Application Note

GLP-1 assays optimized for the Gyrolab® platform compare favorably to Mesoscale Discovery (MSD) assays for GLP-1

Glucagon-like peptide 1 (GLP-1) is a gut peptide and incretin hormone that stimulates insulin production in response to glucose and reduces appetite and food intake. As of 2015, six GLP-1 receptor agonists were either on the market or in development for the treatment of type 2 diabetes[1].

GLP-1 is found in multiple active forms, GLP-1 (7-36) amide, GLP-1 (7-37), GLP-1 (1-36) amide, and GLP-1 (1-37)[2]. The enzyme, dipeptidyl peptidase IV (DPP IV) processes the active peptides into GLP-1 (9-36) amide and (9-37), which make up the majority of the GLP-1 isoforms in circulation[3]. While only GLP-1 7-36 (amide) and GLP-1 (7-37) stimulate insulin production, the other isoforms have been shown to have extrapancreatic functions[4].

Because of the importance of GLP-1 and because of how widely it is used as a biomarker in drug development programs, we developed analytically validated, commercially available assays for quantifying total and active GLP-1 on the nanoliter-scale, high-throughput Gyrolab® xP Workstation platform, namely GyroMark™ HT assays.

The Gyrolab® xP Workstation is an automated, compact, disc-based nanotechnology that uses microfluidic technology in a sandwich ELISA format using a biotinylated capture antibody and a fluorescence-labeled detection antibody. The technology has several advantages, including small sample volume (1000 nL), high sensitivity, broad dynamic range, and short assay time (1 hour per assay).

Given the low levels of GLP-1 circulating in the body (0–15 pmol/L for active GLP-1 (7-36 and 7-37) and 5–80 pmol/L for total GLP-1), it can be challenging to develop an assay for GLP-1 that is sufficiently specific and sensitive to enable effective downstream decisions in research or drug development[5]. Previously, we showed that the newly developed GLP-1 assays for the Gyrolab® platform were well-correlated to our widely published and cited GLP-1 ELISAs (data presented at the Gyrolab® Users Meeting, 2014). In this study, we compared our Gyrolab® platform assays to commercially available GLP-1 assays for the Mesoscale Discovery (MSD) platform in order to demonstrate assay specificity, sensitivity and precision.

Materials and Methods

To determine the changes GLP-1 levels with respect to food intake, blood samples were collected from normal subjects (n=20) after overnight fasting or two hours after eating a standard breakfast. The protease inhibitors (DPPIV inhibitor, AEBSF, and protease inhibitor cocktail) were immediately added in the collected blood samples. The serum and plasma samples were separated and measured with the following assays: GyroMark™ HT GLP-1 Active Assay (Cat. No. GYGLP1A-35K), GyroMark™ HT GLP-1 Total Assay (Cat. No. GYGLP1T-36K), the MSD

Active-GLP-1 (7-36) Amide Kit (Mesoscale Discovery, Cat. No. K150HYC-4), the MSD Total GLP-1 (v2) Kit (Mesocale Discovery, Cat. No. K150JVC-1), and the MSD Active GLP-1 (ver. 2) Kit (Mesocale Discovery, Cat. No. K150JWC-1). MSD assays for GLP-1 have been previously cited in published studies involving GLP-1[6,7,others].

General method for GyroMark™ HT Assays

Every GyroMark™ HT assay includes a biotin-labeled capture antibody in buffer, bound to streptavidin coated particles in a Gyrolab Bioaffy™ CD.

All experimental manipulations were performed by programming the Gyrolab® XP workstation using the Bioaffy™ 1000 3-step C-A-D wash station wiz v2. Serial dilutions of standard prepared in buffer and diluted samples were first bound to the capture antibody by loading onto the CD. A dye-labeled detection antibody diluted in buffer was then applied to the bound standards and samples. The resulting fluorescence signal was detected and quantified by the fully automated workstation.

Buffers used:

Wash Buffer 1:
Phosphate-buffered saline with 0.01% Tween® 20

Wash Buffer 2:
Gyros® pH 11 buffer

Results

A recent study showed that GLP-1 assays from various manufacturers exhibited high intra-assay variability and also showed discrepancies between published assay specifications and actual performance[5]. In particular, published specifications frequently omitted cross-reactivity information for the various GLP-1 isoforms. We initiated the comparison between the MSD GLP-1 assays and our new GyroMark™ assays by first comparing the published specifications (Table 1). While the MSD Active GLP-1 (ver. 2) Kit was marketed to recognize all forms of insulinogenic GLP-1, cross-reactivity with the 7-37 was only reported to be 31%.

