Relationship between adiponectin levels, acylated ghrelin levels, and short-term body mass index changes in children with diabetes mellitus type 1 at diagnosis and after insulin therapy

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Abstract
Objective: To determine the effect of the initial metabolic imbalance and its restoration after insulin therapy on adiponectin and acylated ghrelin levels in children with type 1 diabetes mellitus (T1DM).
Study design: Twenty prepubertal children with newly diagnosed T1DM were prospectively studied at diagnosis and after 1 and 4 months of therapy. Body mass index (BMI) and serum levels of adiponectin, resistin, total and acylated ghrelin, leptin, tumor necrosis factor α (TNF-α), and interleukin-6 (IL-6) were determined. The control group comprised 40 healthy prepubertal children.
Results: BMI was decreased at diagnosis, normalized at 1 month, and remained so thereafter. Adiponectin levels at diagnosis were similar to controls, increasing significantly after 1 month and normalizing at 4 months. Acylated ghrelin levels were lower at diagnosis, with a significant increase at 1 month and normalizing at 4 months. Resistin levels were normal at all time points. Leptin levels were decreased, while TNF-α and IL-6 were increased at diagnosis and normalized at 1 month.
Conclusions: These findings suggest that BMI is not the main predictor of acylated ghrelin or adiponectin levels in newly diagnosed T1DM subjects and that these peptides may play an important role in the metabolic adaptation in this disease.

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Introduction
The improper regulation of glucose and lipid metabolism due to the lack of insulin in type 1 diabetes mellitus (T1DM) leads to an increased lipolysis rate and decreased stored fat tissue, which is reversible after insulin administration (1). Adipose tissue has been discovered to be an endocrine organ; hence, changes in fat mass could have a relevant influence on its hormonal and metabolic role (2).

Adiponectin, a hormone produced specifically in adipocytes, acts mainly on skeletal muscle and liver, increasing insulin sensitization, and inhibiting key gluconeogenic enzymes (3, 4). Its levels are negatively correlated with body mass index (BMI) and fat content (5), and tend to normalize when weight control is achieved (6). Resistin has also been associated with obesity and insulin resistance in murine models, although its role in humans remains unclear (3). Hyperglycemia and hyperketonemia induce proinflammatory cytokine production (7), which is modified after insulin treatment (8). Adipose tissue produces interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α), which are related to BMI and fat stores, modulating adiponectin gene expression and increasing insulin resistance (9).

Circulating levels of leptin and ghrelin correlate with body fat content throughout normal childhood development, as well as in obesity and anorexia (10, 11). Both peptides play a role in glucose metabolism and we previously reported reduced total ghrelin levels in T1DM children, as well as after oral glucose overload in obese children (12, 13). Later, T1DM patients were reported to have low acylated ghrelin levels (14), after the discovery of this form as the specific ligand of the growth hormone secretagog type 1a (GHS1a) receptor, and as having important physiological roles such as stimulation of glucose output from hepatocytes, whereas non-acylated ghrelin has the opposite effect (15).

Due to their relationship with fat stores and insulin sensitivity, these peptides could be involved in carbohydrate and lipid metabolism in children with T1DM. Therefore, the aims of this study were to analyze the circulating levels of adiponectin, resistin, total and acylated ghrelin, leptin, TNF-α, and IL-6 in T1DM children at diagnosis and after 1 and 4 months of insulin therapy and determine the relationship between these variables, as well as with metabolic and anthropometric data.
Subjects and methods

Subjects

Twenty prepubertal (Tanner stage I) children (ten boys and ten girls) with newly diagnosed T1DM, mean age 7.34 ± 0.88 years, admitted to the Hospital Infantil Universitario Niño Jesús from January to July 2004, were studied for 4 months. All patients had abrupt diabetes onset with classical symptoms, although they were not severely impaired (pH 7.31 ± 0.02). None of the patients entered puberty during the study.

Patients were treated with i.v. fluids and either continuous i.v. insulin infusion (n = 10, doses between 0.05 and 0.1 IU/kg per h) or short acting s.c. insulin every 4 h (n = 10, doses between 0.6 and 1 IU/kg per day). After 48–60 h, they were started on a split-mix regimen of s.c. NPH and short-acting insulin analog (Lis-pro).

Forty healthy Tanner stage-, age- and sex-matched children (20 girls and 20 boys) composed the control group. Their mean age was 8.16 ± 0.46 years. BMI SDS −0.1 ± 0.35, height SDS 0.0 ± 0.13, and weight SDS −0.16 ± 0.13 according to Spanish standards (16).

