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RAPID COMMUNICATION

# Post-prandial decrease of human plasma ghrelin in the absence of insulin

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**ABSTRACT. Ghrelin is the most powerful orexigenic hormone in mammalian physiology. Ghrelin plasma concentrations increase prior to meal onset, but decrease post-prandially. We and others reported previously that insulin reduces circulating ghrelin levels and might therefore be a driving force for post-prandial suppression of ghrelin. To test the influence of insulin on post-prandial ghrelin regulation, a patient with Type 1 diabetes with complete insulin deficiency received a low glycemic index meal and subsequently an additional high glycemic index meal in the absence of insulin substitution. Subsequently, a sc injection of 0.08 IU Lispro insulin per kg body weight was given. Results were compared to those of a healthy control subject matched for sex, age and body mass index, which was undergoing the same test series (without Lispro bolus) in the presence of endogenous post-prandial insulin secretion. A substantial decrease of plasma ghrelin levels was observed in the insulin-deficient patient following low glycemic index carbohydrate load (27% plasma ghrelin decrease). The subsequent exposure to a high glycemic index meal resulted in a slight additional reduction of ghrelin levels (32% from baseline), while Lispro bolus did not induce further changes in circulating ghrelin (27% of baseline at termination). This post-prandial response was comparable to that of the healthy control subject (33% reduction after the first meal, 40% after the second meal). These data tentatively suggest that post-prandial secretion of ghrelin is not exclusively regulated by plasma insulin or plasma glucose but may depend on other metabolic factors yet to be identified.**

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## *INTRODUCTION*

Ghrelin is the endogenous ligand of the GH secretagogue receptor (GHS-R1a) (1, 2) and has been shown to induce a positive energy balance in rodents via an increase of caloric intake and a reduction of energy expenditure (3, 4). A comparable role for ghrelin as an appetite-inducing factor has been shown in humans (5). Ghrelin is the only peripherally secreted orexigenic and adipogenic hormone and available data suggest that ghrelin is essentially involved at several levels in the neuroendocrine regulation of energy homeostasis (6). Ghrelin is released predominantly by cells in the stomach in response to the absence of caloric intake (1), and its circulating levels decrease rapidly following nutrient ingestion (7, 8). As a recent study suggested that ghrelin concentration increases before a meal, it is now also frequently termed the "hunger" hormone (8). However, this observation of a pre-prandial elevation of ghrelin is not consistently seen by others and awaits confirmation by further studies (9).

Several recent studies demonstrated that insulin reduces circulating ghrelin levels (10-14), suggesting that insulin might mediate the post-prandial reduction of ghrelin. However, two studies yielded controversial results (15, 16). In addition, hyperinsulinemic clamp studies are based on either supraphysiological insulin concentrations or involve hours long states of hyperinsulinemia, both of which are not occurring naturally. Thus, even if euglycemic hyperinsulinemia showed a decrease of serum ghrelin it is yet unclear whether the physiological insulin response after a normal meal exclusively triggers the post-prandial ghrelin reduction. Type 1 diabetes is caused by severe insulin deficiency that typically results from autoimmune destruction of pancreatic β-cells (17). In later stages of the disease, patients

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usually have a complete loss of insulin secretion and insulin levels of patients are undetectable accordingly. Based on the putatively decisive role of plasma insulin as for the mediation of post-prandial suppression of human ghrelin secretion, we hypothesized that patients with Type I diabetes should not exhibit a reduction of plasma ghrelin after meals.