Form	MSD Active GLP-1 v2 Assay	MSD GLP-1 (7-36) Amide Assay	GyroMark™ HT Active GLP-1 Assay
GLP-1 (7-36) amide	100%	100%	100%
GLP-1 (9-36) amide	< 0.1%	< 0.1%	< 0.1%
GLP-1 (1-36) amide	< 0.1%	< 0.1%	< 0.1%
GLP-1 (7-37)	31%	< 0.1%	100%
GLP-1 (1-37)	< 0.1%	< 0.1%	< 0.1%

Table 1. Reactivity of GLP-1 (active) assays with various isoforms of GLP-1, as reported by manufacturer.

Good correlation between GyroMark™ HT Active GLP-1 vs. MSD Active GLP-1 (ver. 2) Assays

Levels of active GLP-1 in 32 samples as measured by GyroMark™ HT and MSD assays correlated very well between platforms (r=0.98; Figure 1). The MSD GLP-1 standard (included with the assay kit) was 3-4 times more potent than the GyroMark™ HT standard, with samples demonstrating 3-4 fold higher sample concentration in the GyroMark™ HT assay compared to the MSD assay. This concentration difference was due to the difference in standard potency and also the cross-reactivity variation described in Table 1. The GyroMark™ HT assay cross-reacted with the 7-36 (amide) isoform more than did the MSD Active GLP-1 (ver. 2) assay.

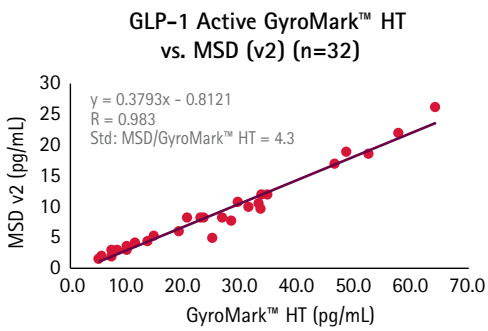

Figure 1.
Active GLP-1 measured in 32 samples, using either the MSD Active GLP-1 (ver. 2) assay or the GyroMark™ HT Active GLP-1 assay.

Poor correlation: MSD Active GLP-1 (7-36) Amide Assay vs. assays for active GLP-1

Given the difference in assay cross-reactivity, we did not expect to measure similar GLP-1 levels using the MSD Active GLP-1 (7-36) Amide Assay and other assays for active GLP-1 (both MSD and GyroMark™ HT assays) which reacted with more than one isoform of active GLP-1. Accordingly, comparative data showed poor correlation between the MSD Active GLP-1 (7-36) Amide Assay and both the GyroMark™ HT and MSD assay kits for active GLP-1 (Figure 2).

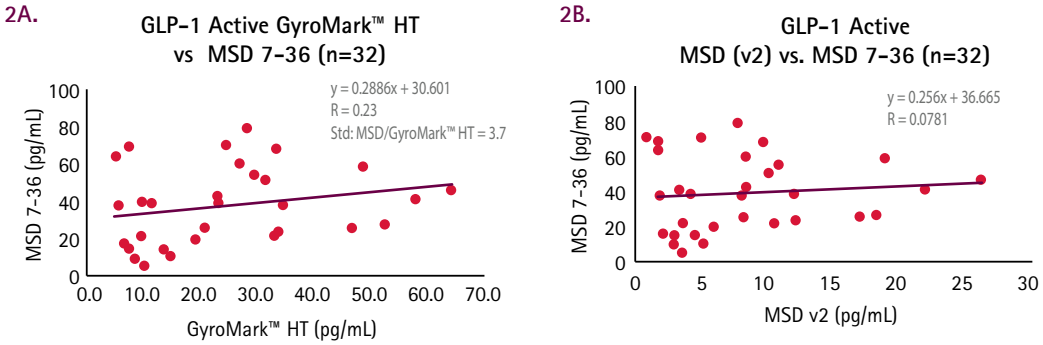

Figure 2.

Levels of GLP-1 (7-36) amide measured in 32 samples using the MSD kit were compared to GLP-1 levels measured using the GyroMark™ HT Active GLP-1 Assay (2A) and the MSD Active GLP-1 (ver. 2) Kit (2B).

Total GLP-1 Assay Kits: comparing published specifications

The MSD Total GLP-1 assay kit, marketed to be suitable for determination of GLP-1 total, was accompanied by published specifications stating that GLP-1 (9-36) amide cross-reactivity was 38% and that the assay showed less cross-reactivity with other isoforms. This meant that the true total GLP-1 levels would be underestimated, because the (9-36) amide isoform was the major circulating form of GLP-1 (Table 2).

Form	MSD Total GLP-1 (v2) Kit	GyroMark™ HT Total GLP-1 Assay
GLP-1 (7-36) amide	100%	96%
GLP-1 (9-36) amide	38%	100%
GLP-1 (1-36) amide	25%	10%
GLP-1 (7-37)	34%	100%
GLP-1 (1-37)	15%	100%

Table 2. Reactivity of GLP-1 (total) assays with various isoforms of GLP-1, as reported by manufacturer.

