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# Effects of ghrelin, growth hormone–releasing peptide–6, and growth hormone–releasing hormone on growth hormone, adrenocorticotropic hormone, and cortisol release in type 1 diabetes mellitus

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**Abstract**

In type 1 diabetes mellitus (T1DM), growth hormone (GH) responses to provocative stimuli are normal or exaggerated, whereas the hypothalamic-pituitary-adrenal axis has been less studied. Ghrelin is a GH secretagogue that also increases adrenocorticotropic hormone (ACTH) and cortisol levels, similarly to GH-releasing peptide–6 (GHRP-6). Ghrelin's effects in patients with T1DM have not been evaluated. We therefore studied GH, ACTH, and cortisol responses to ghrelin and GHRP-6 in 9 patients with T1DM and 9 control subjects. The GH-releasing hormone (GHRH)–induced GH release was also evaluated. Mean fasting GH levels (micrograms per liter) were higher in T1DM (3.5 ± 1.2) than in controls (0.6 ± 0.3). In both groups, ghrelin-induced GH release was higher than that after GHRP-6 and GHRH. When analyzing Δ area under the curve (ΔAUC) GH values after ghrelin, GHRP-6, and GHRH, no significant differences were observed in T1DM compared with controls. There was a trend ($P = .055$) to higher mean basal cortisol values (micrograms per deciliter) in T1DM (11.7 ± 1.5) compared with controls (8.2 ± 0.8). No significant differences were seen in ΔAUC cortisol values in both groups after ghrelin and GHRP-6. Mean fasting ACTH values were similar in T1DM and controls. No differences were seen in ΔAUC ACTH levels in both groups after ghrelin and GHRP-6. In summary, patients with T1DM have normal GH responsiveness to ghrelin, GHRP-6, and GHRH. The ACTH and cortisol release after ghrelin and GHRP-6 is also similar to controls. Our results suggest that chronic hyperglycemia of T1DM does not interfere with GH-, ACTH-, and cortisol-releasing mechanisms stimulated by these peptides.


## 1. Introduction

Growth hormone (GH) secretion is modulated by an interplay between GH-releasing hormone (GHRH) and somatostatin. Growth hormone secretagogues (GHSs) might also have a role in this process, acting at both pituitary and hypothalamic receptors [1,2]. Ghrelin, the endogenous ligand of GHS-receptor (GHS-R), was discovered in the stomach; but is also present in the hypothalamus, mainly in the arcuate nucleus [3,4]. The chemical structure of this acylated peptide is different from GHSs [3]. Ghrelin and GH-releasing peptide–6 (GHRP-6), a GHS, induce GH, adrenocorticotropic hormone (ACTH), and cortisol release [5]. Their main site of action is the hypothalamus, as demonstrated in hypothalamic-pituitary disconnection studies in which these effects are reduced or abolished [6-9]. It has been shown that GHRH and ghrelin/GHS act through different receptors and intracellular mechanisms at pituitary level. Ghrelin might stimulate several interdependent pathways of GH release, as demonstrated recently [10,11].

Ghrelin and GHS are also able to stimulate ACTH and cortisol release in humans, but the magnitude of ACTH response with ghrelin is higher [5,12,13]. This effect is due to a hypothalamic action of these peptides because they do not increase ACTH release from pituitary fragments in vitro [3] and normal corticotrophs do not express GHS-R [14]. In hypothalamic-pituitary disconnection, the effects of GHS on cortisol release are markedly reduced [7,8]. Although

Institutional approval: The experimental protocol was approved by the Ethics Committee of Universidade Federal de São Paulo, and all subjects were studied after giving informed consent.

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controversial, GHS and ghrelin stimulate ACTH secretion in humans probably through hypothalamic arginine vasopressin (AVP)–releasing-pathways [15,16].

In patients with type 1 diabetes mellitus (T1DM), basal GH levels are either normal [17-22] or elevated [23-25], depending on glycemic control; and insulin-like growth factor–I (IGF-I) levels are normal [20] or reduced [19,21,24-26]. It has been suggested that the enhanced GH secretion might be due to a decrease in IGF-I feedback on the hypothalamic-pituitary unit [27].

