Activation of GLP-1 and Associated Genes in L-cells Using the MindBody GLP-1 System™*§

Objective: To evaluate and optimize the effects of MindBody GLP-1 System on targeted gene activation and GLP-1 secretion in an *in vitro* L-cell model.

Sponsor: LifeVantage Corp.

Principal Investigator: LifeVantage Corp.

Reference: LifeVantage Corp., Lehi, Utah 84043, LV-57

Introduction

The right nutrition is essential for life. Modern society has made it easy to gather and overconsume food that is high in calories, sugar, and unhealthy fat but low in nutritional value, which can result in adding extra pounds to the human frame in the form of fat.

The ease of obtaining excess food and a lack of physical exercise is contributing to the epidemic of overweight that can be observed throughout the US and the world.

Our body evolved to take plant and animal components and convert them into nutrients the body uses to elicit various chemical reactions pertinent to our survival. This process requires all our organs to function as effectively as possible in order to break down nutrients into meaningful cell signaling molecules.

Our digestive tract is responsible for metabolizing food into usable forms. The cells in our digestive tract have highly specialized functions that are in a constant state of communication. This can be in the form of direct connections via nerves, signaling throughout the body by hormones and other signaling molecules, or through an intricate network of internal cellular communication referred to as cellular signaling.

These highly specialized cells in the gastrointestinal (GI) tract are called enteroendocrine cells (EC) and the GI tract is lined with many types of EC cells, one of which is called an L-cell. These EC cells release hormones or signaling peptides to initiate digestive actions or protective responses. Bacteria and other microorganisms in our microbiome also play a critical role in these responses because of their short chain fatty acid fermentation products, which act as stimuli to some of the cells. For healthy digestion, we require the function and interaction of all these cell types.

One of the hormones produced by cells in the digestive tract is GLP-1 (glucagon-like peptide-1). Like a lot of things in our body, GLP-1 production slows down due to a variety of factors such as age, environment, stress, and diet. All of these can negatively impact GLP-1 production. One downside of an imbalanced GLP-1 response in our bodies is that the metabolism slows down, leading to overeating because the brain isn't getting the message that enough food has been consumed.

WHAT IS GLP-1?

The hormone GLP-1 is part of the metabolic hormones called incretin. Incretins are released in response to nutrients, primarily glucose and fats, and they elicit an insulin response. GLP-1 has a short half-life of about 1–2 minutes once released into the hepatic portal system due to its breakdown by an enzyme called dipeptidyl peptidase-4 (DPP-4).

Some GLP-1 is also produced in the brain. GLP-1 can easily cross the blood-brain-barrier by simple diffusion and becomes an important factor in the gut-brain axis, a 2-way communication system between the gut and the brain.

GLP-1 binds to the GLP-1 receptor GLP1R, which is expressed in a variety of tissues such as pancreas, heart, kidneys, stomach, intestines, pituitary, hypothalamus, and Vagus nerve where it elicits various chemical signaling processes. Its main functions are as listed below:

- *Decrease blood sugar concentration:* GLP-1 enhances the secretion of insulin from pancreatic beta-cells as a response to increased blood glucose levels, and this enhances glucose-dependent insulin secretion.

§ Results based on a cell culture study on active ingredient blends in the MindBody GLP-1 System.

- *Inhibits glucagon release:* GLP-1 suppresses glucagon secretion from pancreatic alpha-cells, further reducing glucose production in the liver.

- *Slows gastric emptying*: GLP-1 delays gastric emptying of the stomach, which helps reduce postprandial blood glucose spikes.

- *Promotes satiety:* GLP-1 interacts with central nervous system receptors via the gut-brain axis to promote feelings of fullness and reduce appetite.

Another hormone produced by the L-cells is peptide YY (PYY). It is closely related to the pancreatic peptide family and shows structural similarities to pancreatic polypeptide Y (PPY) and other neuropeptides that bridge the brain-gut axis and, thus, talk to the brain regarding food cravings and hunger.