Good correlation between GyroMark™ HT Total GLP-1 vs. MSD Total GLP-1 (ver. 2) Assays

Levels of total GLP-1 in 32 samples as measured using GyroMark™ HT and MSD assays for total GLP-1 correlated very well (r=0.97). As in Figure 1, the MSD total GLP-1 standard was 3 times more potent than the GyroMark™ HT standard, resulting in 3-4 fold higher calculated GLP-1 levels in GyroMark™ HT assays compared to MSD. This difference was not only due to the difference standard potency but also due to the cross-reactivity variation shown in Table 2. Namely, it was likely that the MSD Total GLP-1 (ver. 2) Kit did not detect the total level of GLP-1 present in each sample because of low cross-reactivity of GLP-1 (Table 2).

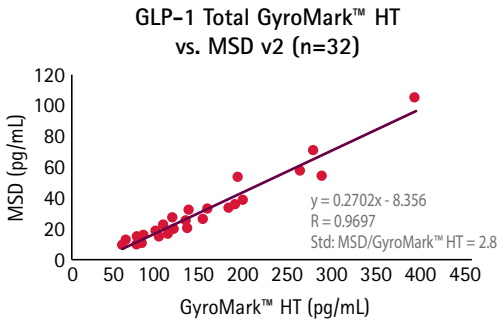

Figure 3.

Total GLP-1 measured in 32 samples, using either the MSD Total GLP-1 (ver. 2) assay or the GyroMark™ HT Total GLP-1 assay.

Comparing active vs. total GLP-1: confounding results using MSD assays

To assess the physiological relevance of each set of GLP-1 assays (GyroMark™ HT assays and MSD assays), we compared the average calculated active GLP-1 levels and total GLP-1 levels of the 32 samples. As expected, levels of active GLP-1 were lower than total GLP-1 levels using the GyroMark™ HT kits (Figure 4A). Levels of active GLP-1 were also lower than total GLP-1 levels using the MSD Active GLP-1 (ver. 2) and MSD Total GLP-1 (v2) Kits (Figure 4B).

However, the MSD assay results showed that GLP-1 active (7-36) amide levels were unusually high compared to GLP-1 levels obtained using the MSD Active GLP-1 (ver. 2) and Total GLP-1 (v2) Kits (Figure 4B). If the (7-36) amide assay is claiming to be more specific than the MSD Active GLP-1 (ver.2) Kit, GLP-1 (7-36) amide levels should be lower. Moreover, GLP-1 Active levels should never be higher than GLP-1 total levels. The fact that this is the case indicates that the results of one or more MSD kits may not be relevant to physiological studies of GLP-1.

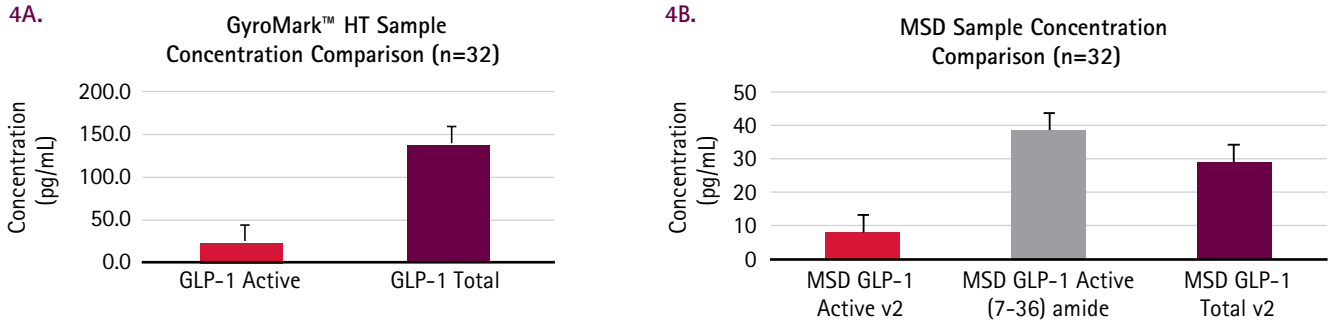

Figure 4.

Levels of active and total GLP-1 as measured using GyroMark™ HT kits (4A) and MSD kits (4B).

Comparing GLP-1 in fasting and postprandial subjects: less significant difference using MSD GLP-1 (7-36) Amide Kit

Another way to biologically qualify a GLP-1 assay is to examine the measured difference in active GLP-1 in postprandial subjects compared to fasting subjects. As expected, in serum and plasma samples obtained from nine fasting and postprandial subjects, levels of active GLP-1 were elevated (as measured using the GyroMark™ HT Active GLP-1 Assay and using the MSD Active GLP-1 (ver. 2) Kit; Figures 5A and 5B). However, there was a much less significant difference in active GLP-1 levels observed in postprandial vs. fasting subjects when the MSD Active GLP-1 (7-36) Amide Kit was used (Figure 5C).