There are controversial findings in the literature about stimulated GH values in patients with T1DM. Both normal responsiveness and enhanced responsiveness to several stimuli, such as GHRH and hexarelin, have been reported [17-19,22,23,26,28]. We have previously shown that GH responses to GHRH and GHRP-6 are similar in T1DM and in healthy subjects [20]. However, Catalina et al [21] reported enhanced GH responses to these peptides in diabetic patients with higher hemoglobin $A_{1c}$ ($HbA_{1c}$) levels.

It has been suggested that patients with T1DM have hyperactivity of the hypothalamic-pituitary-adrenal axis. Although controversial, it has been shown that these patients have an increase in circulating cortisol values. A change in the pattern of 24-hour cortisol secretion has also been described, and glucocorticoid negative feedback is impaired [29,30]. The hyperactivity of hypothalamic-pituitary-adrenal axis in T1DM could be due to activation of corticotrophin-releasing hormone (CRH)/AVP hypothalamic neurons, changes in ACTH release from corticotrophs, and/or alteration of adrenal sensitivity [31,32]. Interestingly, in experimental studies, hyperglycemia activates pro-opiomelanocortin gene expression [33] and enhances ACTH release from normal anterior pituitary cells [34].

There are no data in the literature about the effects of ghrelin on GH, ACTH, and cortisol release in patients with T1DM.

Therefore, the aim of our study was to evaluate GH, ACTH, and cortisol release after the administration of ghrelin and GHRP-6 in a group of patients with T1DM with poor metabolic control. The GHRH-induced GH release was studied additionally, as it stimulates GH release by different pathways than those of GHS.

## 2. Subjects and methods

### 2.1. Subjects

Nine patients (5 men and 4 women) with T1DM were studied. Their mean age was 24.1 ± 1.5 years (mean ± SE; range, 18-30), with a mean body mass index (BMI) of 21.2 ± 0.8 kg/m$^2$ (range, 18.1-24.5). The duration of diabetes was 5.6 ± 1.3 years (range, 0-10). Mean $HbA_{1c}$ level at the time of evaluation was 11.7% ± 1.3% (reference range, 4.6%-6.5%). All patients had normal thyroid and renal function. Eight patients were receiving NPH and regular insulin, and one was treated with insulin detemir and aspart. None had clinical or laboratory evidence of nephropathy or neuropathy. Two of them were taking angiotensin-converting enzyme inhibitors. None of the patients had other associated diseases. Patients with hypoglycemia episodes were excluded from the study group.

Nine healthy subjects (6 men and 3 women) were also studied as a control group. Their mean age was 28.4 ± 1.5 years (range, 20-35), and their mean BMI was 23.3 ± 0.8 kg/m$^2$ (range, 18.8-25.9). Mean $HbA_{1c}$ was of 5.0% ± 0.1%. All subjects had normal thyroid function and were free of any medication at the time of the study. The women were tested in the early follicular phase of the menstrual cycle.

### 2.2. Study protocol

The experimental protocol was approved by the Ethics Committee of Universidade Federal de São Paulo, and all subjects were studied after giving informed consent. The tests were performed after an overnight fast, and the subjects remained recumbent throughout it. The patients did not receive insulin on the morning of the study. Each subject underwent 3 tests, randomly, with an interval of at least 48 hours between them. Forty-five minutes before starting the test, an indwelling catheter was inserted into an antecubital vein and kept patent by slow 0.9% saline infusion. After the first blood sample, each subject received ghrelin (Neosystem, Strasbourg, France) at a dose of 1 $\mu$g/kg, GHRP-6 (Bachem, San Carlos, CA) at the same dose, or GHRH(1-29) $NH_2$ (Clinalfa, Laufelfingen, Switzerland) at a dose of 100 $\mu$g, intravenously, in bolus. Blood samples were collected every 15 minutes until 120 minutes for hormonal determinations. Baseline blood samples were also obtained for IGF-I and glucose measurements.