The Mindbody GLP-1 System™ In Vitro Study

LifeVantage approached developing the MindBody GLP-1 System from the understanding that there are different ways to activate the GLP-1 hormone and its pathway. One from the direct stimulation and activation of the L-cells and the genes involved to produce GLP-1 and the second via stimulating the microbiome to produce nutrients required for L-cells to activate the GLP-1 hormone production.*

METHODS

The MindBody GLP-1 System, which consists of two different formulations, MB Core™ (capsule) and MB Enhance™ (powder), was investigated for its ability to activate various genes involved in the GLP-1 pathway using an *in vitro* L-cell model. Total GLP-1 production was also measured together with the visualization of GLP-1 using immunofluorescence imaging.*§

We investigated several genes involved in GLP-1 production and regulation:

- The first gene, GCG, encodes proglucagon, the precursor to GLP-1, which is cleaved by the enzyme prohormone convertase, encoded by the PCSK1 gene (Table 1).

- Given GLP-1's role in the gut-brain-axis and hunger regulation, we also examined neuropeptide genes such as PPY and PYY for activation.

- Because GLP-1 is rapidly degraded by dipeptidyl peptidase-4 (DPP-4), encoded by DPP4, we assessed its gene activity.

- Finally, we investigated GLP1R, which encodes the GLP-1 receptor.

Together, these genes suggest a potential mechanism for GLP-1 activation in L-cells by the MindBody GLP-1 System.*§

Table 1. Genes involved in the conversion of proglucagon to GLP-1 and other peptides/intermediaries.

Gene	Function
GCG	proglucagon, the precursor protein that ultimately is broken down into GLP-1 and other proteins
PCSK1	prohormone convertase, which is the enzyme involved in breaking proglucagon into GLP-1 and other proteins
GLP1R	GLP-1 receptor, where GLP-1 binds to
DPP4	dipeptidyl peptidase-4, the enzyme that breaks down GLP-1 in the blood
PPY	pancreatic polypeptide Y, a neuropeptide that talks to the brain
PYY	peptide YY, a neuropeptide that talks to the brain

*These statements have not been evaluated by the Food and Drug Administration. These products are not intended to diagnose, treat, cure or prevent any disease.
§ Results based on a cell culture study on active ingredient blends in the MindBody GLP-1 System.

RESULTS AND DISCUSSION

a. Gene Expression and Mechanism of Action

The results show the key genes activated when using the MindBody GLP-1 System as compared to the individual MB Core and MB Enhance products alone. The MindBody GLP-1 System showed dual and synergistic activation of all genes listed in Table 2.*§

Table 2. Change of gene expression in L-cells after nutrient stimulation using the MB GLP-1 System.

Gene	MindBody GLP-1 System
GCG	+95%
PCSK1	+32%
GLP1R	+53%
DPP4	-38%
PPY	+1280%
PYY	+55%

A potential mechanism of action was observed where all gene expressions showed a synergistic increase using the MindBody GLP-1 System as compared to the individual products alone:

- L-cells are activated and stimulated by the nutrients from the MindBody GLP-1 System to express GCG (proglucagon gene). GCG gene expression increased by 95% as compared to unstimulated L-cells.*§
- Proglucagon is then cleaved by PC 1/3, which is activated by the gene PCSK1, and ultimately produces GLP-1. PCSK1 is activated by 32% allowing for more proglucagon to be cleaved into GLP-1.*§
- GLP-1 is then secreted into the body and transported to various organs that have the GLP-1 receptor, which is expressed by the GLP1R gene. We saw a 53% increase in receptor expression, which allows for more binding of GLP-1 to elicit its beneficial responses.*§
- We saw a 38% decrease in the expression of the DPP4 gene expression, which encodes for the enzyme that breaks down GLP-1. Therefore, more GLP-1 will be available to elicit its beneficial responses for longer, further allowing the GLP-1 protein to remain active.*§
- Other neuropeptide genes, PPY and PYY, were also significantly expressed by 1280% and 55% respectively, and thus more signals are available to work through the gut-brain axis to influence hunger signals.*§