## CASE REPORT

We report on a 35-yr-old patient [male, height 1.82 m, weight 73 kg, $HbA_{1c}$ 8.3%, islet cell antibody (ICA) and glutamic acid decarboxylase 65 antibody (GAD65) positive, otherwise healthy] with a 33-yr history of Type I diabetes at time of investigation. The patient was treated with Lispro insulin (Humalog, Lilly, Indianapolis) using continuous sc insulin infusion (CSII; H-TRON plus V100, Disetronic Medical Systems, Burgdorf, Switzerland). Clinical examination and baseline laboratory were without further major pathological findings. A 35-yr-old control person (male, height 1.93 m, weight 82 kg, $HbA_{1c}$ 4.9%) was compared to the patient with Type I diabetes. Oral glucose tolerance test was performed one week before test meals to exclude impaired glucose metabolism in the control person. Seventy-two h before the testing phase neither individual starved, overate, nor exercised. Daily basal infusion rate of Lispro insulin 72 h prior to the study was 23.1 IU/24 h in the diabetic patient. Average daily bolus infusion was 27.3 IU/24 h. Both individuals were exposed to a stress-free environment and refrained from physical activity for the duration of the experiment. At 08:00 h, following a 10-h overnight fast, the basal Lispro insulin infusion rate of the patient was fixed at 0.9 IU/h. This rate was previously shown to set the blood glucose constant in this individual patient in case of fasting during morning hours. The first test meal was applied 2 h later. The meal was ingested within 5 min and contained 36.6 g carbohydrate, 40.6 g fat and 40.2 g protein resulting in a total energy intake of 2868 kJ. Less than 0.6 g of the initial meal were monosaccharides. Ninety min after the start of the study, an additional high glycemic index meal (150 ml of Dextro O.G-T, Roche, Mannheim containing 37.5 g carbohydrate, no substantial fat or protein content) was given to the patient and the control and was again ingested within 5 min. The diabetic patient was treated with a sc bolus of 6.0 IU Lispro insulin after 120 min of the experiment. The run-out period was finished after 180 min for the control and 195 min for the patient with Type I diabetes.

Blood samples were drawn from a forearm vein at time points 0 and subsequently every 15 min until the end of the test. Blood was centrifuged and plasma samples were frozen at –80 C immediately. Capillary blood glucose was measured every 15 min using the glucose oxidase method on Dr. Müller Super GL, Freital, Germany. Insulin was measured using a commercially available ELISA, which does not detect Lispro insulin (Mercodia, Uppsala, Sveden, intra-assay CV 3.2%). Total human ghrelin was measured with a commercially available radioimmunoassay (RIA; Phoenix Pharmaceuticals, Belmont, CA) using $^{125}$I-labeled bioactive ghrelin as tracer and a polyclonal antibody raised in rabbits against human ghrelin (intra-assay coefficient of variation 4.5%).

The study was approved by the local Ethics Committee of the University of Potsdam.

The control person had a baseline ghrelin of 450 pg/ml. The precise course of ghrelin concentrations is displayed in Figure 1A. Basically the observed changes in the control person during the experiment were comparable to previously published data on the post-prandial course of ghrelin levels in healthy individuals (7, 8). Insulin plasma levels (Fig. 1B) increased about 8-fold compared to baseline level (7.59 mU/l) with a first maximum at 45 min after the initial meal and a second peak after the high glycemic index meal (135 min; 91.85 mU/l). Baseline glucose concentration (Fig. 1C) was 5.1 mmol/l, changed moderately after the ini-

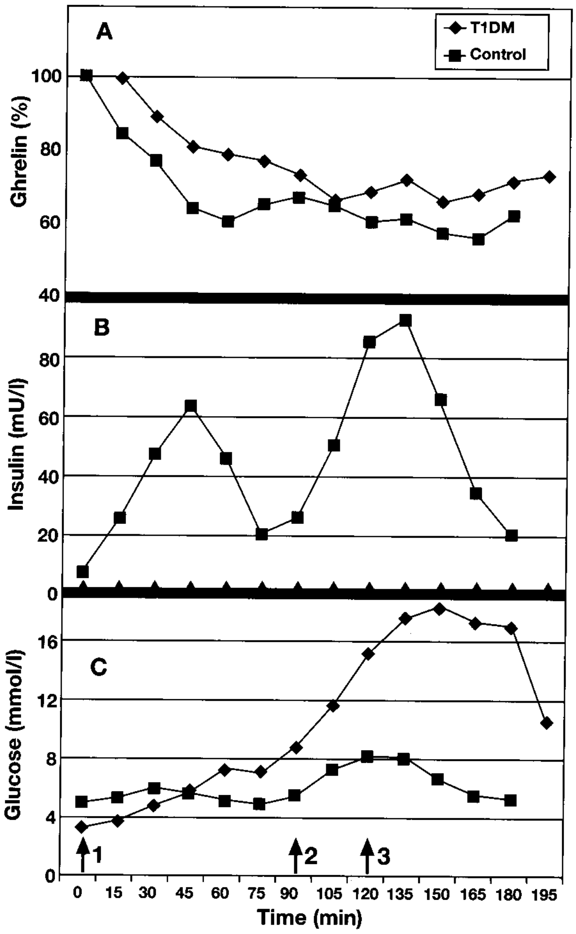


Fig. 1 - *Time course of total plasma ghrelin (A), plasma insulin (B) and blood glucose concentration (C) in a patient with Type I diabetes (T1DM) as compared to a healthy control individual.*
*(1) Mixed meal with low glycemic index; (2) high glycemic index meal; (3) Lispro insulin bolus (0.08 IU per kg body weight) in the diabetic patient.*