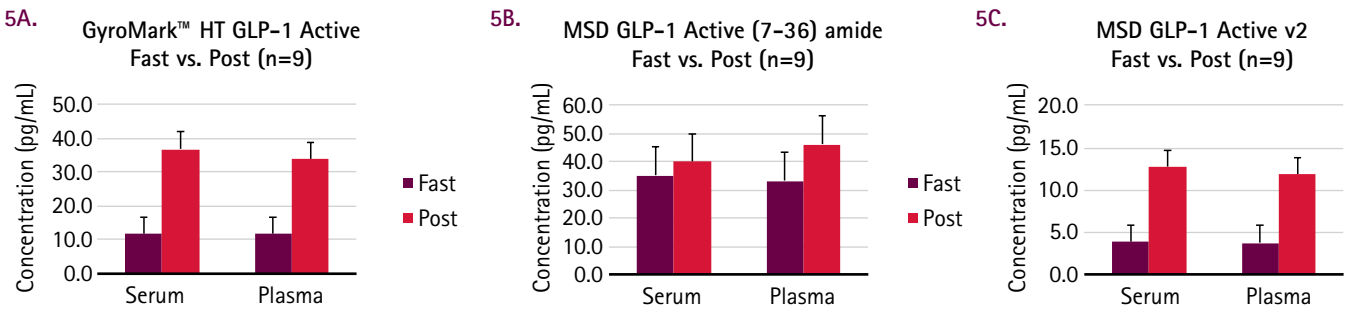

Figure 5.

GLP-1 levels in fasting and postprandial subjects compared using the GyroMark™ HT Active GLP-1 Assay, the MSD Active GLP-1 (ver. 2) Kit, and the MSD Active GLP-1 (7-36) Amide Kit. The MSD GLP-1 Active (7-36) Amide Kit showed a less significant difference in active GLP-1 levels between the two experimental conditions.

Conclusion

The new GyroMark™ HT assays for total and active GLP-1 enable any laboratory to take advantage of the high throughput Gyrolab® platform to quantify this important endocrine biomarker in extremely small sample volumes. Our analytical and biological validation data have demonstrated that this GLP-1 assay for the Gyrolab® platform is sensitive, accurate, and reproducible. Comparing the data with GLP-1 levels quantitated using the Meso Scale Discovery platform assays for GLP-1 revealed that users of commercially available assays should carefully review product specifications and performance claims, and they should consider the stringency with which manufacturers validate their products.

References

1. Trujillo JM, Nuffer W, Ellis SL. GLP-1 receptor agonists: a review of head-to-head clinical studies. Ther Adv Endocrinol Metab. 2015 Feb; 6(1):19-28.
2. Koole C, Wootten D, Simms J, Miller LJ, Christopoulos A, Sexton PM. Differential Impact of Amino Acid Substitutions on Critical Residues of the Human Glucagon-Like Peptide-1 Receptor (GLP-1R) Involved in Peptide Activity and Small Molecule Allostery. J Pharmacol Exp Ther. 2015 Jan 28.
3. Deacon CF. Circulation and degradation of GIP and GLP-1. Horm Metab Res. 2004 Nov-Dec; 36(11-12):761-5. Review.
4. Sharma R, McDonald TS, Eng H, Limberakis C, Stevens BD, Patel S, Kalgutkar AS. In vitro metabolism of the glucagon-like peptide-1 (GLP-1)-derived metabolites GLP-1(9-36) amide and GLP-1(28-36) amide in mouse and human hepatocytes. Drug Metab Dispos. 2013 Dec; 41(12):2148-57.
5. Bak MJ, Wewer Albrechtsen NJ, Pedersen J, Knop FK, Vilsbøll T, Jørgensen NB, Hartmann B, Deacon CF, Dragsted LO, Holst JJ. Specificity and sensitivity of commercially available assays for glucagon-like peptide-1 (GLP-1): implications for GLP-1 measurements in clinical studies. Diabetes Obes Metab. 2014 Nov; 16(11):1155-64.
6. Tolhurst G, Heffron H, Lam YS, Parker HE, Habib AM, Diakogiannaki E, Cameron J, Grosse J, Reimann F, Gribble FM. Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. Diabetes. 2012 Feb; 61(2):364-71.
7. Villareal DT, Robertson H, Bell GI, Patterson BW, Tran H, Wice B, Polonsky KS. TCF7L2 variant rs7903146 affects the risk of type 2 diabetes by modulating incretin action. Diabetes. 2010 Feb; 59(2):479-85.

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Lit No. AN5736ENEU BS-GEN-15-11155 4/2015 Printed in the USA.