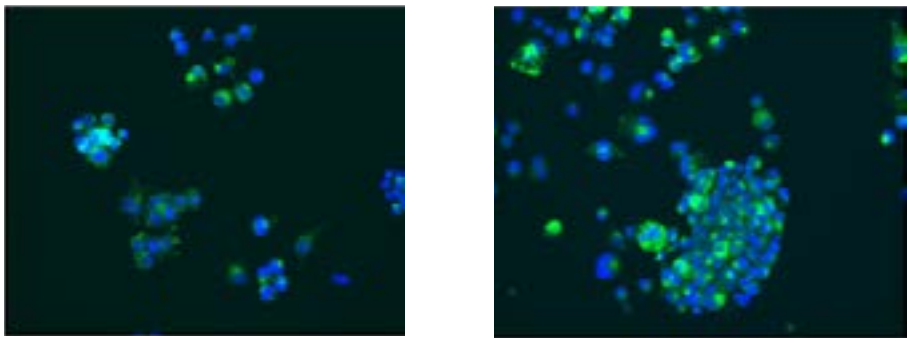
The results confirmed synergistic activation of GLP-1 by the MindBody GLP-1 System. The GCG and PCSK genes both showed increased activation with the two products together allowing for more proglucagon and ultimately more GLP-1 to be produced. We call this dual activation due to the different synergistic approaches of the two products: direct activation for MB Core and indirect activation via the microbiome for MB Enhance.*§

We also saw an increase of the gene GLP1R as well as a decrease in the DPP4 gene, both complementary actions allowing for more GLP-1 to bind to receptors and remain active in the body. We call this dual amplification because the system amplifies the effects of the GLP-1 hormone that it first activates.*§

b. GLP-1 Quantification and Imaging

GLP-1 production was also quantified after stimulating L-cells with MB Core, MB Enhance, or the MindBody GLP-1 System. The system showed an astounding synergistic 54% increase in total amounts as compared to either product alone. To confirm this, we looked under a microscope and verified these findings (Figure 1).*§

*These statements have not been evaluated by the Food and Drug Administration. These products are not intended to diagnose, treat, cure or prevent any disease.
† System helps maintain blood sugar levels already in the normal, healthy range.
§ Results based on a cell culture study on active ingredient blends in the MindBody GLP-1 System.



Control MB System

Figure 1. Green fluorescence indicates the GLP-1 protein and its accumulation in L-cells. After treatment with the MindBody GLP-1 System there is a brighter and denser fluorescence signal observed as compared to control.

CONCLUSION

MindBody GLP-1 System showed a dual synergistic activation mechanism by stimulating specialized gut cells, L-cells, in two different ways to express GCG and PCSK1—key genes involved in the production of GLP-1. A dual amplification mechanism for allowing more GLP-1 to circulate and bind to receptors occurred at the same time by decreasing DPP4 gene activation and increasing GLP1R receptor gene activation. An interesting observation was the increase in PYY and PPY gene expression. Because they talk to the brain, the increase in these neuropeptides further established a link to the involvement of the gut-brain-axis.*§

MindBody GLP-1 System uses a dual activation and dual amplification mechanism, allowing for increased production and secretion of the GLP-1 hormone. This activity helps directly reduce hunger and increase satiety. It also supports a sustained and balanced blood glucose response†, the health of essential organs such as kidney, liver, pancreas, brain, and heart, and proper nerve function.*§

*These statements have not been evaluated by the Food and Drug Administration. These products are not intended to diagnose, treat, cure or prevent any disease.
† System helps maintain blood sugar levels already in the normal, healthy range.
§ Results based on a cell culture study on active ingredient blends in the MindBody GLP-1 System.