

# Final Answer: Ghrelin Can Suppress Insulin Secretion in Humans, but Is It Clinically Relevant?

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**G**hrelin is a 28-amino acid peptide initially isolated from rat and human stomachs as an endogenous ligand for the growth hormone secretagogue receptor type 1a (GHS-R1a) (1) using an intracellular calcium influx assay on a stable cell expressing rat GHS-R. At the time of its discovery in 1999, ghrelin was known to increase growth hormone release from the pituitary gland by binding to GHS-R1a, and 1 year later, its role in food intake and body adiposity was described (2). Since then, numerous additional effects of ghrelin have been demonstrated, including regulating cell proliferation and survival, apoptosis, inflammation, angiogenesis, development and reproduction as well as metabolism. Recently, it has become of great research interest whether ghrelin also has a role in  $\beta$ -cell function. This research area is particularly important given its potential to lead to the development of novel strategies to increase insulin secretion for the treatment of type 2 diabetes.

In humans, circulating ghrelin levels surge immediately before meals and fall to a nadir within 1 h after food intake, which results in a two- to threefold variation in plasma levels. Circulating ghrelin is primarily produced in the stomach, but smaller amounts are also produced in the brain, heart, lung, kidney, placenta, gastrointestinal tract, and pancreas. Within the islets of Langerhans of the pancreas, ghrelin production has been found in  $\alpha$ -cells,  $\beta$ -cells, and the more recently discovered  $\epsilon$ -cells (3). Ghrelin peptides exist in two major forms, unacylated ghrelin and acylated ghrelin, and only the latter binds to GHS-R1a. Acylation is catalyzed by ghrelin O-acyltransferase (GOAT), which is, interestingly, highly expressed in the stomach and the pancreas in humans (4). Consistent with the multitude of effects of ghrelin, GHS-R1a is expressed in variety of tissues. Most importantly, with regard to insulin secretion, GHS-R1a partially colocalizes with insulin-positive  $\beta$ -cells in human pancreatic islets (5), suggesting that human  $\beta$ -cell might be directly responsive to ghrelin stimulation.

To date, the role of ghrelin in the regulation of insulin secretion remains controversial. Perhaps because of differences in the experimental conditions, ghrelin was found to inhibit insulin secretion in the majority of studies but to

stimulate insulin secretion in others. For example, ghrelin increased cytosolic free  $\text{Ca}^{2+}$  concentrations in rat  $\beta$ -cells and increased insulin secretion in isolated rat pancreatic islets under glucose-stimulated conditions (6). Furthermore, ghrelin increased insulin secretion from the pancreas of both normal and diabetic rats (7,8). In contrast, GHS-R1a blockade by specific antagonists or ghrelin inactivation using an antiserum enhanced glucose-induced insulin release and intracellular  $\text{Ca}^{2+}$  concentrations in isolated rat islets (9). Further, exogenous ghrelin decreased insulin secretion in isolated mouse pancreata (10), in mouse and rat isolated islets (11), and in  $\beta$ -cell lines (12). In humans, data regarding the effects of ghrelin on insulin secretion are scant and inconsistent. Although intravenous injection of ghrelin decreased plasma insulin and increased plasma glucose concentrations in overnight-fasted humans in some studies that raised plasma ghrelin at least 4.5-fold (13,14), no changes in insulin and glucose were observed in another that raised plasma ghrelin 2- to 3-fold (15).

Tong et al. (16) in a recent issue of *Diabetes* have, therefore, revisited the effects of ghrelin on  $\beta$ -cell function in humans using a more sensitive approach. To this end, acyl ghrelin (0.3, 0.9, or 1.5 nmol/kg/h) or saline was infused in 12 healthy, young, nonobese subjects on four separate occasions in a counterbalanced fashion after an overnight fast. After reaching steady-state plasma ghrelin levels, an intravenous glucose tolerance test was performed on each occasion to determine the acute insulin response to intravenous glucose ( $\text{AIR}_g$ ), the acute C-peptide response to intravenous glucose ( $\text{ACR}_g$ ) as well as the glucose disappearance constant as an index of intravenous glucose tolerance. The authors found that ghrelin infusion did not alter fasting insulin or glucose concentrations but significantly decreased  $\text{AIR}_g$  ~30–45% in a dose-dependent manner. Similar suppressions were seen in the C-peptide release in response to intravenous glucose, indicating that the ghrelin-induced reduction in plasma insulin levels was not due to increased hepatic insulin clearance. In addition, ghrelin infusion at 0.3 and 1.5 nmol/kg/h significantly decreased the glucose disappearance constant by ~30%. These responses to ghrelin infusion were associated with marked and dose-dependent increases in plasma growth hormone and cortisol concentrations but unaltered plasma concentrations of glucagon, epinephrine, and norepinephrine. The authors conclude that exogenous ghrelin reduces glucose-stimulated insulin secretion and glucose disappearance in humans. Moreover, they put forward that endogenous ghrelin may have a role in physiological insulin secretion and that ghrelin antagonists could improve  $\beta$ -cell function.

