

Ghrelin is not suppressed in hyperglycemic clamps by gastric inhibitory polypeptide and arginine

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Abstract

Systemic ghrelin concentration falls rapidly after nutrient ingestion *in vivo*. The effect incretins on ghrelin secretion in humans remains unclear. We quantified circulating ghrelin concentrations under hyperglycemic conditions combined with infusion of gastric inhibitory polypeptide (GIP) and arginine.

Methods: Eight healthy volunteers were studied with a hyperglycemic clamp followed by addition of GIP (2 pmol · kg⁻¹ · min⁻¹, 60–115 min) and an arginine-bolus and -infusion (10 mg · kg⁻¹ · min⁻¹, 90–115 min).

Results: Hyperglycemia alone increased circulating insulin concentrations ($p < 0.01$), and decreased ghrelin concentrations to 89.8% of basal ($p = 0.208$). GIP-infusion resulted in circulating insulin concentration of 1109 ± 942 pmol/l ($p < 0.02$) and no further decrease of ghrelin (86.2% of baseline, $p = 0.050$). Under arginine- and GIP-infusion together, insulin concentrations increased progressively to 3005 ± 1604 pmol/l ($p < 0.01$) without further decreasing in ghrelin concentrations (98.9% of baseline, $p = 0.575$).

Conclusions: Hyperglycemic hyperinsulinemia and further increases of hyperinsulinemia to supraphysiological and high supraphysiological concentrations under GIP- and arginine-infusion do not significantly decrease ghrelin concentrations in healthy subjects. Moreover, there is no dose-dependent suppression of ghrelin by insulin in the hyperglycemic condition. Neither GIP nor arginine affected ghrelin release.

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1. Introduction

Ghrelin is a 28-amino acid peptide with a fatty acid side chain produced and secreted mainly by the X/A-like endocrine cells in the gastric fundus [1,2]. Ghrelin is a natural ligand of the growth hormone-secretagogue receptor through which it strongly stimulates GH secretion both in animals and in

humans [1,3,4]. In addition, ghrelin has emerged as an important factor in the control of energy homeostasis, which stimulates food intake and promoting obesity [5,6]. Cross-sectional studies show circulating ghrelin to be raised in anorexic individuals and suppressed in the obese [7].

Plasma ghrelin levels are tightly linked to meal intake. Ghrelin levels rise in fasting animals and humans [8,9] and decline sharply following meals [10]. The mechanism controlling ghrelin secretion during fasting and postprandial states remains unclear. Hyperinsulinemic euglycemia reduces circulating ghrelin levels [11–14], suggesting that insulin might mediate the fasting suppression of ghrelin. Recent studies suggested that increased postprandial con-

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centrations of insulin mediate the postprandial decrease in circulating ghrelin [14,15]. However, two studies yielded controversial results and observed no decrease of ghrelin after either simulation of postprandial insulin levels by i.v. application of insulin in normal subjects [16] or in a subject with type 1 diabetes mellitus who had a decrease of ghrelin without postprandial changes of insulin levels [17]. Moreover, recent observations on patients with type 1 diabetes mellitus suggested that insulin is required but not the deciding regulator of postprandial suppression of ghrelin [18,19]. On the other hand, hyperglycemia induces rapid decreases in plasma ghrelin concentrations after an intravenous or oral glucose loading [15,20]. Thus, the postprandial regulation of ghrelin secretion appears not to be predominantly regulated by changes in insulin or glucose concentrations only and several other regulatory factors should be involved.

Among these candidates, incretins, the gastrointestinal regulatory peptides, are believed to mediate the insulin release induced by the ingestion of glucose and other nutrients and play a physiological role in maintaining glucose homeostasis [21]. Glucose-dependent insulinotropic polypeptide (GIP) is a key incretin hormone, released postprandially into the circulation in response to feeding [21] and produces a glucose-dependent stimulation of insulin secretion [22]. In addition to stimulating insulin release, GIP like ghrelin has shown anabolic properties [23] and has been involved in the development of obesity in rodents [24]. A recent study on isolated rat stomach demonstrated a direct effect of GIP on ghrelin secretion, which reduced the postprandial decrease of ghrelin [25].

