Mercodia

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White Paper
Important aspects to consider when measuring GLP-1

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It is amazing how fast it happened. From initial reports about the incretin Glucagon-Like Peptide (GLP-1) with glucose lowering effects in the late 1980's, until the time point when the first GLP-1 receptor agonist exenatide to treat Type 2 Diabetes Mellitus (T2DM) was launched onto the market in 2005. The story is continuously developing as we learn more about the fascinating, multi-tasking incretin GLP-1.
But to acquire more knowledge, it is crucial to design relevant studies and to use measurement methods that ensure scientists know exactly what form(s) of GLP-1 they are measuring. This white paper addresses some of the important analytical factors to consider when measuring circulating GLP-1, from degradation to the different forms of GLP-1.

An incretin hormone

Incretin hormones are released in the gut and stimulate glucose-dependent insulin secretion in response to food intake. The two major incretin hormones are GLP-1 and Glucose-dependent Insulinotropic Peptide (GIP). They execute their actions by binding to their respective receptors (GLP-1R and GIP-R) and activating signaling inside target cells. Both hormones stimulate insulin secretion, but a major difference between them is that GIP enhances the postprandial glucagon response, while GLP-1 suppresses it. Because of the ability of GLP-1 to decrease blood sugar levels in a glucose-dependent manner by enhancing the secretion of insulin and suppressing the glucagon response, GLP-1 receptor agonists have attracted attention for their potential to treat T2DM patients.

GLP-1 is also a satiety-signaling hormone[1]. Obese patients can have high levels of GLP-1 and still not feel satisfied, indicating that they may suffer from GLP-1 insensitivity, much like T2DM patients are insensitive to insulin[2-4]. GLP-1 satiety-related signaling is likely to be mediated through the nervous system via the vagus nerve[5,6].

Processing of proglucagon to GLP-1

GLP-1 is derived from proglucagon, which is also the precursor for several other peptides such as glucagon, GLP-2, oxyntomodulin, glicentin, and major proglucagon fragment (MPGF). MPGF shares sequences with both GLP-1 and GLP-2 and is a product released mainly from pancreatic cells. Depending on the type of prohormone convertase (PC) present in the cell, proglucagon takes different paths in its posttranslational processing. The L-cells in the gastrointestinal tract predominantly express PC 1/3 and process proglucagon into GLP-1 as well as GLP-2, oxyntomodulin and glicentin. Expression of PC 2, as in pancreatic alpha-cells, results in MPGF and glucagon. (Fig. 1)

The fact that MPGF shares sequence similarity with GLP-1 must be considered when measuring GLP-1. Cross-reactivity to MPGF should be evaluated to determine if GLP-1 measurement is specific enough. Assays detecting amidated GLP-1 have an advantage in this respect, since they are less likely to suffer from cross-reactivity to MPGF due to the C-terminal specificity of the antibodies.

