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GLUCAGON
METABOLIC REGULATOR CRITICAL FOR PATHOPHYSIOLOGY AND PHARMACOLOGY

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LOOK INSIDE FOR ANSWERS TO THESE QUESTIONS:

- Why don't changes in insulin fully explain changes in glycemic control?
- How is glucagon affected by therapies currently approved or under investigation?
- During studies in which a metabolic marker(s) is included, why consider glucagon?

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A Primary Role in Glucose Homeostasis

Despite the fact that glucagon was first identified and named in the 1920's (1), eventually purified and characterized around the 1950's (2, 3) and subsequently determined to play a fundamental role in the pathophysiology of diabetes in the 1970's (4), attention has largely focused on the glucose-lowering effects of insulin.

Insulin replacement has long been the established treatment for individuals with type 1 diabetes mellitus (T1DM) and while it has undoubtedly saved lives, treatment with exogenous insulin has not completely alleviated poor glycemic control and the risk of complications in these patients. As our understanding of metabolic physiology has evolved, so has our appreciation for pancreatic alpha cells. These cells are an integral part of the islets of Langerhans and secrete the hormone glucagon.

"treatment with exo-genous insulin has not completely alleviated poor glycemic control and the risk of complications"

Glucagon circulates at very low levels, approximately 10 pmol/L (35 pg/mL). The physiological range, including hyper- and hypoglycemic conditions, is reported to be 0 – 30 pmol/L (0 – 105 pg/mL). (5) The half-life of glucagon in circulation is approximately 7 minutes in humans, and is 2 and 5 minutes in rats and dogs, respectively (6). The glucagon receptor is a G-coupled protein receptor, highly expressed in the liver. Studies in rats have also shown the glucagon receptor to be expressed in stomach, lung, adrenal gland, kidney, small intestine, brain, heart, spleen, as well as brown and white adipose tissues (7, 8). Activation of the glucagon receptor leads to increases in cyclic AMP (cAMP) and activation of phospholipase C, both of which trigger complex signaling events leading to important actions in target tissues.

Glucagon's primary role is to stimulate hepatic glucose production (Figure 1) and it does so by activating glycogenolysis and gluconeogenesis, as well as inhibiting glycogenesis and glycolysis (6). Through this primary action, glucagon can prevent or rescue hypoglycemia. However, balance in this system is extremely important and glucagon must be regulated so as not to cause dangerously elevated levels of endogenous glucose production.

Glucagon is released from alpha cells in response to peripheral cues (e.g., plasma glucose and amino acid concentrations), as well as signaling from neighbors, beta (e.g., insulin, GABA and zinc ions) and delta (e.g., somatostatin) cells. Cross-talk with neighboring cells is not inconsequential as paracrine signaling by intra-islet insulin acts to suppress glucagon secretion from alpha cells following a sufficient rise in blood glucose in healthy individuals. In fact, confocal microscopy of single cells within cultured isolated islets revealed the ability of beta cells to wrap around alpha cells, further evidence of the intricate and functionally important interplay between these two cell types (9). There is no doubt that tight control of the insulin:glucagon ratio is critical for optimal glycemic control.

"Other glucagon-modulating factors have been identified since, including the satiety hormone leptin"

The regulation of glucagon secretion is complex and also involves the brain, autonomic nervous system, gut and even autocrine signaling (including intracellular circadian rhythm pathways) (10, 11). A number of stimulatory and inhibitory factors (including neurotransmitters) that regulate glucagon secretion can be found in a review by Dunning *et al.* (12). Other glucagon-modulating factors have been identified since, including the satiety hormone leptin, secreted by adipoctyes, which has been found to be a potent suppressor of glucagon (13).
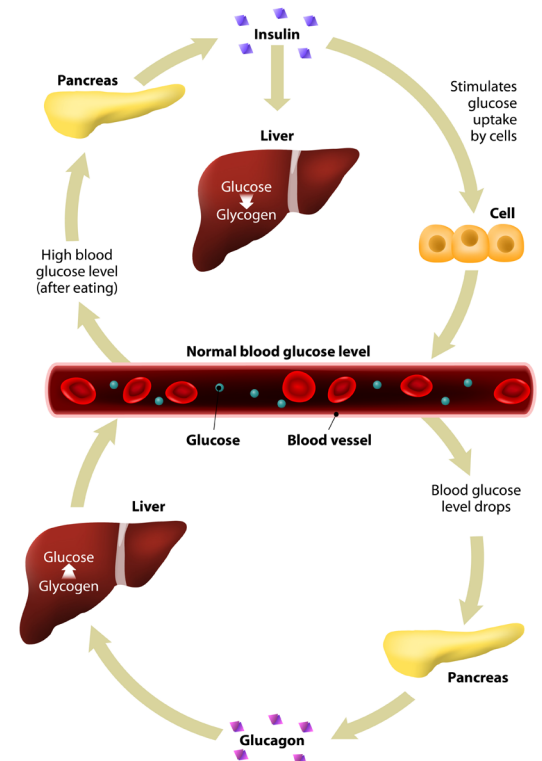

