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# Ghrelin: A New Incretin Enhancer Therapy?

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COMMENTARY

The overarching goal of diabetes therapies is to get patients under good glycemic control using therapies that lower abnormally elevated plasma glucose, thereby reducing glucose toxicity, oxidative stress, and inflammation, conditions that promote cardiovascular disease (CVD) and chronic kidney disease. Unfortunately, some conventional diabetes therapies, although effective at controlling glycemia, induce weight gain and actually increase the risk of hypoglycemic episodes and associated CVD events. To this end, the development of novel, safe, and effective therapies that improve long-term glycemic control, that minimize the risk of hypoglycemia, that do not increase weight gain (or induce therapeutic weight loss), and that promote cardiovascular (CV) health is a better way forward (1). The ongoing development of incretin-enhancing therapies may be an example of such an approach. The gut-derived incretin hormones, glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide, are secreted in response to the postprandial increase in glucose and act to augment glucose-dependent pancreatic insulin secretion, suppress glucagon release, slow gastric emptying, enhance satiety, and modulate the so-called gut-brain axis (2,3). The action of incretin hormones is short-lived due to rapid degradation by the ubiquitous enzyme, dipeptidyl peptidase-4 (DPP-4). Earlier studies showed that the incretin axis in individuals with type 2 diabetes mellitus (T2DM) is impaired (4,5), and this has led to the development of novel incretin-enhancing therapies, such as the stable (i.e., DPP-4–resistant) receptor (GLP-1R) agonists and DPP-4 inhibitors. Accumulating evidence suggests that modulation of incretin signaling by GLP-1R agonists or DPP-4 inhibitors is not only beneficial for improving glycemic control but confers neutral or modest CV protection (6,7). Given the complexity of the incretin axis and interaction with the gut-brain axis, it is likely that additional mechanisms, yet to be discovered, exist to enhance the incretin axis and improve glycemia in T2DM.

In this issue, Gagnon et al. (8) propose a novel mechanism to enhance the GLP-1 secretory response to ingested nutrients (Fig. 1*A*). The focus of their study is the stomach-derived hormone ghrelin, which is key to whole-body energy metabolism (9). Unlike GLP-1, which is secreted following nutrient ingestion, ghrelin levels are highest shortly before regular meal times. The premeal ghrelin surge suggested to the authors that ghrelin acts to prepare the body for the incoming meal. More specifically, it was hypothesized that the ghrelin surge may prime the intestinal L-cells to release GLP-1 in response to the upcoming meal. Given that obese individuals have reduced premeal ghrelin levels (10), it is possible that administration of exogenous ghrelin could serve to enhance GLP-1 secretion.

To test their hypothesis, Gagnon et al. first injected normal male C57BL/6 mice with acylated ghrelin or vehicle solution 15 min prior to performing an oral glucose tolerance test (OGTT). Blood samples were drawn before and after mice received the glucose load to evaluate pre- and postprandial circulating GLP-1, glucose, and insulin. Ghrelin pretreatment did not affect baseline GLP-1 level but lead to an almost twofold increase in GLP-1 levels 10 min after glucose loading compared with vehicle-treated mice. Glucose tolerance was significantly improved in response to ghrelin pretreatment, and this occurred in the absence of a significant effect on insulin levels. At this stage, the authors had their initial proof of concept that ghrelin pretreatment increased GLP-1 secretion and improved glucose handling in normal male mice.

Next, the investigative team tested whether ghrelin receptor antagonism could reduce glucose-stimulated GLP-1 secretion as well as glucose tolerance. The goal was the reverse of their first experiment (i.e., to establish the body's responses to endogenous rather than exogenous ghrelin secretion). In this protocol, mice were pretreated with the ghrelin receptor blocker, D-Lys GHRP-6,

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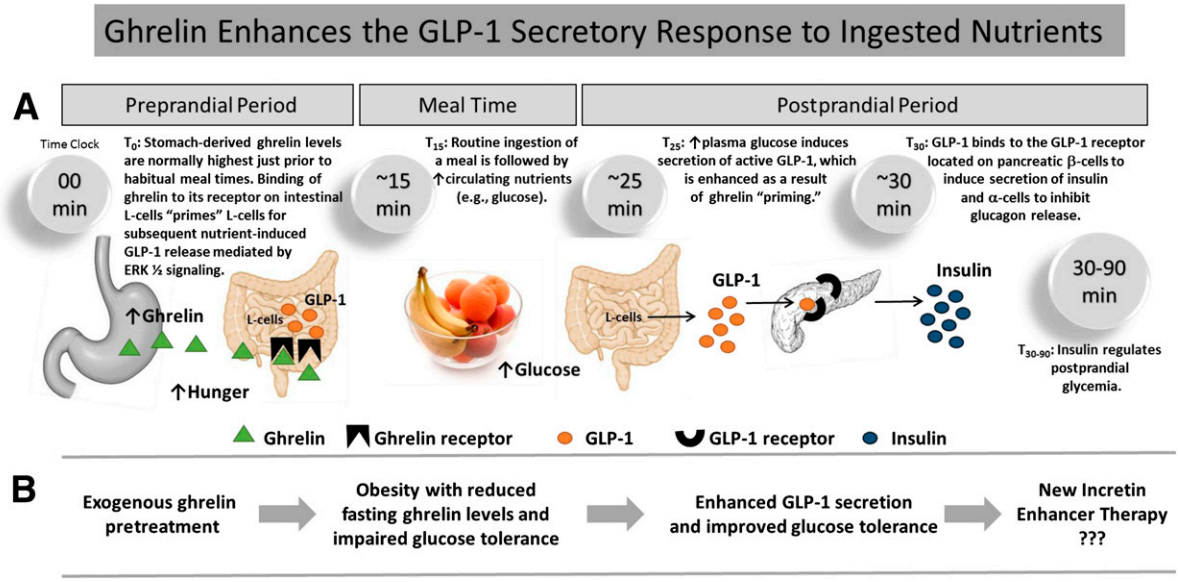