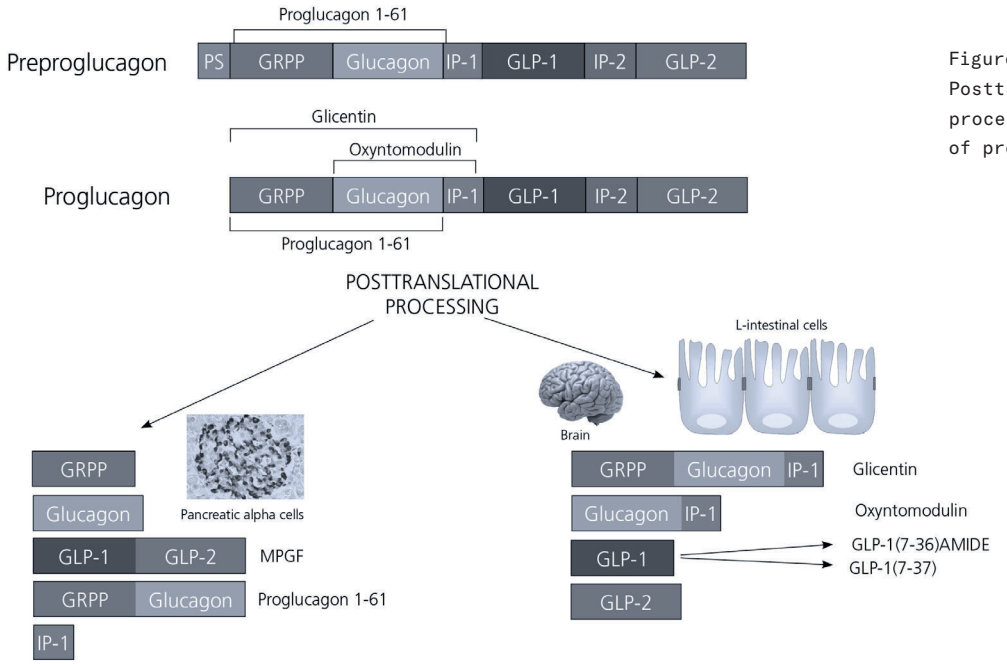

Figure 1. Posttranslational processing of proglucagon

Mercodia has developed a GLP-1 assay that has no cross-reactivity to MPGF (Fig. 2). The use of this assay will help scientists to avoid drawing a wrong conclusion from inaccurate data, which may be generated from other less specific commercially available tests.

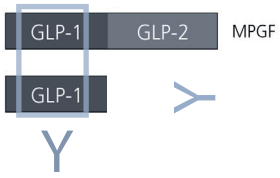

Figure 2. Positional binding of monoclonal antibodies in the Mercodia GLP-1 NL-ELISA

Amidation dominates

GLP-1(1-37) corresponds to amino acid residues 72-108 of proglucagon, but this is an inactive form of GLP-1. GLP-1 needs to be further processed into GLP-1(7-37) or GLP-1(7-36) amide to become active, with the latter version being amidated after removal of the C-terminal glycine residue. Both isoforms have been shown to be equally potent in activating the GLP-1R, and the active forms are sometimes also referred to as "intact" GLP-1[7,8]. GLP-1(7-36) amide is the major secretory isoform, and it has been shown that the levels of circulating amidated GLP-1 change significantly upon stimulation, while the levels of glycine-extended GLP-1(7-37) remain relatively unchanged[7].

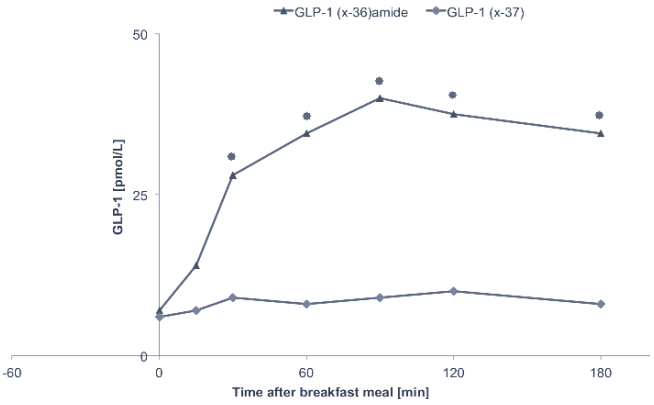

Figure 3. The amidated forms of GLP-1, (x-36) amide, rise in the circulation postprandially, while there is very little change in the glycine-extended isoforms (x-37)1. This means that in order to detect the changes in GLP-1 concentration that occur after a meal, one must measure the amidated isoforms of GLP-1. [7]

In fact, most GLP-1 leaving the gut is amidated (»80%). When GLP-1 secretion needs to be measured, it is important to accurately detect the amidated isoforms, since they predominate. It has been established that some commercially available assays lack specificity to detect the amidated isoforms, and as a consequence these assays will underestimate the true levels of GLP-1[9].

DPP-IV degradation is fast

Upon stimulation, GLP-1(7-37) and GLP-1(7-36) amide is secreted. However, cleavage by the enzyme dipeptidyl peptidase IV (DPP-IV), which is expressed near the site of GLP-1 secretion from the enteroendocrine L-cells, results in a short GLP-1 half-life of only a couple of minutes. DPP-IV is responsible for creating the metabolites GLP-1(9-37) and GLP-1(9-36) amide. Traditionally, the metabolites have been considered inactive, but several studies performed in dogs, rats and mice suggest that GLP-1(9-36) amide have cardioprotective effects[10-12]. Other studies point towards that GLP-1 receptor stimulation by exendin-4[13] and treatment with GLP-1(9-36) amide[14], have neuroprotective effects in Alzheimer's disease models, including both cultured cells and mice.

