Starving for Ghrelin

Mark W. Sleeman[1,2,*] and David C. Spanswick[1]
[1]Department of Physiology
[2]Department of Biochemistry & Molecular Biology
Monash University, Clayton, Victoria 3800, Australia
*Correspondence: mark.sleeman@monash.edu
http://dx.doi.org/10.1016/j.cmet.2014.06.006

The initial discovery of ghrelin as a potent orexigenic hormone raised excitement about a new direction for possibly treating eating disorders. McFarlane et al. (2014) show that with deletion of ghrelin-producing cells from an adult animal, there is little effect on appetitive behaviors but significant implications for glucose homeostasis.

Metabolic disorders, such as obesity and type 2 diabetes (T2D), have become major causes of morbidity and mortality in Western societies, a trend that is not likely to change in the foreseeable future. It is likely that our modern refined food sources and dietary choices as well as sedentary lifestyle are partially to blame, but a genetic component is equally supported. Often postulated in shaping this latter component are the bottleneck periods of famine and starvation providing evolutionary pressure in a variety of adaptive mechanisms necessary to support life. The latest work by McFarlane et al. (2014) suggests that the ghrelin system may have evolved to play more of a defensive role in protecting against starvation-induced hypoglycemia, and it is less important in the regulation of food intake and body weight.

The discovery of ghrelin was founded of the back of selective screening protocols for growth hormone (GH) secretagogues and led to the expression cloning of the GH secretagogue receptor (GHSR). In their search for novel peptides, Kojima and Kangawa (2013) used cell lines overexpressing GHSR to isolate and characterize a natural ligand from stomach extracts that they named ghrelin (*ghre* is the root word for grow). Ghrelin is a 28 amino acid polypeptide product that is principally synthesized from secretory cells in the stomach. It is acylated on its serine 3 residue by a medium-chain fatty acid octanoate mediated by a very specific transferase, later called ghrelin O-acyltransferase (GOAT). In addition to its role in regulating GH secretion, the expression pattern of the GHSR in various neuronal populations including brain stem suggested that it had a broader endocrine function. A model quickly developed after ghrelin levels were observed to fluctuate with feeding/fasting, and a role as a peripheral sensor of nutrition status acting via GHSRs in AgRP/NPY neurons in the basal arcuate nucleus was proposed. These neurons and their associated circuits are believed to be critical for mediating the effects of ghrelin both on food intake and in maintaining glucose homeostasis (Wang et al., 2014).

Germline deletion strategies were quickly developed for a number of levels for the ghrelin pathway. The first reports for total ghrelin knockouts (KO) however failed to observe any robust modification of food intake parameters, and only minor changes in body weight regulation were observed under a number of dietary conditions (Sun et al., 2003). The deletion of GHSR confirmed that this was the biologically relevant receptor mediating exogenous ghrelin stimulation of GH release and food intake; however, body composition and food intake were essentially normal. Ghrelin deficiency was not able to rescue the obese hyperphagic response when mice were crossed to leptin-deficient mice (*ob*/*ob*), but it did significantly impact glucose homeostasis and insulin secretion (Sun et al., 2007). The impact of severe starvation paradigms on ghrelin physiology was first explored in an acyl-ghrelin-deficient model using a newly developed GOAT KO (Zhao et al., 2010). It was clearly demonstrated that under severe caloric restriction paradigms glucose homeostasis could be significantly affected; when mice were placed on a 7-day caloric restriction of 40% of normal food intake, mice became profoundly hypoglycemic and moribund (Li et al., 2012; Zhao et al., 2010). Importantly euglycemic control could be restored by infusion of either GH or ghrelin. There are a number of critical variables needed to elicit this effect, such as the amount of body fat, age, and severity of the stress, as not all investigators have been able to reproduce these findings (Kirchner et al., 2013; Yi et al., 2012).

A dependence on rodent KO models for defining physiological behaviors is not without its critics, and there are valid concerns about developmental compensation with germline deletion strategies masking the true biological role. The recent *Cell Metabolism* report by McFarlane et al. (2014) is important because it provides an additional data set from animals in which ghrelin-producing cells have been deleted in an adult animal. The genetically modified mouse line was developed with a copy of the diphtheria toxin receptor (iDTR) specific to cells that produce ghrelin. Essentially, this is a benign modification until mice are injected with the diphtheria toxin (DTX) ligand. In greater than 90% of the DTX-injected adult mice, all ghrelin-producing cells were ablated in an all-or-none manner, with no partial responders. Deletion of ghrelin cells was validated by following the subsequent reductions in plasma ghrelin levels, ghrelin mRNA, and a histological analysis of ghrelin protein at the major site of production in the stomach, although the fate of GHSR expression in central neural regions controlling food intake and glucose homeostasis was not quantified. Subsequent phenotypic analysis of treated adult mice once again revealed no effects on food intake, fat partitioning, or body

weight under normal or high-fat feeding. However, consistent with their previous studies, adult ghrelin-deficient animals are unable to prevent hypoglycemia developing in response to severe caloric restriction. Importantly these studies also highlight that replacement ghrelin dosing to physiological levels is without effect and that it is not until supraphysiological dosing that changes in food intake are observed. The complexity and extremity of the fasting protocol used may explain why it has not been supported in other animal models and from prior studies in humans.

