CLINICAL STUDY

Increased serum soluble leptin receptor levels in children and adolescents with type 1 diabetes mellitus

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Abstract

Objective: We investigated whether or not serum levels of the soluble leptin receptor (sOB-R) and leptin are related to anthropometric and metabolic changes during pubertal development of children and adolescents with type 1 diabetes mellitus.

Design and methods: Blood levels of sOB-R, leptin and HbA1c, as well as body-mass index (BMI), diabetes duration and daily insulin doses, were determined in 212 (97 girls; 115 boys) children with type 1 diabetes mellitus and compared with the sOB-R serum levels in 526 healthy children and adolescents.

Results: sOB-R serum levels and parallel values of the molar ratio between sOB-R and leptin were significantly higher in children with diabetes than in normal children ($P < 0.05$) in almost all investigated Tanner stages. Furthermore, in the entire group of patients, we demonstrated statistically significant correlations ($P < 0.02$) between sOB-R and the duration of diabetes ($r = 0.30$), HbA1c levels ($r = 0.32$) and the insulin dose ($r = 0.18$). Multiple-regression analysis revealed that HbA1c (12.4%), height (7.9%) and duration of diabetes (8.7%) contributed to 29% variance of sOB-R in diabetic children.

Conclusions: Our data suggest that poor glycemic control in diabetes may lead to increased serum levels of sOB-R. This regulation of sOB-R appears to be independent of leptin, but may have an impact on leptin action. The consequently developing molar excess of sOB-R related to leptin could reduce leptin sensitivity and may, therefore, influence leptin-related anthropometric and metabolic abnormalities.

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Introduction

The *ob* gene product, leptin (1), is mainly secreted by the adipose tissue and acts predominantly by binding to hypothalamic cells. The biologic effects of leptin include suppression of food intake and increase of energy expenditure, and, consequently, the regulation of body weight (2–5). Several studies have suggested that leptin also influences sexual progression through puberty (6–8). Leptin serum levels are related to body fat mass, body-mass index (BMI), gender and pubertal stage. In both sexes, leptin serum concentrations are increasing before the onset of puberty. In girls, leptin rises steadily until the end of sexual maturation. In boys, leptin levels decline in puberty after an initial rise later. This particular pattern can be partially explained by the effect of steroid hormones such as testosterone upon leptin expression (6, 9–12). Impaired growth and pubertal delay have been observed in children with diabetes (13–15). These children are also more inclined to develop obesity, even if they are treated

with modern insulin therapy. The mechanism of weight gain in subjects with type 1 diabetes mellitus (DM) has not yet been examined; however, there are suggestions of an association between weight gain and hyperinsulinemia, which is reflected by correlations between the insulin dose and the frequency of insulin injections (16). Moreover, a recent study revealed that increased levels of leptin, indicating leptin resistance, may explain weight gain, especially in girls with diabetes (17). However, the leptin data of patients with diabetes compared with healthy controls are controversial during pubertal development. There is no unique picture of increased serum leptin levels (18–22). For better understanding of the correlation between the leptin axis and anthropometric as well as metabolic abnormalities of children with diabetes, we assessed the role of the soluble leptin receptor (sOB-R) as a potential modulator of leptin actions. The function of sOB-R has not been fully elucidated so far (23, 24). Like other hormone binding proteins of the cytokine family, sOB-R could prolong the half-life and, therefore, the bioavailability of its ligand

leptin in blood. However, as a molar excess of sOB-R over leptin suppressed leptin bioactivity *in vitro*, the interaction between sOB-R and leptin may be a causal factor in the pathogenesis of weight gain *in vivo* (25). For further examination of this issue, we measured serum sOB-R and leptin levels in a large cohort of children and adolescents with diabetes, and we compared these data with sOB-R and leptin levels of healthy controls.

Subjects and methods

Patients and controls

A total of 212 patients (97 girls and 115 boys, age range: 2.8–19.3 years; median: 12.7 years), diagnosed with type 1 DM by the criteria of the American Diabetes Association (ADA) (26), were included in our study (Table 1). Eighty-two were patients at the Hospital for Children and Adolescents, University of Leipzig, Leipzig, Germany, and 130 patients came from the Children's Hospital of Chemnitz, Germany. All patients had diabetes duration of more than 5 months and were receiving exogenous insulin, administered by 3–5 subcutaneous injections daily. They demonstrated only in very rare cases (<1%) clinical symptoms or signs of neuropathy, nephropathy, retinopathy or severe metabolic decompensation such as ketoacidosis. None of them had additional chronic or acute illness. Apart from insulin, no other permanent medication was used. None of the patients paid special attention to diet apart from the usual diabetes-related nutrition recommendations for a carbohydrate-balanced, lipid-poor and normal-protein diet. Reference values from children with comparable age and pubertal stage were taken from a previous study published by our group ($n = 526$) (24). All patients or their parents gave written, informed consent to participate in the study. The University of Leipzig ethics committee approved this study.