The findings by Tong et al. provide clear proof of concept that ghrelin can reduce glucose-stimulated insulin secretion. The experiments were logically designed, and the data are clear. However, there are at least two reasons

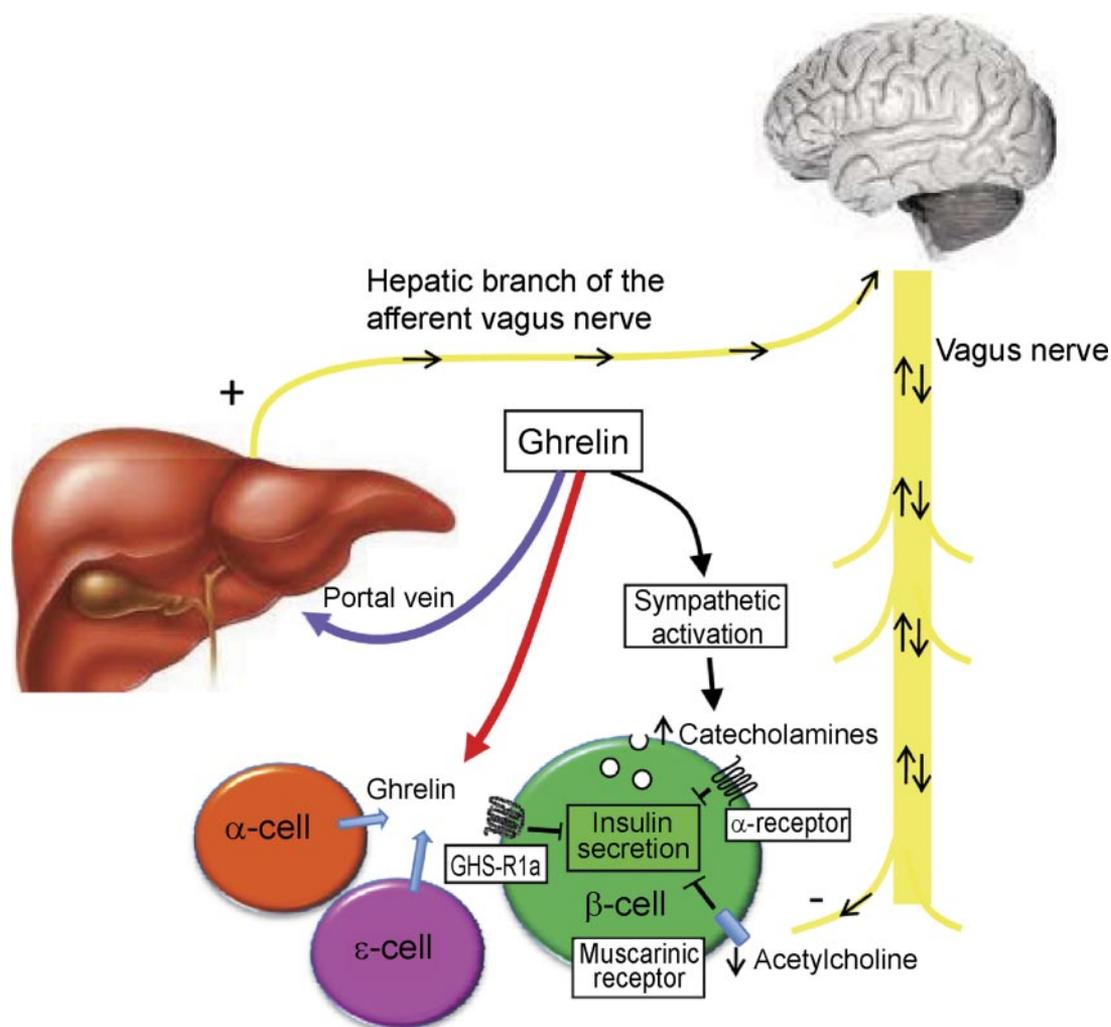
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DOI: 10.2337/db10-1088