### 2.3. Assays

Serum GH was measured in duplicate by a 2-site monoclonal antibody immunofluorometric assay [35]. The sensitivity of the method is 0.02 $\mu$g/L, with mean intra- and interassay coefficients of variation (CVs) of 8.8% and 13.9%, respectively. An immunochemiluminometric assay (DPC, Los Angeles, CA) was used to measure plasma ACTH. The sensitivity of the method is 5 pg/mL, with mean intra- and interassay CVs of 2.8% and 3.6%, respectively. Serum cortisol levels were measured in duplicate by a fluoroimmunoassay assay (Wallac, Turku, Finland) with sensitivity of 0.2 $\mu$g/dL and mean intra- and interassay CVs of 6.2% and 8.2%, respectively. Insulin-like growth factor–I levels were determined by an immunochemiluminometric assay (DPC) with sensitivity of 20 ng/mL and mean intra- and interassay CVs of 3.8% and 5.4%, respectively. Plasma glucose levels were determined by the hexokinase method (Advia 1650, Bayer, Deerfield, IL). Hemoglobin $A_{1c}$ levels were measured by high-performance liquid chromatography (Tosoh, South San Francisco, CA).

Table 1
Basal values and clinical data of T1DM patients (n =9) and control subjects (n =9) (mean ± SE)

| | Controls | T1DM |
|---|---|---|
| Age (y) | 28.4 ± 1.5 | 24.1 ± 1.5 |
| BMI (kg/m$^2$) | 23.3 ± 0.8 | 21.2 ± 0.8 |
| $HbA_{1c}$ (%) | 5.0 ± 0.1 | 11.7 ± 1.3* |
| GH ($\mu$g/L) | 0.6 ± 0.3 | 3.5 ± 1.2* |
| IGF-I (ng/mL) | 179 ± 20.6 | 165.7 ± 12.9 |
| Cortisol ($\mu$g/dL) | 8.2 ± 0.8 | 11.7 ± 1.5† |
| ACTH (pg/mL) | 14.5 ± 2.3 | 19.9 ± 3.4 |
| Glucose (mg/dL) | 84 ± 2 | 200 ± 22* |

* $P < .05$.
† $P = .055$.

### 2.4. Statistical analysis

Friedman analysis of variance was performed to compare GH, ACTH, and cortisol levels after the injection of each peptide. Wilcoxon signed rank sum test was used for comparisons of ACTH and cortisol values within the same group. Mann-Whitney rank sum test was performed for comparisons between patients and controls. The mean basal levels were calculated using all individual values obtained before the injection of each peptide. The responses were also analyzed by the Δ area under the curve (ΔAUC), which was calculated by trapezoidal integration with subtraction of basal levels. Spearman correlation coefficient was calculated when appropriate. For statistical purposes, undetectable GH values were considered to be equal to 0.02 $\mu$g/L. Results are shown as mean ± SE. $P < .05$ was considered statistically significant.

## 3. Results

### 3.1. Basal values and clinical data

There were no differences in age and BMI between the 2 groups. Hemoglobin $A_{1c}$ values were significantly higher in T1DM patients (Table 1).

Mean fasting GH levels (micrograms per liter) were 3.5 ± 1.2 in diabetic patients, and these values were significantly higher than those of controls (0.6 ± 0.3). Insulin-like growth factor–I levels (nanograms per milliliter) were similar between the 2 groups (T1DM, 165.7 ± 12.9; controls, 179.3 ± 20.6). There was a trend to higher mean fasting cortisol levels (micrograms per deciliter) in diabetic patients (11.7 ± 1.5) compared with controls (8.2 ± 0.8) ($P = .06$). Mean fasting ACTH values (picograms per milliliter) were 19.9 ± 3.4 in T1DM and did not differ significantly from those observed in controls (14.5 ± 2.3). Mean fasting glucose values (milligrams per deciliter) were higher in T1DM patients (200 ± 22) than in controls (84 ± 2).