Methods

Clinical examination In all patients, clinical and anthropometric data were recorded on a structured data-sheet during routine examination. Weight was measured with light underwear to the nearest 0.1 kg on a calibrated balance-beam scale. Height was measured to the nearest 0.1 cm with a standardized measuring device (Keller system 2). BMI was calculated as body weight (kg) divided by height (m) squared. BMI S.D. Scores (SDS) levels were calculated according to the German Reference Data of Kromeyer–Hausschild from 2002 (27). Pubertal development was assessed according to Tanner (28). The whole set of data is demonstrated in Table 1.

Biochemical analysis Blood samples for leptin, sOB-R and HbA1c were collected from a cubital vein at 0800–1200 h. Samples were centrifuged at 4000 U for 20 min and stored at -25°C until analysis. HbA1c was measured by the Tina-quant technique (Roche Diagnostics Mannheim, Germany).

Serum parameter of the leptin axis

Leptin was measured by a specific 'in-house' radioimmunoassay (RIA) (29). The sensitivity of the RIA was 0.2 ng/ml (2 S.D. of the leptin-free standard matrix, $n = 12$). The RIA proved to be precise, as its intra-assay and interassay coefficients of variation were both below 12.5% in the range 1–8 ng/ml leptin.

sOB-R was measured with a newly developed in-house ligand-immunofunctional assay (LIFA) (24). The lowest detectable sOB-R concentration in the assay was calculated to be less than 2 ng per ml. The intra-assay and interassay coefficients of variation for two control samples were lower than 11.7% ($n = 10$). The molar sOB-R/leptin ratio was calculated by the measured levels of sOB-R divided by the leptin values and multiplied by 0.123, the ratio between the molecular weights of both parameters (25).

Table 1 Anthropometric, biochemical and clinical data characterizing the patients with type 1 diabetes mellitus. Data were expressed as mean $\pm$ S.D. or median and quartiles (BMI).

Tanner stage	Sex	n	Age (years)	BMI SDS	HbA1c (%)	Insulin dose (mU/kg)	Duration of diabetes (years)
1	M	44	8.58 $\pm$ 2.66	0.07 (–0.49/0.59)	8.05 $\pm$ 1.75	0.63 $\pm$ 0.23	3.24 $\pm$ 2.47
	F	26	9.65 $\pm$ 3.03	–0.17 (–0.83/0.21)	7.82 $\pm$ 1.52	0.80 $\pm$ 0.28	3.78 $\pm$ 2.01
2	M	14	13.3 $\pm$ 1.45	–0.25 (–0.65/0.70)	9.46 $\pm$ 4.46	0.79 $\pm$ 0.27	7.53 $\pm$ 4.08
	F	6	11.6 $\pm$ 0.97	0.13 (–0.15/0.76)	6.99 $\pm$ 1.26	0.86 $\pm$ 0.15	3.84 $\pm$ 2.83
3	M	13	13.7 $\pm$ 0.96	0.15 (0.04/0.43)	8.79 $\pm$ 2.17	0.75 $\pm$ 0.16	3.89 $\pm$ 2.50
	F	9	12.3 $\pm$ 1.48	–0.05 (–0.44/0.26)	9.69 $\pm$ 3.30	0.72 $\pm$ 0.21	5.93 $\pm$ 3.91
4	M	17	15.0 $\pm$ 1.47	0.13 (–0.17/1.0)	9.18 $\pm$ 1.85	0.87 $\pm$ 0.19	7.18 $\pm$ 3.61
	F	17	14.2 $\pm$ 1.45	0.29 (–0.23/0.58)	9.29 $\pm$ 1.65	0.75 $\pm$ 0.29	4.51 $\pm$ 2.85
5	M	26	16.7 $\pm$ 1.22	0.15 (0.02/1.24)	8.58 $\pm$ 1.93	0.74 $\pm$ 0.20	5.12 $\pm$ 2.16
	F	38	15.8 $\pm$ 1.55	0.68 (0.24/1.20)	8.98 $\pm$ 2.73	0.76 $\pm$ 0.26	5.06 $\pm$ 3.85

M, boys; F, girls.