L-arginine elevates intracellular concentrations of calcium and stimulates insulin secretion as a consequence of its electrogenic transport into the beta-cell [26]. L-arginine is the biological precursor of nitric oxide, which serves as an important signal and effector molecule in humans and animals. Nitric oxide synthase, an enzyme which catalyzes the generation of nitric oxide from L-arginine, is present in the oxyntic cells of the stomach [27]. Thus, L-arginine is a possible regulator of ghrelin secretion from oxyntic cells either by depolarization or via the NO-signalling pathway.

In the present study, we tested the effect of a dose-dependent stimulation of insulin secretion by three different secretagogues under clamped hyperglycemia on ghrelin release in health subjects. Based on recent studies *in vitro*, we hypothesized that GIP and L-arginine may be involved in regulation of ghrelin release *in vivo*.

2. Subjects, materials and methods

2.1. Subjects

Eight healthy young volunteers (age [mean±S.E.]: 27.5±7.8 years.; BMI: 24.2±4.2 kg/m²; sex: 7 male/1 women) were studied. They did not take any medication

known to affect glucose tolerance, insulin sensitivity or insulin secretion. All subjects had been instructed to maintain their usual diet and physical activity before the study. In all participants, diabetes mellitus was excluded according ADA criteria [28].

2.2. Protocol

The study protocol was approved by the ethical committee of the Ruhr-University, Bochum, Germany. Before the study, informed written consent was obtained from all participants.

All subjects underwent a modified hyperglycaemic clamp (11.1 mmol/l) combined with GIP- and arginine-infusion. The clamp test was performed in the morning after 12 h overnight fast. A distal forearm vein was punctured with a Teflon cannula (Moskito 123, 18 g, Vygon, Aachen, Germany), and kept patent using physiological saline. At the same time, an antecubital vein of the contralateral arm was cannulated for infusions. Both ear lobes were made hyperaemic using Finalgon® (Nonivamid 4 mg/g, Nicoboxil 25 mg/g). After baseline samples had been obtained, a hyperglycaemic clamp [29] was carried out. An intravenous bolus of 40% glucose was given over 1 min to instantaneously raise capillary blood glucose to 11.1 mmol/l [bolus glucose=body weight (kg)×0.3 g glucose]. Subsequently, the glucose infusion was adjusted to maintain capillary blood glucose at 11.1 mmol/l for 2 h. After 60 min GIP [human GIP amide (PolyPeptide Laboratories, Wolfenbüttel, Germany)] was given as a continuous infusion (2 pmol×kg⁻¹×min⁻¹) during the next 60 min. At 90 min, a bolus (12% of infusion dose) of arginine hydrochloride (L-arginine-hydrochloride; 21% in water, Braun, Melsungen, Germany) and a continuous infusion (10 mg×kg⁻¹ body weight×min⁻¹) of arginine was given during the next 30 min.

Synthetic GIP was purchased from PolyPeptide Laboratories and processed for intravenous infusions as described [30]. In all subjects, GIP from the same batch (C-0229) was used.

Blood samples were collected at baseline and 60, 90 and 115 min of the clamp test. After centrifugation, serum for hormone analyses was kept frozen at -20 °C.

2.3. Laboratory determinations

Blood glucose concentrations were determined at bedside every 5 min with a glucose oxidase based method (HemoCue glucose photometer, HemoCue, Ängelholm, Sweden).

Serum insulin was measured using an enzyme-linked immunosorbent assay (Insulin ELISA, Mercodia, Uppsala, Sweden) with negligible cross-reaction with C-peptide of <0.01%. The sensitivity of this assay was <1 mU/L. The cross-reaction with intact human proinsulin was <0.01%. Intra- and interassay coefficients of variation (CV) were 4%.

Serum C-peptide was measured by ELISA (C-peptide ELISA, Mercodia), with a cross-reaction with insulin of

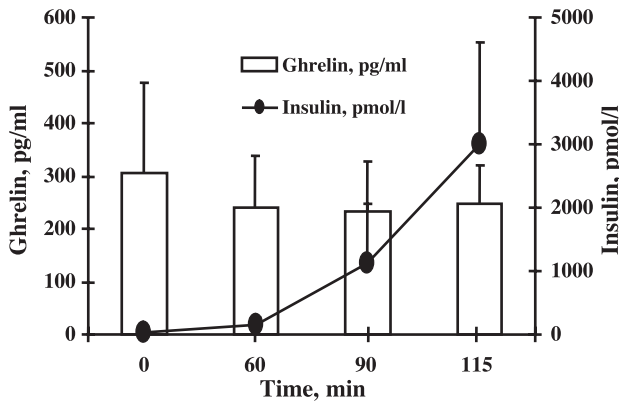


Fig. 1. Changes in serum ghrelin (columns) and insulin (points) concentrations during hyperglycemic clamp combined with GIP- and arginine infusion. Data are means±S.E.

