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Short communication

Insulin increases serum leptin concentrations in children and adolescents with newly diagnosed Type I diabetes mellitus with and without ketoacidosis

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Abstract

Aims/hypothesis. The aims of this study were to analyse the changes of serum leptin in newly diagnosed children and adolescents with Type I (insulin-dependent) diabetes mellitus after insulin treatment and to examine the possible impact of ketoacidosis on these changes.
Methods. Baseline serum leptin concentrations were measured in 28 newly diagnosed Type I diabetic patients [age 8.75 ± 4.05 years (means ± SD); BMI 15.79 ± 2.47 kg/m^2; HbA_{1c} 11.3 ± 1.9 %] with (n = 18) and without (n = 10) ketoacidosis before commencement of insulin treatment, at the time of diagnosis. Thereafter, during a 4-day course of continuous intravenous insulin injection to gain and maintain euglycaemia, serum leptin concentrations were assessed.
Results. Baseline serum leptin concentrations, adjusted to age, BMI, sex and pubertal stage, differed among these patients. There was, however, an increase of leptin in all subjects from 1.37 ± 0.56 ng/ml (mean ± SD) up to 2.97 ± 1.52 ng/ml by 117 % ($p < 0.0001$) after insulin therapy. On average, peak serum leptin concentration was obtained after 42 h of insulin treatment. Further, there was no difference in the mean increase of serum leptin concentrations in the two groups, namely with and without ketoadicosis, of insulin-dependent diabetic children and adolescents. In addition, there was no correlation between serum leptin concentrations and correction of ketoacidosis during insulin treatment.
Conclusions/interpretation. Insulin increases serum leptin, within 1 day, in children and adolescents with newly diagnosed Type I diabetes. Ketoacidosis does not influence this interaction between insulin and leptin. [Diabetologia (1999) 42: 1067–1070]

Keywords Type I diabetes mellitus, ketoacidosis, fat, leptin, children.

Leptin, the *ob* gene product [1] is predominantly expressed in white adipose tissue and acts as a signalling factor regulating energy balance through specific receptors located in the central nervous system and in peripheral tissues. Serum leptin concentrations in healthy humans are related to body fat mass, body mass index, sex and pubertal stage. Circulating leptin concentrations decrease in response to short-term fasting in both humans and rodents [2–3] and increase after refeeding or insulin treatment [4–5], also in patients with Type II (non-insulin-dependent) diabetes mellitus [6]. Other than this pulsatile diurnal secretory pattern of leptin related to food intake and insulin secretion or insulin-stimulated glucose metabolism, respectively [7], plasma leptin shows a nocturnal increase. Furthermore, changes in leptin concentrations regulate hypothalamic neuropeptide Y (NPY), which is a potent stimulator of food intake, by negative feedback [8].

Bearing in mind the physiological interaction between food intake and insulin secretion, the aim of this study was to analyse the possible impact of insulin concentration on the serum leptin concentration in a group of patients with absent or negligible endo-

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genous insulin production. Therefore, the influence of insulin on serum leptin concentrations in children and adolescents with newly diagnosed Type I diabetes mellitus, with and without ketoacidosis at diagnosis, was investigated. We hypothesised that serum leptin concentrations in these children lacking physiological insulin production should be low at diagnosis and increase within 24 h of insulin treatment. Whether or not ketoacidosis plays a part in the stimulation of leptin secretion was also examined in this study.

Subjects and methods

Subjects and study design. We recruited 28 children and adolescents with newly diagnosed Type I diabetes mellitus [13 girls, 15 boys; age (means ± SD): 8.75 ± 4.05 years, range 1.32–14.37 years; BMI 15.79 ± 2.47 kg/m^2; HbA_{1c} 11.3 ± 1.9 %] for the study. All subjects were of normal weight before diagnosis and did not receive any treatment, especially no insulin. Informed consent was obtained from patients and their parents and investigations done in accordance with ethical principles of the Declaration of Helsinki. History, physical examination and blood tests were taken to establish the diagnosis of Type I diabetes and exclude any other disease before treatment of metabolic disturbances was started as it is usually done in any institution looking after diabetic children.

Insulin regimen. Patients were started on insulin therapy (insulin Infusit U-100, Hoechst Marion Roussel, Zürich, Switzerland) by continous intravenous injection (H-Tron plus V100 pump system, Disetronic, Burgdorf, Switzerland), to decrease the plasma glucose by 5.5–8 mmol/l an hour, until euglycaemia was established. Thereafter, insulin was infused at a rate of 0.02–0.08 IU/kg an hour to maintain plasma glucose concentrations between 4 and 8 mmol/l. Within the first 24 h a quantitative and qualitative diabetes regimen, which was adjusted to age and weight, was started.