Statistical analysis

For statistical analysis, we used the STATISTICA 6.0 software program (StatSoft, Tulsa, USA). As data for BMI SDS, leptin, sOB-R and the sOB-R/leptin ratio were non-normally distributed, nonparametric statistical tests were used. Comparison of BMI SDS in individual Tanner stages was performed by analysis of variance as well as by the post-hoc Mann–Whitney U-test. Adjustment of parameters of the leptin axis for BMI was performed by analysis of covariance.

Results

sOB-R and leptin serum concentrations in children with type 1 DM during puberty

A significant inverse relationship between sOB-R and Tanner stage was detectable in both genders ($r = -0.30$, $P < 0.01$ in boys; $r = -0.26$, $P = 0.01$ in girls). The changes of sOB-R between the individual stages are shown in Table 2. However, serum concentrations of sOB-R did not reveal gender dependence in patients with diabetes as calculated by correlation analysis according to Spearman ($P = 0.97$). Furthermore, in the whole group of patients, we demonstrated statistically significant correlations ($P < 0.02$) between sOB-R and the duration of diabetes ($r = 0.30$), HbA1c levels ($r = 0.32$) and insulin dose ($r = 0.18$). In the subgroup of pubertal patients ($n = 140$, Tanner stage of >1), these coefficients of correlation increased to $r = 0.43$ (diabetes duration); $r = 0.46$ (HbA1c level) (Fig. 1) and $r = 0.28$ (insulin dose), with a statistical significance of $P < 0.001$ in all comparisons. Multiple-regression analysis revealed that HbA1c (12.4%),

height (7.9%) and duration of diabetes (8.7%) contributed to 29% variance of sOB-R in the diabetic children. Interestingly, leptin was not a significant predictor of sOB-R. Leptin levels were significantly higher in girls with diabetes than in boys ($P < 0.0001$), with the exception of patients with Tanner stage 2. In girls, serum leptin concentrations increased during pubertal development (Table 2). In boys, leptin levels increased only until Tanner stage 2 and decreased until the end of puberty (Table 2). Therefore, the relationship between leptin and Tanner stages was distinctly different between girls ($r = 0.59$; $P < 0.001$) and boys ($r = -0.20$, $P < 0.05$). Consequently, compared with boys ($r = 0.28$; $P < 0.01$), serum leptin levels demonstrated a stronger correlation with BMI SDS in girls ($r = 0.60$, $P < 0.001$). In our cohort of patients, only HbA1c ($r = 0.15$, $P = 0.03$) was significantly correlated to leptin levels, but not the duration of diabetes or the individual insulin dose. In the multiple-regression analysis including age, BMI, height, Tanner stage, sOB-R and our diabetes-associated variables, only BMI, in boys, and BMI and Tanner stage, in girls, were significant independent predictors of the variation of leptin levels.

Comparison of sOB-R and leptin between healthy children and children with diabetes

To investigate the differences in sOB-R, leptin and the sOB-R/leptin ratio between healthy children and children with diabetes, we compared these parameters of our cohort with 526 healthy children aged 6–20 years (Table 2 and Figs 2–4). In almost all Tanner stages, sOB-R serum levels (Fig. 2) and sOB-R/leptin ratio data (Fig. 4) were significantly higher in children

Table 2 Data of the leptin axis (median, quartiles) from patients with type 1 diabetes mellitus (DM) by Tanner stage (TS) and compared to levels of healthy controls. Significant differences between both groups were demonstrated as * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