Figure 1. Insulin and Glucagon Regulate Blood Glucose Levels

Integral Part of Diabetes Etiology

Numerous studies have demonstrated that there is dysregulation of glucagon in all forms of diabetes, with the two most common forms being type 1 and type 2.

T1DM is a chronic disease, largely defined by a lack of insulin secretion leading to hyperglycemia. There is also a deleterious stimulation of glucagon during the postprandial period, rather than the appropriate suppression of glucagon seen in healthy individuals. Dysregulation of glucagon occurs early in the disease process and in a study by Fredheim *et al.*, postprandial glucagon increased 160% over a five year period after T1DM diagnosis in children. This dramatic increase was associated with worsening glycemic control and a deterioration of beta cell function. Results from this study also suggest that the physiological secretion of the incretin GLP-1 (glucagon-like peptide-1) after a meal is not sufficient to suppress glucagon secretion in these individuals. Thus, GLP-1 analogues may be an important addition to insulin therapy early in the T1DM disease process and warrants further investigation. (14)

Significant evidence that glucagon is an important target for T1DM comes from a study by Roger Unger and colleagues. They showed that eliminating glucagon action, either by inhibiting glucagon secretion or signaling, completely prevents hyperglycemia and restores HbA1c in animals with T1DM. Moreover, a glucagon receptor antibody completely suppressed the T1DM phenotype and did so in the absence of insulin. (15)

Autoimmunity plays a significant role in the development and progression of T1DM, with CD8 T cells being major players in the process (16). The activation of these cells contributes to the destruction of insulin-secreting beta cells. With the loss of sufficient and functioning beta cells comes a breakdown in communication within the islets and as a result, alpha cells do not get sufficient cues about current levels of glucose in circulation. Unfortunately, replacement of insulin through subcutaneous injection does not result in the same intra-islet hormone levels seen in healthy individuals. Recently, Mukherjee *et al.* found glucagon-specific CD8 T-cells in NOD mouse islets, raising the possibility that the alpha cell may also be targeted by the destructive autoimmune activation that wreaks havoc in pancreatic beta cells during T1DM progression (17).

In the case of type 2 diabetes mellitus (T2DM), hyperglycemia is a result of hyperglucagonemia in addition to a decrease in insulin sensitivity, and the two do not appear to be mutually exclusive.

In T2DM, insulin-sensitive cells in the periphery are no longer as responsive to insulin and therefore do not take up glucose sufficiently, resulting in more glucose in circulation. Similarly, alpha cells become insulin resistant and thus, do not respond to normal paracrine cues from the beta cells (18, 19). Like with T1DM, in T2DM the paradoxical increase in glucagon following a glucose load causes blood glucose levels to rise even further. This is significant to disease etiology as "hyperglucagonemia may be responsible for up to 50% of the glycemic excursion following an oral glucose challenge in patients with T2DM" (20).

As obesity rates in pediatric populations have increased, so has the incidence of T2DM. This has led scientists to examine whether the disease process in younger individuals resembles that which is seen in adults. A study by Manell et al. demonstrates that not only are fasting levels of glucagon elevated in obese adolescents with T2DM but plasma glucagon is also increased during the first 15 minutes after glucose intake, similar to what has been shown in adults with T2DM (21).