tial meal with a maximum of 5.9 mmol/l after 45 min and returned thereafter to 5.1 mmol/l after 90 min. The second meal with a high glycemic index resulted in a more pronounced increase of glucose levels with a peak of 8 mmol/l at 120 min. Again glucose levels decreased rapidly reaching 5.1 mmol/l after 180 min.

Baseline levels of ghrelin (602 pg/ml) were higher in the patient with Type 1 diabetes mellitus compared to the control individual. The patient with Type 1 diabetes also yielded a substantial reduction of circulating total ghrelin levels despite complete lack of post-prandial insulin secretion (27% plasma ghrelin decrease after 90 min). The further course of ghrelin is shown in Figure 1A. As expected, endogenous insulin levels were undetectable during the whole experiment in the diabetic patient (Fig. 1B). Glucose concentration of the diabetic patient increased from 3.5 mmol/l at baseline to 18 mmol/l at 150 min. After the Lispro insulin bolus at 120 min, glucose was decreasing, but was still 10.3 mmol/l at the end of the experiment (Fig. 1C).

## DISCUSSION

Ghrelin is a recently discovered naturally occurring peptide hormone that acts at the GH secretagogue receptor in the pituitary, the hypothalamus and to a lesser degree at several other sites. Most interestingly ghrelin appears to have a central role in the control of energy homeostasis (4, 6). The highly conserved acylated peptide is predominantly released by the stomach (1). When given to rodents, ghrelin increases food intake, reduces locomotor activity and energy expenditure finally resulting in a sustained increase of body fat mass (4). Ghrelin adipogenic effects in rodents are independent of its stimulating influence on GH-secretion and are mediated via hypothalamic signal cascades including orexigenic neuropeptides neuropeptide Y (NPY) and Agouti-related protein (AgRP) (18).

Ghrelin plasma concentrations decrease rapidly following nutrient ingestion (8). Although feeding suppresses ghrelin production and fasting appears to stimulate ghrelin release (8, 9), the underlying mechanisms controlling this process remain unclear. Recent hyperinsulinemic-euglycemic clamp studies were showing an insulin-induced downregulation of ghrelin secretion *in vivo* suggesting that post-prandial insulin spikes might cause the post-prandial decrease of ghrelin (10-14). While such data have been reported in rodents and humans, partially controversial results (15, 16) cast doubt on the hypothesis that insulin is indeed essentially responsible for post-prandial ghrelin reduction.

We here report on a patient with Type 1 diabetes who still has a substantial post-prandial decline of total ghrelin plasma levels despite complete absence of insulin. Our case report implies that the reduction of circulating ghrelin concentrations after a meal does not necessarily require post-prandial insulin secretion or even the availability of insulin in general. However, the ghrelin baseline levels of the patient with Type 1 diabetes were higher than those of the control person. In addition, the post-prandial ghrelin reduction was slightly more pronounced in the control compared to the patient. These data are in accordance with recently published data on elevated levels of ghrelin in streptozotocin diabetic rats (19). Our observations are compatible with a model of multiple metabolic factors linking caloric intake with the rate of ghrelin release into the circulation. Insulin might participate to a certain degree to post-prandial plasma ghrelin decrease, since this suppressive effect seems to be more pronounced in a healthy volunteer in comparison with an insulin deficient patient. Although it cannot be generalized based on this case report, and controlled studies in large populations of Type 1 diabetic patients are necessary before solid conclusions can be drawn, a more pronounced ghrelin reduction in the control person compared to the patient with Type 1 diabetes may reflect an additive effect of insulin with other determinants of ghrelin secretion involved in the meal-induced downregulation of ghrelin.

Together, post-prandial reduction of ghrelin plasma levels are, at least in major parts, not regulated by endogenous insulin secretion, hence by factors yet to be identified.

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