These data highlight that not only are the peripheral ghrelin-producing cells responsive to glucose levels, but the GHSR cells, predominantly in CNS, are likely to be highly sensitive to glycemic levels to provide necessary feedback. This is also consistent with existing CNS glucose-sensing neural networks that respond to release hormones and contribute to maintaining energy requirements. Currently, NPY/AgRP neurons are generally considered to be inhibited when glucose levels rise (Chalmers et al., 2014) and activated by ghrelin, suggesting that NPY/AgRP neural function and glucose homeostasis are maintained by a balance between these two energy status signaling pathways. Whether function-specific NPY/AgRP subgroups and circuits or specific neurons with convergent signaling pathways are responsible for coordinating this behavior is currently unclear. In addition, other functions ascribed to ghrelin on the reproduction, stress, and immune system response have not been tested under these extreme environmental conditions. Given that under such extreme conditions behaviors geared toward seeking and consuming food to contribute to restoring glucose levels would be prioritized, understanding how ghrelin acts at the level of the motivation and reward pathways to control food intake also needs to be addressed.

REFERENCES

Chalmers, J.A., Jang, J.J., and Belsham, D.D. (2014). Mol. Cell. Endocrinol. *382*, 262–270.

Kirchner, H., Heppner, K.M., Holland, J., Kabra, D., Tschöp, M.H., and Pfluger, P.T. (2013). PLoS ONE *8*, e61822.

Kojima, M., and Kangawa, K. (2013). Endocr. Dev. *25*, 1–4.

Li, R.L., Sherbet, D.P., Elsbernd, B.L., Goldstein, J.L., Brown, M.S., and Zhao, T.J. (2012). J. Biol. Chem. *287*, 17942–17950.

McFarlane, M.R., Brown, M.S., Goldstein, J.L., and Zhao, T.-J. (2014). Cell Metab. *20*, this issue, 54–60.

Sun, Y., Ahmed, S., and Smith, R.G. (2003). Mol. Cell. Biol. *23*, 7973–7981.

Sun, Y., Asnicar, M., and Smith, R.G. (2007). Neuroendocrinology *86*, 215–228.

Wang, Q., Liu, C., Uchida, A., Chuang, J.-C., Walker, A., Liu, T., Osborne-Lawrence, S., Mason, B.L., Mosher, C., Berglund, E.D., et al. (2014). Mol Metab *3*, 64–72.

Yi, C.-X., Heppner, K.M., Kirchner, H., Tong, J., Bielohuby, M., Gaylinn, B.D., Müller, T.D., Bartley, E., Davis, H.W., Zhao, Y., et al. (2012). PLoS ONE *7*, e32100.

Zhao, T.-J., Liang, G., Li, R.L., Xie, X., Sleeman, M.W., Murphy, A.J., Valenzuela, D.M., Yancopoulos, G.D., Goldstein, J.L., and Brown, M.S. (2010). Proc. Natl. Acad. Sci. USA *107*, 7467–7472.

Rejuvenation: It's in Our Blood

Alessandro Bitto[1] **and Matt Kaeberlein**[1,*]
[1]Department of Pathology, University of Washington, Seattle, WA 98195-7470, USA
*Correspondence: kaeber@uw.edu
http://dx.doi.org/10.1016/j.cmet.2014.06.007

It has been known for some time that blood from young mice can positively impact aged animals, while blood from old mice has the opposite effect. Recent studies report that rejuvenating effects of young blood extend to multiple tissues and have identified GDF11 and CCL11 as factors mediating these effects.

Parabiosis is a surgical technique that involves joining the circulatory system of two animals such that they continuously exchange blood and other circulating factors. About a decade ago, this method was used to test whether the age of one animal has effects on the health of its partner through heterochronic parabiosis, where a young mouse shares its circulatory system with an old mouse. Strikingly, muscle stem cells and liver cells from the young mouse functioned less well, while the same cells from the old mouse showed molecular and functional evidence for rejuvenation (Conboy et al., 2005). Since then, similar effects have been demonstrated in other tissues including spinal cord (Ruckh et al., 2012), heart (Loffredo et al., 2013), and brain (Villeda et al., 2011). Recently, this work has been extended by the finding that injecting plasma from young mice is sufficient to enhance cognitive function and synaptic plasticity in aged mice (Villeda et al., 2014), and the identification of two molecules as key mediators of the beneficial and negative consequences from heterochronic parabiosis (Figure 1), growth differentiation factor 11 (GDF11) (Katsimpardi et al., 2014; Sinha et al., 2014), and C-C motif chemokine 11 (CCL11) (Villeda et al., 2011).

GDF11, a member of the TGF-β superfamily, declines in blood with age (Loffredo et al., 2013), and restoration of youthful levels of GDF11 are sufficient to