Many patients with T2DM must add insulin therapy to their treatment plans to compensate for the decline in beta cell function that can occur over time. In an interesting study by Ahlkvist *et al.*, chronic infusion of a stable glucagon analogue resulted in decreased insulin secretion after an oral or intravenous glucose challenge in glucose intolerant mice. The authors suggest that while indirect mechanisms cannot be ruled out, chronic elevation of glucagon during type 2 diabetes may directly contribute to the progressive beta cell dysfunction seen in this disease. (22)

Dynamic changes in both insulin and glucagon are fundamental to the progression of diabetes and thus, determination of the insulin:glucagon ratio has been used for many years in research as a more comprehensive metabolic index than measuring insulin alone (4, 23, 24, 25, 26). This ratio is not only important to the study of diabetes but provides critical insight into the storage and utilization of nutrients in normal as well as catabolic states such as infection, trauma, cancer, etc. (4). Furthermore, it has been suggested that "preclinical and clinical development programs for modern drugs should include detailed study of their effects on the insulin:glucagon axis: this will help to unravel new facets of human biochemistry and physiology" (27).

Research has shown that a beta cell-centric focus on glycemic control and diabetes is much too simplistic. This is also the case for other conditions that involve disturbances in glucose metabolism and fasting hyperglucagonemia such as end stage renal disease (ESRD) in nondiabetics, nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) (28, 29, 30). Understanding glucagon alterations under various pathophysiological conditions provides critical insight into what is driving changes in glycemic control and how those processes might be altered to benefit patients.

Connection to Therapeutics

There are numerous direct and indirect (and occasionally unexpected) connections between glucagon and a variety of therapeutic strategies.

Examples from the T1DM field highlight glucagon's critical counter-regulatory role.
For many years now, emergency glucagon kits have been available to help rescue diabetic patients from extreme hypoglycemia. Due to stability issues, these kits include lyophilized glucagon that must be reconstituted before an injection can be given. More recently, work has been done to develop more stable and/or less invasive modes of glucagon delivery including ready-to-use mini-dose glucagon pens for treatment of mild-to-moderate hypoglycemia (31) and glucagon powder that can be delivered via intranasal doses (32).

Some research groups developing a bionic or artificial pancreas have added glucagon to their automated delivery systems to address the issue of hypoglycemia and more closely mimic actions of the whole endocrine pancreas, not just the beta cells. Regulation of the delicate balance between insulin and glucagon in these carefully coordinated dual hormone systems is resulting in better glycemic control and fewer hypoglycemic events in patients with T1DM in clinical trials (33, 34).

"The importance of targeting glucagon in the treatment of type 2 diabetes mellitus (T2DM) is underscored by the fact that multiple efficacious therapies exert effects on this hormone"

Regulation of glycemia may not be glucagon's only role that is exploited in the treatment of T1DM. Great strides have been made in an effort to replace beta cells, particularly in the case of islet transplantation, but also in regenerative medicine. A recent study by Ye *et al.* demonstrated that the glucagon gene plays a critical role in mechanisms that govern cell fate and glucagon/GLP-1 signaling is required for transdifferentiation of alpha cells to beta cells. The authors conclude that their results support the notion that "alpha cells constitute an endogenous reservoir of new beta cells that is pharmacologically exploitable". (35)

The importance of targeting glucagon in the treatment of T2DM is underscored by the fact that multiple efficacious therapies exert effects on this hormone (Figure 2).
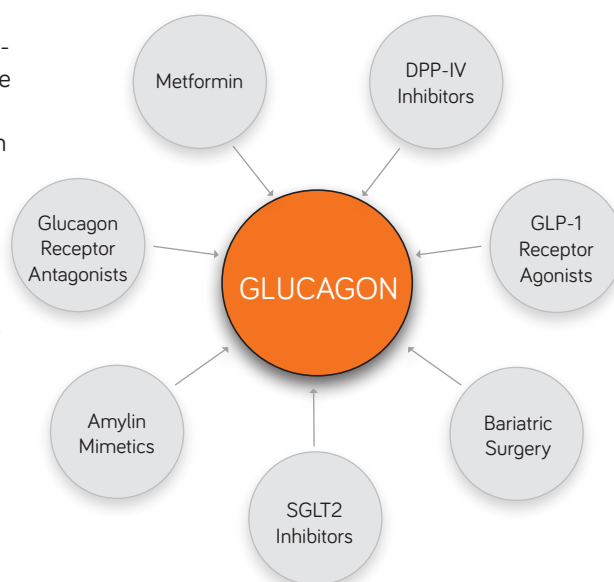