Blood samples were collected at diagnosis before insulin therapy (Dx), and after 1 (1M) and 4 months (4M). All samples were obtained after overnight fasting, except at the onset of the disease. None of the patients enrolled had eaten for 3 h prior to diagnosis. All patients and their parents gave informed consent as required by the local ethics committee, which had previously approved the study.

Biochemical measurements

Adiponectin, and total and acylated ghrelin levels were measured by RIA (LINCO Research, Inc., St Charles, MO, USA). Intra- and interassay coefficients of variation (CV) were 3.9 and 8.5% for adiponectin, 6.4 and 16.3% for total ghrelin, and 7.4 and 13.4% for acylated ghrelin respectively. Resistin was determined by ELISA (LINCO Research, Inc.). Intra- and interassay CV were 4.5 and 7.4% respectively. Ultrasensitive ELISAs were used for TNF-α and IL-6 determination (Quantikine HS; R&D Systems, Inc., Minneapolis, MN, USA). Intra- and interassay CV were 6.7 and 13.3% for TNF-α and 7.4 and 7.8% for IL-6 respectively.

C-peptide was determined by RIA (Diagnostic Systems Laboratories-7000) and islet cell antibodies (ICA) by indirect immunofluorescence at 1M (17). Glucose, insulin, leptin, and HbA1c were measured as previously reported (12).

Statistical analysis

Data are reported as the mean ± s.d. Analysis was performed by one way ANOVA with Bonferroni and Dunnett’s tests for post hoc comparisons, followed by Student’s t-test or Wilcoxon 2-sample test. The relationships between variables were determined by linear regression analysis after log transformation. A value of P < 0.05 was chosen as the level of significance. Statistical analyses were performed using SPSS 12.0 software for Windows (MapInfo Corporation, Troy, NY, USA).

Results

As no differences were found between sexes in any variable, values were analyzed together. Every diabetic patient had detectable circulating ICA, ranging from 20 to 320 juvenile diabetes foundation units, as well as C-peptide levels below the detection limit of the assay (0.5 ng/ml).

Serum glucose levels were higher at diagnosis (365 ± 124 mg/dl; P < 0.001) than at later time points (1M, 133 ± 47 mg/dl; 4M, 151 ± 64 mg/dl). The insulin dose was higher at diagnosis (0.72 ± 0.21 IU/kg per day; P < 0.001) than at 1M or 4M (0.35 ± 0.21 and 0.48 ± 0.13 IU/kg per day respectively). No differences were found in HbA1c. The BMI increased at 1M (Dx −0.85 ± 0.45 vs 1M −0.16 ± 0.37, P < 0.05) remaining unchanged at 4M (−0.14 ± 0.56; Fig 1A).

At diagnosis, adiponectin levels were similar to controls (9.94 ± 3.69 (median = 9.82, range = 8.10) and 10.30 ± 3.22 μg/ml (median = 9.57, range = 11.40) respectively). However, at 1M adiponectin increased (12.43 ± 5.59 μg/ml (median = 11.40, range = 9.38); P < 0.05), normalizing at 4M (9.89 ± 4.08 μg/ml (median = 10.27, range = 8.84); Fig. 1B).

Total ghrelin remained decreased and unchanged throughout the study (Dx 912 ± 451 (median = 852, range = 1311), 1M 888 ± 253 (median = 890, range = 675) and 4M 905 ± 503 (median = 871, range = 1128) versus controls 1350 ± 638 pg/ml (median = 1270, range = 1246); P < 0.05; Fig. 1C).

Acylated ghrelin was decreased at diagnosis compared with controls (50.2 ± 20.2 (median = 47.4, range = 52.5) vs 80.0 ± 37.2 pg/ml (median = 79.6, range = 60.3); P < 0.05), rose at 1M (1M 92.4 ± 86.8 pg/ml (median = 79.2, range = 120.9); P < 0.05 versus Dx and P < 0.05 versus controls) and normalized at 4M (64.4 ± 37.0 pg/ml (median = 73.2, range = 113.1); Fig. 1D).

Leptin levels were decreased at diagnosis compared with controls (2.7 ± 1.7 (median = 2.5, range = 4.3) vs 5.1 ± 3.7 ng/ml (median = 4.6, range = 6.3); P < 0.001), normalizing afterwards (1M 5.2 ± 3.8 ng/ml (median = 4.8, range = 5.0); 4M 6.1 ± 3.4 (median = 5.6, range = 5.4); Fig 1E).