**Figure 1**—*A*: Proposed mechanism to explain a novel role for ghrelin in the regulation of oral glucose-stimulated GLP-1 secretion preprandial release of ghrelin that acts directly on ghrelin receptors located on enteroendocrine L-cells. This "priming" effect of ghrelin on nutrient-stimulated GLP-1 secretion requires signaling through the MAPK pathway. *B*: Obese mice pretreated with exogenous ghrelin exhibit enhanced GLP-1 secretion and normal glucose tolerance.

or a vehicle 15 min prior to OGTT. Compared with pretreatment with vehicle, ghrelin receptor blockade resulted in lower levels of circulating GLP-1. Moreover, the insulin response to glucose load was impaired as was the clearance of glucose. This experiment also provided proof of concept by demonstrating a role for endogenous ghrelin and the ghrelin receptor in the regulation of GLP-1 secretion and glucose handling.

Next, the authors tested whether the effects of ghrelin on glucose tolerance were mediated through the GLP-1R pathway. This experiment was important because we know that GLP-1 can exert effects through the GLP-1R, as well as through GLP-1R–independent pathways. Two protocols were performed to test this concept. The first involved simultaneous pretreatment of C57BL/6 mice with acylated ghrelin and the GLP-1R blocker, exendin-4, or a vehicle. In the second protocol, GLP-1R knockout mice were used in place of exendin-4. Similar to their first experiment described above, the authors demonstrated in these two protocols that ghrelin pretreatment stimulated secretion of GLP-1 during OGTT, despite the presence of the GLP-1R blocker. The downstream effects of exogenous ghrelin on insulin secretion and glucose handling during OGTT, however, were completely eliminated in the presence of exendin-4. These experiments provided proof of concept that GLP-1R is required for ghrelin's effects on glucose metabolism.

The final in vivo test of the hypothesis was designed to determine whether ghrelin pretreatment would sufficiently stimulate GLP-1 secretion to improve glucose handling in a model of diet-induced obesity caused by high-fat–diet feeding (Fig. 1*B*). Ten weeks of high-fat–diet feeding induced substantial weight gain, abnormally low preprandial ghrelin levels, and impaired glucose tolerance, similar to what is observed in obese humans (10). Thus, the investigators used a preclinical model of obesity that has high translational relevance for testing the efficacy of novel therapies to treat the metabolic complications of obesity and diabetes. In the setting of obesity, ghrelin pretreatment normalized glucose tolerance, and this was likely due to increases in GLP-1 secretion and GLP-1R activation. Collectively, these in vivo results validate the hypothesis that ghrelin pretreatment induces GLP-1 secretion and improves glucose tolerance. Moreover, the hypothesis was validated in glucose-intolerant obese mice. These results will undoubtedly lead to further preclinical testing and are likely to be sufficient to warrant consideration of a phase one trial.

Finally, Gagnon et al. explored mechanisms to explain how preprandial ghrelin induces secretion of GLP-1. They verified expression of ghrelin receptor transcripts in both murine and human L-cell lines and demonstrated increases in GLP-1 secretion in response to the addition of acylated ghrelin to culture media, an effect that could be prevented by pharmacological blockade of mitogen-activated protein kinase (MAPK)/extracellular signal–regulated kinase (MEK)/extracellular signal–regulated kinases (ERK)1/2 activation. These in vitro studies indicate that ghrelin acts directly on L-cells via activation of the MAPK pathway. More details on mechanisms linking MEK/ERK activation to GLP-1 secretion are likely to emerge soon.

The results of the study by Gagnon et al. (8) suggest the potential for a third pharmacological strategy for enhancing the impaired incretin system in the obese population with diabetes. The authors have very elegantly identified ghrelin as a GLP-1 secretagogue. Prior to this

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report, the only pharmacological agent or diabetes therapy identified as a GLP-1 secretagogue was metformin (11). Unlike ghrelin, metformin does not act directly on L-cells to induce GLP-1 release. Whether acylated ghrelin is ultimately developed as a therapy to correct diabetic dysglycemia remains to be seen. Like all newly developed therapies to treat diabetes, arduous work of rigorous testing will be required to determine its effects on body weight, risk for hypoglycemia, and CV health.

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