Figure 2. Glucagon is affected by a number of metabolic therapies

Glucagon action is being targeted directly through development of glucagon receptor antagonists, such as the previously mentioned glucagon receptor antibody (15). These antagonists, either alone or in combination with other compounds, have been very productive in reducing plasma glucose through reducing hepatic glucose output. One antagonist, a fully human monoclonal antibody that inhibits glucagon receptor signaling (REGN1193), produces beneficial effects on glycemia that may also be mediated through decreases in body weight and increases in GLP-1 secretion, in diabetic mice and cynomolgus monkeys (36).

"SGLT2 inhibitors have been shown to increase glucagon levels"

Metformin (part of the biguanide class of drugs) is the most commonly prescribed medication used to treat T2DM and is the "optimal drug for monotherapy" according to the Management of Hyperglycemia in Type 2 Diabetes, 2015 Position Statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) (37). With long-term administration, it may also be a beneficial adjunctive therapy for glycemic control in individuals with T1DM, as demonstrated by studies in pediatric and adult subjects (38, 39).

A study by Miller *et al.* demonstrated metformin's ability to antagonize glucagon signaling, uncovering a key mechanism by which metformin reduces hepatic glucose output (40).

More recently developed drug classes like DPP-4 inhibitors, GLP-1 receptor agonists and amylin mimetics work to re-establish normoglycemia in part through their ability to decrease glucagon secretion. However, the system may not be so cut-and-dry. Data from the LIraglutide and Beta-cell RepAir (LIBRA) trial demonstrated that chronic treatment with the GLP-1 receptor agonist liraglutide resulted in a paradoxical increase in post-challenge glucagonemia in patients with early T2DM (20). Conversely, GLP-1 receptor agonists have been combined with glucagon receptor agonists resulting in reduced adiposity and impro-ved glucose tolerance (41).

Glucagon has also been involved in off target effects of drugs. One very interesting and perhaps unexpected example comes from the study of a more novel class of diabetes drugs called SGLT2 inhibitors. Based on its predominant role in kidney-based glucose reabsorption, SGLT2 has become an attractive drug target and inhibitors of this pro-tein have been very productive at lowering blood glucose by increasing urinary excretion of glucose in diabetic patients. A number of drugs in this class have been approved or are in development pipelines (42).

Figure 3. Glucagon is regulated by gut hormones and peptides

So, what does a drug that targets a renal transporter have to do with glucagon? Through a variety of studies, SGLT2 inhibitors have been shown to increase glucagon levels (25, 26). Furthermore, SGLT2 expression in human pancreatic alpha cells has been reported (43). Could these drugs have a direct effect on alpha cells? Could their effect on glucagon be counteracting their potential for maximum effect in diabetic patients? These and other related questions are being exami-ned by research groups in order to gain a more complete understanding of how this class of drugs exerts its effects in the body. Additionally, SGLT2 inhibitors have been examined in combination with other diabetic drug classes, in an effort to approach glycemic control from multiple angles. Some of the combinations include drugs that directly or indirectly lower glucagon so it is of great importance to understand and monitor changes in glucagon during preclinical and clinical studies.
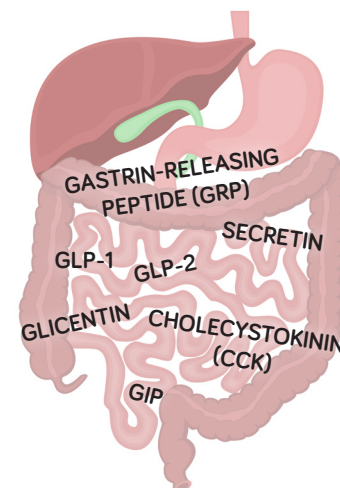
A very important non-pharmacological treatment has been shown to elicit effects on glucagon. Bariatric surgery is being extensively studied due to the dramatic improvements in weight and metabolic status following this type of procedure. Its efficacy to treat obesity has been proven but it has also been shown to have significant beneficial effects on co-morbidities such as diabetes. Gastric bypass, the most commonly performed bariatric surgery in those with morbid obesity, results in stimulation of GLP-1 secretion and sig-nificant effects on both beta and alpha cell function, with distinct changes in glucagon responses (44, 45).