<0.1% and with proinsulin of 100%. Intra- and interassay CVs were 5%. The sensitivity limit was <0.045 ng/ml.

Total human ghrelin was measured with a commercially available radioimmunoassay (RIA; Phoenix Pharmaceuticals, Belmont, CA) using ¹²⁵I-labeled bioactive ghrelin as tracer and a polyclonal antibody raised in rabbits against human ghrelin (intra-assay coefficient of variation 4.5–5.3%).

2.4. Statistical analysis

Data are presented as mean±S.E. Student’s two-tailed *t*-test for paired variables and Wilcoxon-test for not normally distributed variables were performed. Relationships between variables were assessed by the Pearson’s correlation test using the SPSS for Windows program (Version 11, SPSS, Chicago, IL, USA).

3. Results

Steady state glucose concentrations of 11.2±0.2 mmol/l were achieved during the period 40–60 min of the clamp.

The mean coefficient of variation at 1.8% for changes in the capillary blood glucose was achieved during the period 40–120 min.

In the first hour of the hyperglycaemic clamp, circulating insulin concentration increased to 166±98 pmol/l (*p*<0.01), but did not affect ghrelin concentrations, which were 306±194 pg/ml at baseline and 241±112 pg/ml (89.8% of basal; *p*=0.208). An additional exogenous GIP-infusion increased circulating insulin concentration to 1109±942 pmol/l (*p*<0.02) and suppressed ghrelin to 233±107 pg/ml (86.2% of baseline, *p*=0.050 vs. baseline and 96.9% of ghrelin concentration at 60 min; *p*=0.263 vs. 60 min of the clamp). During administration of arginine and GIP together, insulin concentration further increased progressively to 3005±1604 pmol/l (*p*<0.01). Ghrelin concentration remain unchanged at 247±84 pg/ml (98.9% of baseline; *p*=0.674 vs. baseline and 112.9% of ghrelin concentration at 90 min of the clamp; *p*=0.575 vs. 90 min of the clamp) (Fig. 1).

No correlation was seen between the ghrelin and insulin responses in the three periods of the hyperglycemic clamp (Table 1).

4. Discussion

This study was designed to test whether hyperglycemic hyperinsulinemia induced by co-infusion of GIP and arginine affect ghrelin secretion in lean healthy subjects.

We observed a borderline suppression of ghrelin under glucose- and GIP-co-infusion. It is not possible to separate GIP effects from insulin effects in the hyperglycemia. Given previously reported suppressing effect of insulin on ghrelin secretion, our data suggested loss of additional suppressive effects of GIP on ghrelin secretion. Data from experiments in the isolated rat stomach suggested that GIP could attenuate the postprandial decrease of ghrelin production in the stomach [25]. Thus, it is possible that the loss of pronounced suppression of ghrelin by hyperinsulinemia in our experiment may be explained via a stimulatory effect of

Table 1
Correlation analysis of circulating ghrelin and insulin concentrations in the hyperglycemic clamp with GIP and arginine

	Ghrelin [60 min]	Ghrelin [90 min]	Ghrelin [115 min]	Insulin [basal]	Insulin [60 min]	Insulin [90 min]	Insulin [115 min]
Ghrelin [basal]	<i>r</i> =0.83 <i>p</i> =0.01	<i>r</i> =0.85 <i>p</i> =0.007	<i>r</i> =0.85 <i>p</i> =0.007	<i>r</i> =−0.19 <i>p</i> =0.66	<i>r</i> =0.18 <i>p</i> =0.67	<i>r</i> =0.26 <i>p</i> =0.53	<i>r</i> =0.14 <i>p</i> =0.74
Ghrelin [60 min]		<i>r</i> =0.99 <i>p</i> <0.001	<i>r</i> =0.94 <i>p</i> =0.001	<i>r</i> =−0.19 <i>p</i> =0.65	<i>r</i> =0.27 <i>p</i> =0.52	<i>r</i> =0.47 <i>p</i> =0.24	<i>r</i> =0.07 <i>p</i> =0.07
Ghrelin [90 min]			<i>r</i> =0.93 <i>p</i> =0.001	<i>r</i> =−0.17 <i>p</i> =0.69	<i>r</i> =0.31 <i>p</i> =0.46	<i>r</i> =0.51 <i>p</i> =0.20	<i>r</i> =0.09 <i>p</i> =0.83
Ghrelin [115 min]				<i>r</i> =−0.20 <i>p</i> =0.64	<i>r</i> =0.25 <i>p</i> =0.55	<i>r</i> =0.36 <i>p</i> =0.38	<i>r</i> =0.14 <i>p</i> =0.74
Insulin [basal]					<i>r</i> =0.75 <i>p</i> =0.03	<i>r</i> =0.65 <i>p</i> =0.08	<i>r</i> =0.85 <i>p</i> =0.007
Insulin [60 min]						<i>r</i> =0.89 <i>p</i> =0.003	<i>r</i> =0.90 <i>p</i> =0.003
Insulin [90 min]							<i>r</i> =0.70 <i>p</i> =0.054