TS (sex)	Group	n	sOB-R (ng/ml)	Leptin (ng/ml)	sOB-R/leptin ratio
1 (boys)	DM	44	59.3 (50.3/76.8)	3.00 (2.18/3.59)*	2.68 (1.91/3.30)
	Control	58	57.6 (44.4/69.3)	1.96 (1.31/3.56)	3.39 (1.69/6.29)
1 (girls)	DM	26	68.4 (58.8/78.6)***	3.99 (2.93/5.42)	1.89 (1.53/2.92)
	Control	53	53.7 (48.0/60.6)	3.35 (1.72/5.98)	1.95 (1.24/4.10)
2 (boys)	DM	14	55.0 (47.3/75.5)**	4.36 (2.19/5.57)	2.08 (1.28/2.66)**
	Control	49	42.0 (33.9/49.8)	4.96 (3.68/10.9)	1.15 (0.42/1.48)
2 (girls)	DM	6	42.6 (33.5/43.7)	2.95 (2.16/10.1)*	1.76 (0.53/3.11)
	Control	49	43.2 (36.6/50.1)	6.84 (4.81/11.9)	0.66 (0.42/1.21)
3 (boys)	DM	13	48.2 (38.4/61.5)**	2.15 (1.92/3.65)*	2.10 (1.66/3.12)**
	Control	49	33.3 (27.0/39.0)	3.58 (2.44/8.56)	1.08 (0.41/1.91)
3 (girls)	DM	9	57.0 (39.9/72.8)**	3.68 (3.19/8.66)	1.11 (0.68/2.87)*
	Control	50	34.1 (29.1/37.8)	6.77 (4.89/9.40)	0.55 (0.40/0.87)
4 (boys)	DM	17	55.1 (44.2/74.2)***	2.52 (1.64/4.21)	3.08 (2.30/3.80)***
	Control	50	27.8 (24.0/32.7)	2.91 (1.68/4.37)	1.15 (0.68/2.20)
4 (girls)	DM	17	50.9 (41.9/60.8)**	7.33 (6.10/9.83)	0.84 (0.68/1.24)*
	Control	50	37.8 (30.0/41.7)	6.82 (5.60/11.3)	0.58 (0.43/0.88)
5 (boys)	DM	26	40.0 (34.2/54.7)***	2.05 (1.58/3.27)	2.51 (1.49/4.42)***
	Control	56	29.1 (23.0/38.0)	1.87 (1.21/2.77)	2.07 (1.17/3.19)
5 (girls)	DM	38	53.0 (34.6/61.9)***	13.1 (8.45/17.3)	0.50 (0.29/0.93)*
	Control	62	29.4 (24.3/38.7)	9.04 (6.50/14.8)	0.36 (0.22/0.56)

Figure 1 Comparison between levels of sOB-R in serum and HbA1c in blood of pubertal patients with type 1 diabetes mellitus ($n = 140$, Tanner stage of > 1). The coefficient of correlation according to Spearman was calculated as $r = 0.46$ ($P < 0.001$).

with diabetes than in healthy children ($P < 0.05$). In contrast to the group of healthy children, which demonstrated decreasing sOB-R levels and ratio data through puberty, sOB-R levels remained high in children with diabetes throughout this period. The

distinctly increased values of the sOB-R/leptin ratio of children with diabetes was predominantly due to their already mentioned high sOB-R levels, but also to their approximately unchanged leptin levels in comparison with healthy children. Only in Tanner stage 1 did boys with diabetes (Fig. 3A) have significantly higher leptin levels than our controls ($P < 0.05$). In contrast, leptin levels of boys with Tanner stage 3 were significantly lower in the diabetic patients than in healthy subjects ($P = 0.015$). Female type 1 DM patients (Fig. 3B) demonstrated lower leptin values in serum of Tanner stage 2 ($P = 0.02$). All these differences remained significant if leptin levels were adjusted for the BMI values of our subjects.

Discussion

In this study, we show for the first time that important parameters characterizing the metabolic state of type 1 DM, like HbA1c levels, insulin dosage and the duration of diabetes, are correlated with the serum sOB-R level. Therefore, children with poor glycemic control reveal a massive increase in the sOB-R/leptin ratio. The latter parameter reflects the molar excess of sOB-R

Figure 2 sOB-R levels of male (A) and female (B) children and adolescents with type 1 diabetes mellitus compared with the 5th (dotted line), 50th (solid line) and 95th (dotted line) percentiles of healthy children by pubertal stage according to Tanner stage.

Figure 3 Leptin levels of male (A) and female (B) children and adolescents with type 1 diabetes mellitus compared with the 5th (dotted line), 50th (solid line) and 95th (dotted line) percentiles of healthy children by pubertal stage according to Tanner stage.