Since GLP-1(7-36) amide is rapidly degraded, its concentration in plasma is very low and small meals resulting in minor changes in GLP-1 concentrations can be difficult to detect. However, measuring both the concentration of (7-36) amide and the metabolite (9-36) amide, together called "total" GLP-1, provides a better chance of detecting small changes. In fact, for most applications it is best to measure total GLP-1, since it will not only reflect the levels of secretion but also provide a good picture of the actions already performed upstream. Detecting circulating GLP-1(9-36) amide tells us that GLP-1 was secreted and that the brain received its signal via the vagus nerve. Thus, we are studying both secretion and actions in an easy and comprehensive way. Certainly, for some applications, specific (7-36) amide assays may be needed, e.g., when studying DPP-IV inhibitors.

Several commercial assays measuring GLP-1 were evaluated in a publication by Bak et al in 2014, both examining performances of the assays and the implications for clinical studies.[9] The results showed that the specificity and sensitivity of commercially available kits for the analysis of GLP-1 levels varied considerably. One assay detected none of the tested synthetic GLP-1 isoforms (GLP-1(1-36) amide, (7-36) amide, (9-36) amide, (1-37), (7-37) and (9-37)). Other assays had low recovery of non-active forms in plasma, some only detected amidated GLP-1. This indicates that the variable performance of the tested assays should be taken into account when selecting which assay to use and when comparing data from different studies.

The molecule (7-36) amide will have the same chance to be detected as the molecule (9-36) amide. This is, of course, only true if the specificity to GLP-1(7-36) amide is the same as to GLP-1(9-36) amide. If the assay specificity is only 50% to the metabolite, the advantage is gone. GLP-1 levels will then be underestimated and any changes could go unnoticed, making such an assay suboptimal. When measuring total GLP-1 with the Mercodia Total GLP-1 NL-ELISA, the risk of pre-analytical errors is minimized, since degradation of GLP-1 (7-36) will not affect the results.

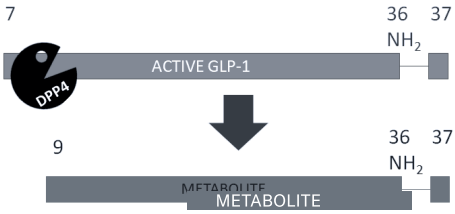

Figure 4. Active GLP-1 is degraded within minutes.

When to measure total GLP-1?

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Wide range of physiological actions

GLP-1 is being studied in many different research settings due to the fact that it seems to affect a variety of physiological processes. Although the pancreatic islet cells are the major targets for GLP-1, it also inhibits gastrin-induced acid secretion[15], slows gastric emptying, inhibits motility of the stomach[16,17], and affects food intake and body weight[18]. All of these actions combined with GLP-1's role in insulin stimulation and glucagon inhibition have established GLP-1 as a critical metabolic hormone. It has also been shown that GLP-1 improves flow mediated dilation (a phenomenon in which a vessel dilates or expands when blood flow increases through it) and has cardiovascular protective effects19, as well as other functions such as expansion and preservation of pancreatic β-cell mass20-22, bone metabolism[23] and neuroprotection[24]. GLP-1 is a versatile hormone whose function needs further exploration within many research areas.

Different physiological levels of GLP-1

GLP-1 circulates at low concentrations in the blood, typically in the range of 0.9-18 pmol/L7. Since the levels of GLP-1 in healthy individuals are low, there is a strong need for sensitive methods to accurately detect GLP-1 as a biomarker in human plasma, with a starting point below 1 pmol/L.

In certain patient groups, GLP-1 is present in abnormal concentrations. Patients which have undergone bariatric surgery show highly increased levels of GLP-1 after a meal; concentrations up to 300 pmol/L have been reported[25,26]. Considering the unquestionable effect of bariatric surgery on GLP-1 levels in these individuals, combined with the fact that insulin levels normalize in most patients within days after surgery, long before any significant weight loss has occurred, it will be interesting to follow research in this field as more facts about GLP-1 and its actions are revealed. These findings will contribute to the understanding of how to develop better drugs for treatment of both diabetes and obesity, emphasizing the importance of accurate measurement of GLP-1.