Serum leptin concentrations. Blood for baseline serum leptin concentration was drawn before any therapy was initiated, at the time of diagnosis, as well as every morning (0700 to 1000 hours) for the ensuing 4 days during continuous insulin injection. Serum leptin concentrations were determined by radioimmunoassay using a commercially available kit (Mediagnost, Tübingen, Germany). The detection limit of this assay was 0.04 ng/ml. The intra-assay coefficient of variation was lower than 5 % and the inter-assay variation 7.6 %, respectively.

Other methods. Plasma glucose was measured by the glucose oxidase method. Glycated haemoglobin A_{1c} (reference range, 4.1–5.7 %) was quantified by latex immunoagglutination inhibition methodology (DCA 2000 Analyzer, Bayer Corporation, Elkhart, USA) and blood gas analysis done by Radiometer ABL 625, Copenhagen Ind., Denmark. For each patient the body mass index (BMI) was calculated by dividing weight (kg) by the height (m^2). Insulin need per body weight and day (IU/kg per day) was calculated as the mean of requirement during the full second and third day of treatment.

We defined patients as having ketoacidosis, when their initial base excess was more than 2.5 SD below normal [range –2.5 to + 2.3 mmol/l (means ± 2 SD)].

Statistical analysis. For analysis of changes in serum leptin concentration Wilcoxon's signed rank test was used. By multivariate regression the influence of base excess, sex, age, body weight, pubertal stage and HbA_{1c} on leptin were studied. Age at diagnosis, body mass index, leptin, insulin requirement and HbA_{1c} were compared between groups with and without ketoacidosis by Student's *t* test. All data are expressed as means ± SD. Statistical significance was assigned to a value of $p < 0.05$.

Results

The two groups of patients with newly diagnosed Type I diabetes mellitus, with and without ketoacidosis, were not statistically significantly different for age, body mass index, pubertal stage, insulin requirement, HbA_{1c} and baseline serum leptin concentrations. There was a difference in sex distribution, with more girls in the group of patients with ketoacidosis compared to the non-ketoacidotic group (Table 1). Insulin treatment, required to maintain euglycaemia, resulted in an increase of serum leptin concentration by 117 % from baseline 1.37 ± 0.56 ng/ml (means ± SD) up to 2.97 ± 1.52 ng/ml ($p < 0.00001$) at the end of day 3 on intravenous infusion (Fig. 1A). There was no statistically significant difference in mean serum leptin concentrations over time on continuous intravenous insulin injection between patients with and without ketoacidosis (leptin day 0: 1.47 ± 0.57 vs 1.18 ± 0.50; leptin day 1: 2.28 ± 1.35 vs 1.89 ± 1.81; leptin day 2: 3.04 ± 1.78 vs 1.89 ± 1.29; leptin day 3: 3.18 ± 1.76 vs 2.60 ± 0.92 ng/ml). Furthermore, although the baseline leptin concentrations were either low, normal or high compared with the normal concentrations adjusted for age, BMI, sex and pubertal stage, there was a constant increase of serum leptin concentration in all subjects after insulin treatment. Leptin concentrations increased from low to normal in four, from low to high in two, from normal to high in seven and from high to even higher in three diabetic children. In 12 subjects leptin increased but within the normal range. The peak in the serum leptin concentration was obtained during the third day of insulin treatment (on average after 42 h).

Base excess was restored to normal in patients with ketoacidosis within 33.1 ± 9.8 h (Fig. 1B).

In multivariate regression analysis no relation was found between the increase in serum leptin concentrations and the correction of ketoacidosis ($p = 0.846$) nor the insulin requirement ($p = 0.841$) in each subject. In contrast, leptin increment, weight ($p = 0.055$) and sex ($p = 0.079$) tended to show positive correlation.

Discussion

In our study we have shown that insulin is a potent stimulator of serum leptin in newly diagnosed insulin-dependent diabetes mellitus with and without ke-

Table 1. Characteristics of patients at diagnosis

		Newly diagnosed Type I Diabetes mellitus		
		with ketoacidosis $n = 18$	without ketoacidosis $n = 10$	total $n = 28$
Age (years)	means ± SD	8.29 ± 3.97	9.59 ± 4.28	8.75 ± 4.05
	range	1.32–13.95	3.10–14.38	1.32–14.38
Sex (male, female)		7 m, 11 f	8 m, 2 f	15 m, 13 f
Body mass index (kg/m^2)	means ± SD	15.39 ± 2.59	16.30 ± 2.01	15.79 ± 2.47
	range	11–20	13–19	11–20
Pubertal stage	prepubertal	11	6	17
	pubertal	7	4	11
Insulin requirement ($IU \cdot kg^{-1} \cdot 24\ h^{-1}$)	means ± SD	1.19 ± 0.21	0.91 ± 0.16	1.8 ± 0.23
	range	0.86–1.73	0.68–1.16	0.68–1.73
Glycated haemoglobin A_{1c}(%)	means ± SD	12.0 ± 2.0	10.1 ± 1.1	11.3 ± 1.9
	range	5.9–14	8.1–11.2	5.9–14
Leptin (ng/ml)	means ± SD	1.47 ± 0.57	1.18 ± 0.50	1.37 ± 0.56
	range	0.53–3.02	0.47–2.0	0.47–3.02