GIP on ghrelin secretion. Recent studies about GLP-1, another important incretine, reported both, suppression or no suppression, of ghrelin in humans [31,32].

The lack of ghrelin suppression under supraphysiological concentrations of insulin and glucose confirms results of a recent study in which intravenous glucose loading failed to suppress systemic ghrelin concentrations [16,17]. In contrast, another study has reported a rapid and transient decrease in plasma ghrelin after a high-dose glucose bolus [15,20]. Although it is possible that changes in ghrelin concentrations within periods shorter than 30 min could have been missed in our experiment, it is unlikely that hyperglycemia combined with hyperinsulinemia in the high supraphysiological range results in ghrelin suppression in healthy individuals. Some recent observations on patients with type 1 diabetes mellitus suggested that a minimal amount of insulin is essential for the meal-induced ghrelin suppression [18,19]. In the absence of insulin, hyperglycemia per se did not affect systemic ghrelin concentrations [19]. One can speculate that clamped hyperglycemia may have hindered the suppressive effect of insulin on circulating ghrelin concentrations. An indirect confirmation of this hypothesis is an effective suppression of circulating ghrelin concentrations by supraphysiological insulin concentrations in the euglycemic clamp [13,16]. Therefore, the insulin effect with respect to ghrelin might be different in the hyperglycemic state compared to the euglycemic conditions.

We observed the step-wise hyperinsulinemia via stimulation of insulin secretion by three different secretagogues. High supraphysiological insulin concentrations were measured under co-infusion of three secretagogues together in the last 30 min of the clamp. L-arginine is an essential amino acid and a well known potent GH and insulin secretagogue in man [33,34]. Arginine was proposed to stimulate growth hormone secretion by suppressing endogenous somatostatin secretion [33], but obviously had no effect on ghrelin release.

Apart from glucose, insulin or incretins, several other regulatory mechanisms appear to be involved in the regulation of ghrelin secretion in the postprandial state. For example, the carbohydrate meal had a greater suppressive effect on postprandial ghrelin secretion compared to a fat meal [18,35] and protein intake stimulates postprandial ghrelin secretion [36]. Thus, intraluminal nutrients are an obvious regulatory candidate, and it appears that direct exposure of the oxyntic cells to these nutrients mediates suppression of ghrelin secretion. On the other hand, ghrelin-producing cells in the stomach might be under control of circulating hormones or other substances via capillary connection with gastric mucosa [2].

Taken together, hyperglycemic hyperinsulinemia and further increases of hyperinsulinemia to supraphysiological and high supraphysiological concentrations under further GIP- and arginine-infusions did not decrease ghrelin concentrations significantly in lean healthy subjects. There-

fore, the insulin effect with respect to ghrelin might be different in the hyperglycemic state compared to the euglycemic conditions described previously. Since postprandially both insulin, GIP and glucose levels increase, the meal-related suppression of ghrelin is not directly regulated by hyperglycemia, insulin or GIP.

In summary, hyperglycemic hyperinsulinemia and further increases of hyperinsulinemia to supraphysiological and high supraphysiological concentrations under GIP- and arginine-infusion do not significantly decrease ghrelin concentrations in healthy subjects. Moreover, there is no dose-dependent suppression of ghrelin by insulin in the hyperglycemic condition. Neither GIP nor arginine affected ghrelin release under hyperglycemia.

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