Another patient group with reported abnormal GLP-1 levels is T2DM patients. However, the deviation, from normal levels, is far from what has been reported in the bariatric surgery patient population and not always consistent across studies. It has been suggested that it is not the fasting GLP-1 levels that differ between diabetic and normal glucose tolerant patients, but that the postprandial GLP-1 levels are significantly lower in the diabetic group [27,28]. This implies that GLP-1 secretion is impaired in this group of patients.

Treatments addressing GLP-1 function

Due to the blood glucose lowering effect and the impact of GLP-1 on several important metabolic processes, the interest in therapeutically targeting this system has expanded over the last decade. Since both GLP-1 and GIP are degraded by DPP-IV within minutes of secretion, large efforts have also been taken to develop drugs inhibiting the activity of DPP-IV. GLP-1 and DPP-IV based therapies are approved for the treatment of patients with type 2 diabetes, including GLP-1 receptor agonists exenatide, liraglutide, dulaglutide, albiglutide and lixisenatide and DPP-IV inhibitors alogliptin, sitagliptin, vildagliptin, saxagliptin, teneligliptin, gemigliptin and linagliptin[29]. The functional mechanism of all these drugs is to increase the physiological actions of GLP-1. GLP-1 receptor agonists (GLP-1 RA) are also approved for the treatment of obesity, because of GLP-1's satiety-signaling effects.

Additional strategies have been evaluated to further enhance the GLP-1 effect by developing dual agonists (e.g., GLP-1/GIP receptor agonists)[30-32]. Dual incretin agonists are sometimes referred to as "Twincretins", describing the combination of activation of the sister incretins GLP-1- and GIP-receptors from one molecule. Results from studies of GLP-1/glucagon combinational therapies indicate that combining the effects of GLP-1 with the lipolytic effects of sustained glucagon receptor activation lower body weight more than what is seen with a single selective GLP-1 agonist[33].

Could a triple agonist be the optimal treatment? In 2015, Brian Finan and colleagues published a study with a triple agonist acting on the GLP-1-receptor, GIP receptor and glucagon receptor[34]. The triple agonist effectively reduced body weight and diabetic complications in rodent models of obesity, and was proven to be superior to any existing dual coagonists and best available monoagonists[34,35]. The scientists behind this monomeric peptide speculate that it "may provide unprecedented opportunities in the personalized treatment of heterogeneous metabolic diseases"[36].

Conclusion

In the last few years, expansion within the incretin field has led to new insights about GLP-1 function and relevance. The key role of GLP-1 in many metabolic processes and the complex physiological interplay with its sister incretin GIP, open a window for new promising therapeutic approaches to the treatment of diabetes and obesity. To continue these advancements, methods to measure GLP-1 must be chosen carefully and the high demands for specificity and sensitivity must be met.

For more information on GLP-1, please visit the following Mercodia resources.

Mercodia webinars

www.mercodia.com/webinars
Dr. Jens Juul Holst: GLP-1 Secretion in Humans: Recent Advances:

During this webinar, one of the foremost experts on GLP-1 focuses on how the secretion of this gut hormone is regulated, including well-established and novel pathways.

Mercodia Total GLP-1 NL-ELISA

https://www.mercodia.se/mercodia-total-glp-1-nl-elisa

Mercodia Total GLP-1 NL-ELISA provides a chemiluminescent method for the quantitative determination of amidated GLP-1 (glucagon-like-peptide-1) in human serum or plasma samples.

The assay has a low sample volume of 25 µL, a broad measuring range (0.9 - 940 pmol/L) and a low functional sensitivity (LLOQ 1 pmol/L), allowing for precise detection in both fasting samples as well as samples with highly elevated levels.

Mercodia GLP-1 (9-36) amide control - low, medium, high

https://www.mercodia.se/mercodia-glp-1-9-36-amide-control-low-medium-high

Each Mercodia GLP-1 (9-36) amide Control (Low, Medium, High) contains 3 vials of stabilized serum controls. The content of each vial is lyophilized, containing 500 µL after reconstitution.

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