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**See accompanying original article, published in *Diabetes* 2010;59:2145–2151.**



**FIG. 1.** Illustration of the mechanisms by which ghrelin may suppress insulin secretion in humans: a) direct inhibition by activation of the  $\beta$ -cell GHS-R1a where ghrelin may act via either endocrine or paracrine mechanisms; b) indirect inhibition by activation of the hepatic branch of the afferent vagus nerve by ghrelin sensors in the portal system; increased firing of the afferent fibers of the vagus nerve reduces the activity of the efferent nerve fibers in the brain, leading to decreased insulin secretion; c) indirect inhibition by activation of the sympathetic nervous system and subsequent stimulation of the  $\alpha$ -adrenergic receptors on  $\beta$ -cells.

to wonder about the clinical relevance of these findings. First, ghrelin infusion at 0.3, 0.9, or 1.5 nmol/kg/h raised plasma ghrelin levels  $\sim$ 4.5-, 15.4-, and 22.6-fold, respectively, so that these levels were within the pharmacological range even at the lowest infusion rate. Whether plasma ghrelin levels within the physiological range inhibit glucose-induced insulin secretion in humans therefore remains unclear. Admittedly, it is possible that ghrelin, due to its local synthesis and release within the islets of Langerhans, may reach very high concentrations at the  $\beta$ -cell and act via autocrine and/or paracrine rather than systemic mechanisms. The pharmacological plasma concentrations of ghrelin reached in the study thus do not exclude the possibility that endogenous ghrelin suppresses insulin secretion in humans as noted by the authors. Nevertheless, because circulating ghrelin is primarily produced in the stomach, experiments using lower dose ghrelin infusion would provide valuable information. The second reason that makes the clinical relevance of this study uncertain is that glucose tolerance has been determined only in response to intravenous glucose administration but not to an oral glucose load or a mixed meal. In contrast to intravenous glucose tolerance, oral

glucose tolerance is strongly influenced by additional mechanisms, such as gastric emptying and the effects of incretins. For illustration, after a 100 g oral glucose load  $\sim$ 70–90% of the increase in peripheral plasma insulin concentrations can be accounted for by the incretin effect (17,18), suggesting that these mechanisms may play a far more important role in postprandial glycemia than the effects of ghrelin. Whether ghrelin does play an appreciable physiological role in insulin secretion and glycemia after oral glucose ingestion hence needs to be tested.

Aside from these issues, future research will also need to determine the mechanism(s) by which ghrelin suppresses insulin secretion in humans particularly if ghrelin antagonists were to be considered as a prospective novel strategy for the improvement of  $\beta$ -cell function. Besides direct, potentially autocrine and/or paracrine mechanisms, there is evidence to suggest that ghrelin may also indirectly suppress insulin secretion. For example, in rats ghrelin inhibited glucose-stimulated insulin release when infused into the portal vein but not when infused into the femoral vein. Hepatic vagotomy or coinfusion with atropine methyl bromide into the portal vein diminished the inhibitory effect of ghrelin on glucose-stimulated insulin

secretion, suggesting that ghrelin may exert an inhibitory effect on glucose-stimulated insulin secretion via the hepatic portal system and the vagus nerve (19). Furthermore, although not observed by Tong et al. (16), ghrelin has been found to increase plasma epinephrine levels in humans (20), suggesting that it may also inhibit insulin secretion via a sympathetic response.

In summary, the study by Tong et al. (16) provides evidence that ghrelin can inhibit insulin-stimulated insulin secretion in humans beyond question. However, given the uncertainties of its role following oral glucose ingestion and its effects at physiological plasma concentrations, future studies will most importantly need to address whether ghrelin has a physiological role in insulin secretion in humans.

#### ACKNOWLEDGMENTS

This commentary was supported by the Office of Research and Development, Medical Research Service, Department of Veterans Affairs.

No potential conflicts of interest relevant to this article were reported.

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