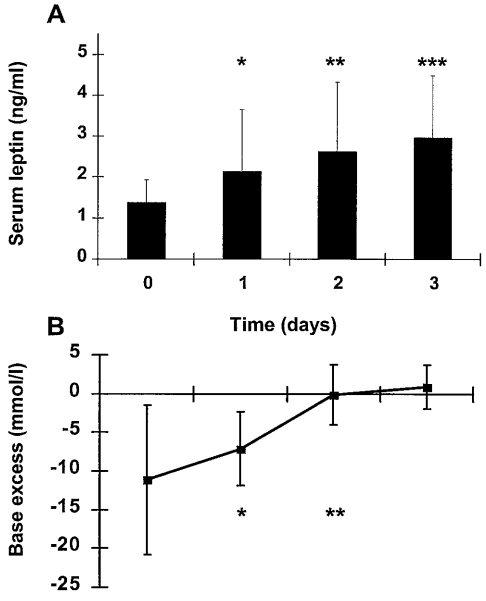

Fig. 1. A Serum leptin concentrations in 28 children and adolescents with newly diagnosed Type I diabetes mellitus with ($n = 18$) and without ($n = 10$) ketoacidosis before insulin treatment (shown as leptin at day 0) and under the influence of insulin treatment during the following 3 days. $*p$: 0.005, $**p < 10^{-4}$, $***p < 10^{-5}$ for change vs leptin at day 0. **B** Correction of ketoacidosis (depicted as base excess) in 18 children and adolescents with newly diagnosed Type I diabetes mellitus under the influence of insulin treatment. $*p < 10^{-6}$, $**p < 10^{-7}$ for change vs base excess at start of insulin treatment

toacidosis. The stimulatory effect of insulin on serum leptin concentrations was documented previously in rats [4], non-insulin-dependent diabetic patients [6], and healthy men [5]. In contrast, in a 4-h euglycaemic hyperinsulinaemic clamp no change in the plasma leptin concentration was observed in patients with established Type I diabetes although the increment of leptin in the matched control group could be clearly shown [9]. Therefore, resistance of leptin synthesis to the short-term effect of insulin in Type I diabetes was postulated [9]. However, unchanged leptin production by short-term insulin stimulation in contrast to long-term has been documented previously in vivo and in vitro [5]. Furthermore, in vitro studies in isolated rat adipocytes showed that leptin secretion is directly proportional to the amount of glucose uptake and metabolism and that inhibition of glucose transport or of glycolysis inhibits leptin secretion also in the presence of insulin [7]. Therefore, the improvement in glycaemic control, presumably resulting in an increase in glucose transport into insulin-sensitive tissues, could partly explain the disparity between short-term and long-term studies. In addition, these data suggest that insulin regulates leptin indirectly [5].

With the results of our study in newly diagnosed Type I diabetic children, in whom glucose uptake in adipose tissue was completely blocked by insulin deficiency, we postulate that treatment with insulin restored glucose transport and stimulated – mediated by improved glucose metabolism – leptin production within 1 day.

Whether or not small changes in body fat mass during treatment played a part in the rise of serum leptin concentrations in our patients is still not clear, since significant weight changes were not seen during the observation period. There was, however, a significant correlation between increment of serum leptin and absolute body weight in each subject.

Notably, evidence that leptin receptors are expressed on the pancreatic islet cells has been reported [10]. This finding opens the possibility that insulin not only has an effect on leptin secretion but also leptin influences insulin production. In pancreatic islets of mice and rodents, recombinant leptin inhibits not only basal insulin release but also the glucose-stimu-

lated insulin secretion, in a dose-dependent manner [10]. This tonic effect of leptin on insulin secretion in a negative feedback loop might be the clue to the difference in leptin increment after insulin treatment in newly diagnosed Type I diabetic subjects compared with long-term treated, mostly Type I diabetic subjects who have received too much insulin.

Ketoacidosis and its correction in newly diagnosed Type I diabetic patients had no influence on serum leptin concentration during insulin treatment. This observation is consistent with results in healthy subjects, in whom artificial ketoacidosis by ketone infusion alone, without fasting, did not alter serum leptin concentrations compared with a decline in leptin in the fasting control group [3].

In summary, our study shows that insulin increases serum leptin concentrations in children and adolescents with newly diagnosed Type I diabetes mellitus within 24 h, reaching a peak after 42 h on average. This effect is not changed by concomitant ketoacidosis. Energy balance regulation by interaction of insulin and leptin seems to be unaffected in Type I diabetic patients.

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