Figure 4 Data of the ratio between sOB-R and leptin levels of male (A) and female (B) children and adolescents with type 1 diabetes mellitus compared with the 5th (dotted line), 50th (solid line) and 95th (dotted line) percentiles of healthy children by pubertal stage according to Tanner stage.

over leptin in blood, a measure of the leptin action as validated by *in vitro* cell experiments (23). A high level of this ratio may demonstrate reduced leptin bioactivity. Whereas the elevation of sOB-R levels as well as of sOB-R/leptin ratios in diabetes is a new finding of our study, the levels of leptin and the relationships between leptin, BMI and Tanner stage are consistent with the findings of other authors. Thus, the gender-specific differences in the leptin course during puberty can partially be explained by the differences in body composition: girls have a higher percentage of fat mass and boys gain more muscle mass. The latter appears to be caused by increased androgen serum levels, which may by themselves inhibit peripheral leptin secretion (6, 7, 11, 30, 31). Furthermore, the leptin concentrations in our study were mainly comparable to the results of healthy controls, except boys in subgroups with Tanner stages 1 and 3 and girls in the subgroup with Tanner stage 2. We speculate that these differences were due to the relatively low number of female subjects in Tanner stage 2 or were related to the degree of individual insulin treatment: insulinopenia, a lowered insulin sensitivity or compliance problems may lead to decreased levels of leptin

(18, 21). On the other hand, hyperinsulinemia caused by exogenous insulin treatment, concurring with a marginal remaining insulin secretion, especially during the first year after diagnosis of type 1 DM, could be associated with an increase of leptin concentrations (15, 18, 22).

In contrast to sOB-R, no important parameters characterizing the metabolic state of type 1 DM, such as HbA1c levels, insulin dosage and the duration of diabetes, were significant independent predictors for serum leptin levels in the multivariate calculations. Therefore, the metabolic situation may have had only a minor influence on the leptin values of these patients. The finding that the leptin concentrations of our cohort were not essentially different from the control data supports this hypothesis. However, as observed for our healthy subjects (data not shown), BMI and Tanner stage are the major independent predictors also in the diabetic state. The distinctly elevated levels of sOB-R and of the sOB-R/leptin ratio in insulin-treated subjects raised the question of whether sOB-R inhibits leptin bioactivity, leading to leptin resistance and possibly weight gain. Although we cannot definitely answer this question, the direct correlation of sOB-R (but not of leptin) with insulin dose, HbA1c and the duration of diabetes, as measured in our study, suggests that metabolic decompensation may have an indirect (mediated by sOB-R) impact on leptin action in type 1 DM. Leptin, the potential regulator for sOB-R shedding in healthy subjects (32, 33), seems to have no relevant effect on sOB-R secretion in diabetes. A possible explanation for the increase of sOB-R observed in our patients may be the well-known induction of protein kinase C (PKC) activity in the diabetic state. PKC can induce A disintegrin and metalloprotease (ADAM) 17 (34), which is able to shed the extracellular domain of cytokine-like hormone receptors, such as growth hormone-receptor and the OB-R, leading to increased levels of the soluble receptor in our patients. Interestingly, Lewandowski *et al.* (35) have found an increased level of sOB-R in pregnant women with diabetes compared with pregnant nondiabetic women. This observation supports our findings. Otherwise, Diamond *et al.* (36) investigated the leptin-binding activity of teenagers with diabetes (8 girls, 10 boys) without detecting any differences from healthy children. The contradiction between the results of this study and our findings may be explained by the low number of subjects, the lack of consideration of pubertal stage and the use of size-exclusion chromatography to quantify leptin binding, which appears to be a relatively unspecific analytic method for the quantification of sOB-R. The biologic consequences of increased sOB-R levels in diabetes are unclear so far. Due to its binding affinity, which is comparable to the binding affinity of the membrane leptin receptor (23, 37), sOB-R may modulate leptin action in two ways. First, sOB-R could inhibit the binding of leptin to specific membrane

receptors by its direct competition for the ligand. In a recent paper, our group was able to show that a 10-fold molar excess of sOB-R over leptin distinctly suppressed leptin bioactivity *in vitro* (23). An up to 20-fold molar excess has been shown by this study. Thus, we hypothesize that this molar excess of sOB-R contributes to leptin insensitivity, which may support weight gain and a decrease of energy consumption in a catabolic condition such as newly diagnosed DM. The second mechanism would involve complexes of leptin and sOB-R inhibiting the renal elimination of leptin, leading to an increased bioavailability of this hormone. However, the latter mechanism would conflict with the increased weight gain of diabetic children during puberty. In conclusion, the finding of elevated sOB-R levels in children and adolescents with diabetes extends to the understanding of the molecular basis for putative derangement of body weight control in diabetes at a young age. To clarify the mechanism of sOB-R secretion and to assess the detailed pathophysiological effect of the molar excess between sOB-R and leptin in diabetes, more detailed investigations should be made. Most interestingly, the question of whether or not patients with leptin insensitivity have a better chance to overcome a state of metabolic decompensation in DM should be a goal for further clinical studies.

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