No differences in serum resistin levels were found between controls (8.0 ± 3.8 ng/ml (median = 7.9, range = 12.1)) and patients at any time point (Dx 7.2 ± 3.8 (median = 7.5, range = 10.6), 1M 7.7 ± 4.3 (median = 7.9, range = 9.4), and 4M 6.5 ± 3.4 ng/ml (median = 5.4, range = 8.8); Fig. 1F).
Circulating TNF-α was increased at diagnosis compared with controls (3.0 ± 3.1 (median = 3.6, range = 6.6) vs 1.4 ± 1.2 pg/ml (median = 1.3, range = 2.3), P < 0.05), normalizing at 1M (1.2 ± 0.9 pg/ml (median = 1.3, range = 2.1)), and remaining stable at 4M (1.1 ± 0.9 (median = 1.1, range = 0.9); Fig. 1G). IL-6 levels exhibited the same pattern, high at diagnosis (4.6 ± 4.7 pg/ml (median = 3.3, range = 8.0) vs controls 1.8 ± 1.8 pg/ml (median = 1.5, range = 4.2); P < 0.01), normal at 1M (1.9 ± 2.1 pg/ml (median = 1.3, range = 4.7)) and 4M (2.0 ± 3.4 (median = 1.3, range = 5.3); Fig. 1H).

A correlation was found between the logarithms of adiponectin and acylated ghrelin levels in patients at diagnosis (r = 0.76, P < 0.01; Fig. 2A), but not after insulin therapy or in controls (Fig. 2B). A positive correlation between BMI and leptin levels was found only in controls (r = 0.59, P < 0.01). No other significant correlations were found.

**Discussion**

Here, we demonstrate that at diagnosis, diabetic patients have decreased BMI serum leptin, and total and acylated ghrelin levels, while IL-6 and TNF-α are increased with no change in resistin or adiponectin. After 1 month, BMI, leptin, TNF-α, and IL-6 normalize, while adiponectin and acylated ghrelin increase above
control values. Total ghrelin remains decreased and resistin is unchanged. After 4 months, only total ghrelin remained decreased.

Acylated ghrelin is reported to stimulate hepatic glucose output (15) and to decrease insulin levels (18) and sensitivity (19). Therefore, the low acylated ghrelin levels found in our patients at diagnosis could be a protective mechanism against hyperglycemia. Their increase after 1 month could be a result of exogenous insulinization due to acylated ghrelin’s ability to favor glucose disposal and to modulate insulin sensitivity. After 4 months, when metabolic stabilization is reached, its levels would not be expected to differ from normal subjects, as observed in our patients, although long-term reduced levels have been postulated as a compensatory mechanism to restrain fat-mass gain after initial refeeding (14). The 3-h fasting prior to diagnosis could be considered insufficient for comparisons with later fasting samples; nevertheless, the chronic hyperglycemia and lipolysis state of these patients should be major metabolic determinants, much more than time of fasting.

Low initial BMI was probably not due to dehydration, as no significant increase in BMI after intensive i.v. rehydration was found, possibly due to the lack of severity in our patients at diagnosis. The parallel recovery of BMI and leptin after 1 month suggests an increase in adipose tissue (1, 20). The decreased BMI and need for increased insulin sensitivity would make one expect increased adiponectin at diagnosis (5, 21); however, the high TNF-α and IL-6 could impair adiponectin gene expression and plasma levels (9, 22). Also, abrupt reduction of caloric intake and weight loss decreases adiponectin levels (23), suggesting that they are not only related to BMI.

The positive correlation between acylated ghrelin and adiponectin at diagnosis that disappears after insulin therapy suggests an adaptive mechanism to metabolic impairment influenced by hyperglycemia, fasting, and proinflammatory cytokines. Normalization of TNF-α and IL-6 and restoration of carbohydrate usage may be responsible for the increment in adiponectin despite initial BMI recovery. Moreover, as insulin downregulates adiponectin receptor expression, insulin replacement could induce adiponectin resistance (24), making increased levels necessary to achieve the physiological effects. The changes in adiponectin and acylated ghrelin after 1 month suggest a shift towards increased glucose disposal and utilization, leading to fat store repletion after a period of tissue starvation. Later, chronic insulin treatment and metabolic equilibrium would normalize their levels.

Recently developed models have failed to find an association between resistin levels, glycemia or insulinemia (25), in agreement with our unchanging resistin levels. Moreover, even the association between resistin, obesity, and insulin resistance is controversial in humans (26, 27).

In conclusion, the relationship of BMI with acylated ghrelin and adiponectin levels in newly diagnosed T1DM patients seems to be influenced by their role in the metabolic adaptation to this disease. In addition, the lack of changes in resistin levels suggests that it is probably not involved in the adaptation to the metabolic changes in T1DM, whereas changes in acylated ghrelin might indicate its potential involvement in glucose homeostasis. Taken together, these results suggest that these peptides might afford additional metabolic information in children with T1DM.

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