### 3.2. GH responses

In controls, mean peak GH (micrograms per liter) and ΔAUC (micrograms per liter · 120 minutes) values after ghrelin administration were 57.6 ± 18.5 and 3228 ± 1036, respectively (Fig. 1). This response was higher than those obtained after GHRP-6 (24.4 ± 2.9, 1271 ± 217) and GHRH (9.3 ± 2.0, 643 ± 178), which were also significantly different. In the diabetic group, GH values after ghrelin (peak, 74.6 ± 10.3; ΔAUC, 3148 ± 427) were higher than those observed after GHRP-6 (peak, 40.3 ± 4.4; ΔAUC, 1428 ± 299) and GHRH (peak, 21.3 ± 4.3; ΔAUC, 885 ± 184). Peak GH values after GHRP-6 were higher than those obtained after GHRH, but ΔAUC values were similar.

When T1DM patients were compared with the control group, no differences were found in GH responsiveness to ghrelin and GHRH. Peak GH response to GHRP-6 was higher in T1DM patients, but no significant differences were seen in ΔAUC values.

### 3.3. Cortisol responses

In controls, there was a trend ($P = .055$) to higher responses in cortisol release with ghrelin (peak, 16.0 ± 1.3 $\mu$g/dL and ΔAUC, 467 ± 86 $\mu$g/dL · 90 min) compared with GHRP-6

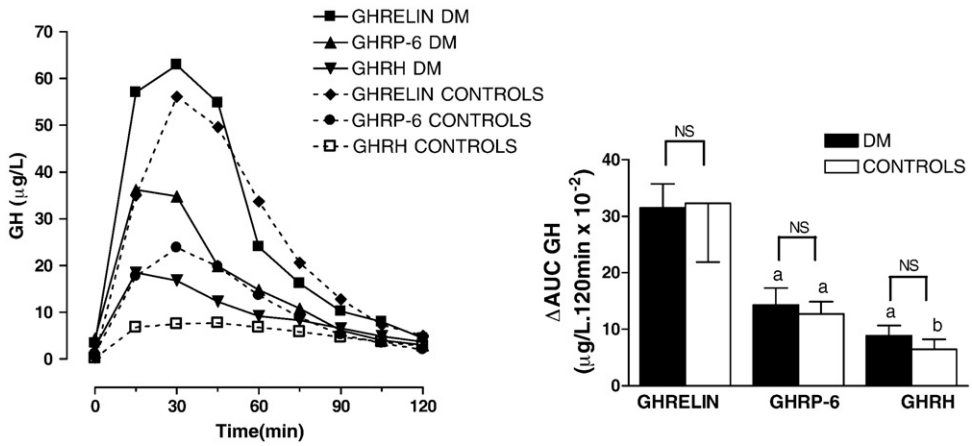


Fig. 1. Mean peak and ΔAUC GH values after ghrelin, GHRP-6, and GHRH administration in diabetic patients and in control subjects (mean ± SE; [a]$P < .05$ vs ghrelin; [b]$P < .05$ vs ghrelin and GHRP-6).

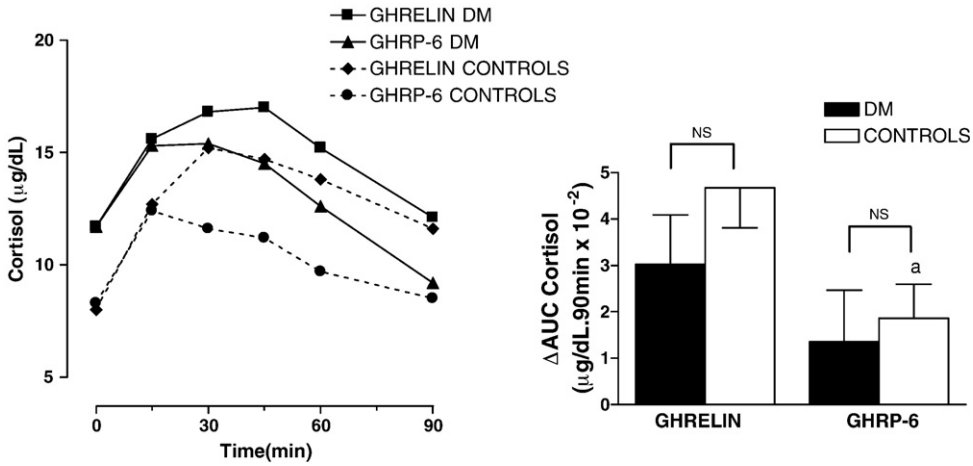


Fig. 2. Mean peak and ΔAUC cortisol values after ghrelin and GHRP-6 administration in diabetic patients and in control subjects (mean ± SE; [a]$P = .055$ vs ghrelin).