The link between bariatric surgery and glucagon is not tangential due to the fact that glucagon is regula-ted by gut hormones (e.g., glicentin, GLP-1, GIP, GLP-2) and other peptides secreted by the gastrointestinal tract such as gastrin-releasing peptide (GRP), cholecystokinin (CCK) and secretin (46, 12, 47) (Figure 3). This may also be a compelling connection for the study of the gut microbiota/microbiome, its regulation by therapies (e.g., bariatric surgery) and endogenous factors (e.g., bile acids) and the metabolic effects thereof.

Beyond Glycemic Control

Glucagon is intimately involved in metabolic status and this association is not strictly related to hepatic glucose production.

It has long been known that glucagon has positive inotropic and chronotropic actions in cardiac muscle, producing effects similar to that of epinephrine (48, 49, 50, 51, 52). In fact, glucagon is used in the clinical treatment of beta blocker overdose (53).

There is research to suggest that under certain conditions, glucagon receptor signaling may exert negative effects on the heart (54) however, since these studies are limited and largely in animal models, further investigation is needed to fully understand the scope of glucagon actions in this organ.

Glucagon has been shown to have a hypocholesterolemic effect in rodents and humans (55, 56, 57) and, in rodents, chronic glucagon administration was shown to increase plasma cholesterol turnover (58). Glucagon has also been shown to regulate levels of PCSK9, an enzyme that is produced in the liver and can bind to and initiate the degradation of the LDL receptor leading to elevated levels of LDL in circulation. PCSK9 inhibitors are a new class of biologic drug that have been hailed, by some, as being revolutionary for the cardiovascular disease arena. These monoclonal antibodies produce dramatic decreases in circulating LDL by blocking PCSK9. Interestingly, in a study by Persson *et al.*, glucagon treatment led to a 70-75% decrease in PCSK9 gene expression in rats and further studies revealed that this may occur through SREBP-2-dependent and -independent pathways (59).

The connection between glucagon and cardiovascular health may be of particular interest to those involved in drug development as well as those studying populations at high risk for developing hypercholesterolemia. This applies to the study of traditional high-risk groups (e.g., obese, diabetic, individuals with metabolic syndrome) and non-traditional high-risk groups such as those with HIV/AIDS (60, 61, 62).

Glucagon has been implicated in a number of other processes including the regulation of food intake, energy expenditure and fat metabolism. For example, its ability to increase production of FGF21 is believed to be the mechanism by which glucagon increases lipolysis (63) and is essential for adaptive thermogenesis in brown adipose tissue (64).

Deterioration of metabolic health is a key component of the aging process and a number of studies have shown that decreased sensitivity to glucagon may play an important role. This is highlighted by research demonstrating that glucagon's regulation of autophagy may be a mechanism behind the anti-aging benefits of caloric restriction. (65, 66, 67)

Conclusion

Glucagon's actions and its regulation are quite complex and intimately linked to metabolism. The recent, renewed interest in glucagon has prompted scientists to refer to it as a long-forgotten hormone, a resurrected hormone and even "the 'new' insulin in the pathophysiology of diabetes" (68).

One thing is certain: Glucagon is a critically important metabolic regulator, an attractive target for therapies, and should be an integral part of the study of diabetes, other metabolic conditions, and related therapies.

For research-based presentations on topics covered in this white paper, visit www.mercodia.com/webinars.

NOTE: Glucagon measurement has been difficult in the past due to multiple analytical obstacles. Mercodia's white paper entitled "Glucagon Measurement: Addressing Long-Standing Analytical Challenges" provides information about those obstacles and novel analytical solutions.

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