucose level in either controls or diabetics (obese and non-obese). This suggests that variations in blood glucose are not an active signal for leptin secretion, in agreement with other physiological studies (11, 12, 15).

The study also shows that increased leptin levels are a feature of juvenile obesity in both diabetic children and young adults: the association between obesity (as measured by BMI) and leptin concentration was similar in diabetic and non-diabetic subjects. The relationship between serum leptin and BMI in our obese subjects is similar to that observed in adults with stable obesity (4, 7, 15–20). In agreement with other reports (5, 9–11), our study shows that leptin concentrations in diabetic patients are similar to those in non-diabetic subjects. On the other hand, Tuominen *et al.* (5) found fasting plasma leptin levels to be higher in patients with IDDM than in controls. These authors suggest that this difference may be due to chronically high insulin concentrations in diabetic patients; it must be remembered that the duration of the disease in these patients was longer than that of our diabetics and this may help to explain the difference between our data and those of Tuominen *et al.* (5).

Some authors have shown an inverse correlation of leptin level with Tanner stage, independent of body fat (19, 21). Our data show a slight increase in leptin level during puberty. Although this increase did not reach statistical significance, these data are in agreement with recent observations (9, 12).

Our study shows that both prepubertal and pubertal obese children have higher leptin levels than non-obese diabetic controls. The differences in leptin concentrations are similar in diabetic and non-diabetic subjects. These data are in agreement with previous reports demonstrating hyperleptinemia in obese people (12, 14, 22). We also confirmed in children the correlation between leptin level and BMI, female sex and age, as reported for adult non-obese populations (1, 6, 7). Although there was a large overlap in the range of serum leptin concentrations between boys and girls, the mean values for the whole group of males was significantly lower than for the females, in diabetics and in controls. In agreement with recent observations, we suggest that the gender differences in serum leptin concentrations, previously described in adult (15, 23, 24) and pediatric (12) healthy subjects, is already evident in young patients with IDDM.

In the present study, obese female adolescents with diabetes mellitus exhibited higher serum leptin concentrations than boys despite comparable BMIs as a relative measure of obesity. This is in agreement with recent studies in healthy children and in adults with and without type 2 diabetes (9, 16).

It is important to underline that metabolic control of our diabetic patients was quite stable and satisfactory; in fact, they were followed carefully at the Center for Childhood Diabetes every 2 months after intensive insulin treatment, and we aimed to reach the best possible HbA_{1c} levels as recommended by the DCCT study (13). This could explain the lack of difference between diabetics and control subjects. In accordance with this hypothesis, it is well known that, in diabetic patients with poor metabolic control, abnormalities of thyroid hormones, growth hormone, sex steroids, growth hormone-binding proteins and other parameters are frequently detected (25–29); in contrast, in diabetic patients with improved metabolic control, normalization of the above abnormalities is generally obtained (30–33). It is possible that in diabetic children (obese and non-obese) with poor metabolic control significant differences in leptin concentrations could have been found.

The elevated leptin levels found in both obese children and adult subjects may suggest a decreased sensitivity to leptin, as reported in the *db/db* rodent model of obesity (3). Alternatively, the high leptin levels may simply reflect the enlarged adipose tissue mass.

Our data demonstrate that diabetes mellitus does not affect leptin levels. In fact, we did not find any significant correlation between daily insulin dose and leptin concentrations. This lack of correlation, which is in agreement with the results of other studies carried out in adults with IDDM (5, 24), may be due to different mechanisms. Although insulin has been shown to have an acute stimulatory effect on serum leptin concentration in both rats (4) and patients with non-insulin-dependent diabetes (9) and a pronounced effect on the regulation of the *ob* gene in adipose cells (34), serum leptin levels did not appear to depend on the amount of exogenous insulin substitution. Moreover, although it has been suggested that insulin could have a direct effect on leptin production and/or leptin expression (33), studies performed in whole rats and in primary rat adipocytes have not always demonstrated a clear insulin effect on *ob* gene expression in streptozotocin-diabetic rats (8, 35). It has recently been documented that euglycemic clamp with steady-state hyperinsulinemia from 80 to 1200 μ U/ml maintained for up to 5 h did not increase serum leptin (8). Accordingly, in humans, postprandial changes in insulin secretion are not accompanied by parallel changes in leptin levels, although the latter show a definite nocturnal rise in lean, obese and diabetic subjects (10, 22, 36). Moreover, in the study of Muscelli *et al.* (36), the acute administration of insulin in physiological amounts and under euglycemic conditions did not change circulating leptin levels in lean or obese subjects. Also the data of Larsson *et al.* (20) confirm that it is unlikely that insulin increases plasma leptin, even at high circulating levels. More recently, Tuominen *et al.* (5) carried out an interesting study on the effect of a 4 h

euglycemic–hyperinsulinemic clamp in 15 men with IDDM and demonstrated that the fasting plasma leptin levels were higher than controls and remained unchanged during hyperinsulinemia; these authors suggest that patients with IDDM are resistant to insulin action on leptin synthesis. We cannot add our data about this because the aim of our study was to evaluate only the leptin levels in diabetic children who received therapeutic doses of insulin, but our lack of correlation between leptin levels and insulin daily dosage are in partial agreement with these reports. Finally, it is important to remember that there is no strict correlation between insulin dose and plasma insulin levels in diabetic children, because many different factors contribute to the modification of the plasma levels (route of administration, variability in absorption, kinetics of circulation, etc.) (37).

In conclusion, IDDM in non-obese patients with satisfactory metabolic control seems to be associated with normal serum leptin concentrations; obese subjects with IDDM show high leptin levels similar to those of obese healthy subjects. In diabetic children and young adults the concentrations of leptin are similar to those of the non-diabetic population.

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