(peak, 13.1 ± 0.8; ΔAUC, 187 ± 73). In T1DM patients, there were no differences between ghrelin- (peak, 18.2 ± 1.2; ΔAUC, 303 ± 106) and GHRP-6–induced (peak, 16.7 ± 0.9; ΔAUC, 135 ± 112) cortisol release (Fig. 2). Cortisol responses to ghrelin were similar between diabetic patients and controls. Peak cortisol responses to GHRP-6 were higher in T1DM, but no significant differences were observed when ΔAUC values were analyzed.

### 3.4. ACTH responses

In controls, mean peak ACTH (picograms per milliliter) and ΔAUC (picograms per milliliter · 90 minutes) values after ghrelin (53.2 ± 10.2, 1394 ± 327) were higher than those after GHRP-6 (28.4 ± 7.6, 423 ± 211) (Fig. 3). In diabetic patients, mean peak ACTH and ΔAUC values after ghrelin (68.2 ± 19.6 and 1372 ± 771) were higher than those observed after GHRP-6 (35.8 ± 6.1, 257 ± 291), but did not reach statistical significance. When T1DM patients and controls were compared, ACTH responses to ghrelin and GHRP-6 were similar.

### 3.5. Adverse effects

One or several of these symptoms (hunger, heat sensation, nausea, transient facial flushing, and sleepiness) were reported in a few patients and controls after ghrelin, GHRP-6, and GHRH administration.

## 4. Discussion

In our study, mean fasting GH levels were significantly higher in diabetic patients compared with healthy subjects, in agreement with previous reports [25,36]. This might be related to metabolic control, as in our study $HbA_{1c}$ levels were higher than those observed in earlier reports that failed to show an increase in basal GH values. Moreover, mean IGF-I levels were unchanged in our patients, which suggests that reductions in circulating levels of this peptide are not related to enhanced GH secretion. Although our experimental protocol only analyzed GH values in the morning, our findings could perhaps reflect the increased frequency of GH pulses and the elevation of interpulse GH

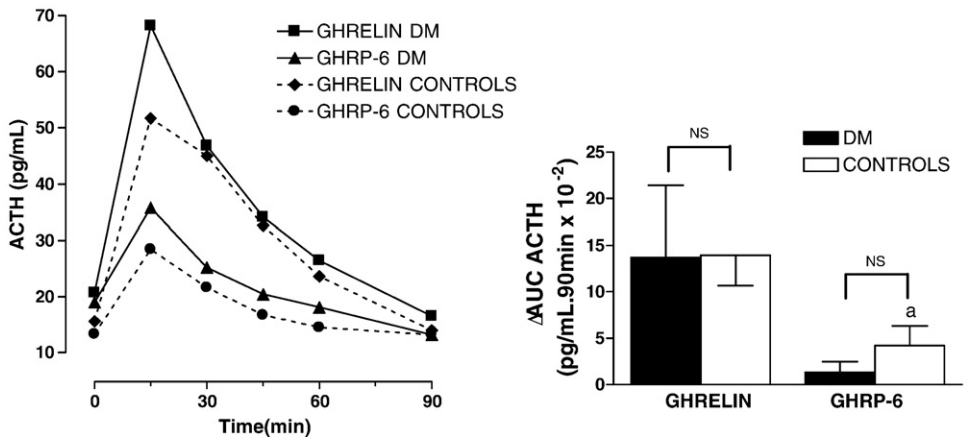


Fig. 3. Mean peak and ΔAUC ACTH values after ghrelin and GHRP-6 administration in diabetic patients and in control subjects (mean ± SE; [a]$P < .05$ vs ghrelin).

concentrations observed in these patients [24]. Moreover, it has been shown that circulating GH clearance is reduced in T1DM [37], which could contribute, at least in part, to the increased GH values.

Interestingly, despite their poor metabolic control, GH release after ghrelin in patients with T1DM was similar to that seen in healthy subjects. Furthermore, like previously reported by us in patients with better glycemic control [20], there were no differences in GH release after GHRP-6 and GHRH in this group of patients with high $HbA_{1c}$ values, which is in contrast to the results of Catalina et al [21]. The lack of effect of metabolic control has also been previously demonstrated for GHRH-induced GH release in patients with T1DM [38]. Moreover, in some of the studies which found enhanced GH release after GHS or GHRH, basal GH values were much higher in diabetes (>5 fold) compared with controls, despite lack of statistical significance. This could eventually contribute to an increase in AUC values and be interpreted as enhanced responsiveness. The normal GH responses to ghrelin, GHRP-6, and GHRH in diabetic patients could be considered inappropriate because it has been reported that oral glucose administration blunts the GH response to ghrelin/GHS and GHRH in healthy subjects [23,39-41].

In anorexia nervosa), another condition associated with high GH levels, a reduced GH response to ghrelin was observed, despite higher GH responsiveness to GHRH [42]. Therefore, chronic malnutrition has a different effect on ghrelin-induced GH release than chronic hyperglycemia.

Interestingly, it has been shown that insulin decreases GHRH-R and GHS-R messenger RNA (mRNA) in pituitary tissue of baboons in vitro. Therefore, theoretically, a decrease in endogenous insulin could enhance pituitary expression of GHRH-R and GHS-R [43] and eventually increase GH responsiveness to both GHRH and GHS. However, as our results demonstrate that the pathways of GH release stimulated by GHRH and GHS/ghrelin are not altered by persistent hyperglycemia, this hypothesis seems unlikely in humans. Moreover, patients with T1DM were receiving treatment with insulin.

In our diabetic patients, a trend to higher fasting cortisol values was observed. Although this was seen in only a single morning sample, our results are in agreement with previous reports of mildly elevated circulating cortisol levels and increased 24-hour urinary free cortisol values described in diabetic patients [29,30]. Moreover, it has also been shown that the circadian pattern of cortisol secretion is altered in these patients, with an increase in trough values [44,45].

Interestingly, both an increase and a reduction in adrenal sensitivity in streptozotocin-induced diabetes in animals have been demonstrated, together with enlargement of the adrenal glands [31,32].

However, in our study, cortisol responsiveness to ghrelin and GHRP-6 was similar in T1DM and controls, which suggests that adrenal responsiveness to these peptides is unchanged in patients with T1DM. This is in agreement with previous reports showing normal cortisol responsiveness to metyrapone, lysine vasopressin, and ACTH in diabetic patients [17,19,22].

In our study, basal ACTH levels in patients with T1DM were similar to those of controls, like previously reported [17,29]. This is in contrast with experimental studies that have shown an increase in basal ACTH values in diabetic rats [46]. Furthermore, there were no differences in ACTH responsiveness to ghrelin and GHRP-6 in patients with T1DM and controls, confirming earlier reports with other stimuli [17,29]. It has been demonstrated that GHRP-6 stimulates AVP release from hypothalamic fragments in vitro [47], whereas ghrelin enhances AVP, CRH and neuropeptide Y release [48], with a predominant action on AVP secretion [49]. Although controversial, it has been shown in experimental models of diabetes that hypothalamic CRH mRNA levels are increased and AVP mRNA values are unchanged [32,46,50]. Therefore, if also true for humans, the lack of changes in AVP mRNA in diabetes could eventually explain our findings, as GHS/ghrelin stimulate ACTH secretion in humans probably through AVP pathways [15,16].

In summary, patients with T1DM have normal GH responsiveness to ghrelin, GHRP-6, and GHRH. The ACTH and cortisol release after ghrelin and GHRP-6 is also similar to controls. Our results suggest that chronic hyperglycemia of T1DM does not interfere with GH-, ACTH-, and cortisol-releasing mechanisms stimulated by these peptides. Further studies are necessary to elucidate these findings.

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