Synergistic Activation Benefits of Using Healthy Weight Stack

Objective: To evaluate the benefits of Mind Body GLP-1 System™ alone and in combination with Protandim® Nrf2 Synergizer®.

Sponsor: LifeVantage Corp.

Principal Investigator: LifeVantage Corp.

Reference: LifeVantage Corp., Lehi, Utah 84043

Introduction

Protandim® Nrf2 Synergizer® is a dietary supplement comprising a synergistic, proprietary blend of standardized extracts that includes milk thistle (*Silybum marianum*), ashwagandha (*Withania somnifera*), turmeric (*Curcuma longa*), Bacopa monnieri, and green tea (*Camelia sinensis*). Its mechanism of action is through activation of the Nrf2 pathway, allowing the Antioxidant-Response-Element region (ARE) of the DNA binding site in the nucleus to become activated, with the result of increasing antioxidant enzymes such as hemeoxygenase-1, superoxide dismutase, catalase, glutathione, etc. Around 30 peer-reviewed studies have been published mentioning Nrf2 Synergizer and its positive and beneficial effects on the oxidative stress response in the body.*

The MindBody GLP-1 System™ comprises MindBody Core™ and MindBody Enhance™. MindBody Core contains a carefully balanced, synergistic blend of 8 naturally derived ingredients that activate specialized L-cells to produce and secrete the GLP-1 hormone: acacia, lemon bioflavonoids, hesperidin, berberine, honeysuckle, yerba mate, matcha, and eggplant. It also contains chromium, which helps the body use sugar properly to regulate blood sugar levels already within a normal healthy range.*

MindBody Enhance contains essential vitamins and minerals along with a GLP-1 Activation Support Blend and Digestive Health Blend, which supply resistant potato starch, resistant tapioca fiber, flaxseed and MCT oils, baobab fruit powder, guar gum, kombucha leaf powder, *Bacillus coagulans* and *Bacillus clausii* spores, licorice, and ginger. These ingredients work together to satisfy your hunger; support the production of short chain fatty acids like butyrate in the colon, which stimulates GLP-1 production; maintain a healthy microbiome balance; and support the health of your stomach and intestinal lining.*

Due to the compelling benefits of both Protandim Nrf2 Synergizer and the MindBody GLP-1 System (also known as the Healthy Weight Stack) on their own, gene expression analysis was performed to determine if synergy exists between them.

Cell culture selection

Selecting the correct cell type was a key first step in developing a protocol for the gene expression study. Epithelial Caco-2 cells derived from the colon were chosen because they have become a standard cell model for a variety of areas, including researching active and passive transport mechanisms across cell membranes, cell permeability of various proteins across intestinal barrier, bioavailability, and absorption of nutrients, because they express a multitude of genes involved in those areas.

As a result, Caco-2 cells have the functional versatility to allow for investigation of a wide array of possible effects when MindBody GLP-1 System and Nrf2 Synergizer are combined.

What Is RNA Sequencing?

RNA sequencing was used to investigate potential synergies.

RNA sequencing (also known as RNA-Seq) is a genomic approach for the detection and quantitative analysis of messenger RNA molecules in a biological sample, and it is useful for studying cellular responses. It shows which genes are upregulated or expressed or downregulated or suppressed at different time points. Heat maps can be generated that tell us what pathways are increased or decreased in intensity.

Essentially, RNA sequencing allows us to look at the entire genome, or the complete set of genetic material of the Caco-2 cell, and see gene expression and, ultimately, which cellular genes and pathways are upregulated or downregulated. A total of around 20,000–25,000 protein coding genes were analyzed.

* These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure or prevent any disease.

METHODS

Global Gene Expression by RNA Sequencing

In the study, we tested MindBody GLP-1 System alone, Nrf2 Synergizer alone, and the combination.

This study was conducted in three parts:

1. A dose-finding study
2. A gene expression study to verify dose timing and concentration
3. Global gene expression study using RNA sequencing with the ultimate heat map generation

After determining the proper dosage and time points for evaluation, the Caco-2 cells were treated with different product combinations. Then mRNA was extracted from the cells and sequenced. Heat maps of upregulation and downregulation of genes as well as influence on metabolic pathways were created.

RESULTS AND DISCUSSION

We focused on analyzing the Healthy Weight Stack since we already knew Nrf2 Synergizer works primarily on oxidative stress pathways and the MindBody GLP-1 System primarily balances a GLP-1 response.* Therefore, only the results observed when both products were used together in the Healthy Weight Stack are presented.

With the large number of genes involved in the Caco-2 cells, we focused on 2 different ways of analyzing this vast amount of information:

1. Gene Ontology, which focuses on categorizing genes based on biological functionality. The top biological functionalities included the following:
    - Metabolic processes, including sphingolipid/ceramide metabolism. These are involved in cell signaling pathways that regulate cell growth, differentiation, and apoptosis in both the cardiovascular and nervous systems, as well as throughout the body
    - Tetratricopeptide repeats crucial for the essential structural integrity and function of proteins and cellular homeostasis
    - Oxidative stress pathways to neutralize free radicals
    - tRNA processing for translating genetic information from DNA into functional proteins

2. Gene Clustering, which focuses on grouping genes based on expression. The top gene expression clusters are listed below:
    - Oxidative stress response/detox
    - Lipid storage and fat cell differentiation
    - Cellular adhesions proteins
    - Complementary pro-survival pathways

Table 1 shows the expected synergistic activation of known pathways that enhanced the benefits delivered by Nrf2 Synergizer and MindBody GLP-1 System alone, as well as new, unexpected beneficial pathways not seen with either product alone.

Table 1. Synergistic benefits of activation of pathways as well as new benefits of the Healthy Weight Stack not observed with either Nrf2 Synergizer or MindBody GLP-1 System alone.

Benefit	Influence of Healthy Weight Stack
Promote antioxidant defense*	ENHANCED: Activated an additional 4 antioxidant pathways as compared to Nrf2 Synergizer alone*
Support fat metabolism*	ENHANCED: Activated an additional 9 fat/fatty acid metabolism pathways as compared to MindBody GLP-1 System alone*
Promote tissue cell strength and Integrity*	NEW: Activated 10 genes involved in cell barrier and tissue strength*
Support Tissue cell regeneration and repair*	NEW: Activated 6 genes involved in the sphingolipid pathway*
Promote coordination, signaling, and impulses with tissue cells and nerves and overall nerve cell health*	NEW: Activated 6 genes involved in neuronal pathways*

* These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure or prevent any disease.

A synergistic activation of both antioxidant and fat/fatty acid metabolism pathways was seen with the Healthy Weight Stack. An additional 4 antioxidant pathways and an additional 9 fat/fatty acid metabolism pathways were activated, which allows a less stressed cell to improve fat metabolism for energy and may lead to a healthier fat distribution throughout the body.*

Based on the surprising and exciting NEW pathways influenced by the Healthy Weight Stack listed above such as cellular adhesion, nerve cell health and neuro- and cardio-protection pathways, we looked at the individual genes involved in those pathways.

A closer look at the genes involved in cellular adhesion protein cluster showed that there were 10 genes significantly influenced that are involved in cell barrier and tissue strength and structure for key organ systems such as the brain (blood brain barrier), cardiovascular (blood vessel integrity), and gut (barrier integrity). These genes were not significantly influenced or not influenced by Nrf2 Synergizer or the MindBody GLP-1 System alone. (Figure 1)

Figure 1.

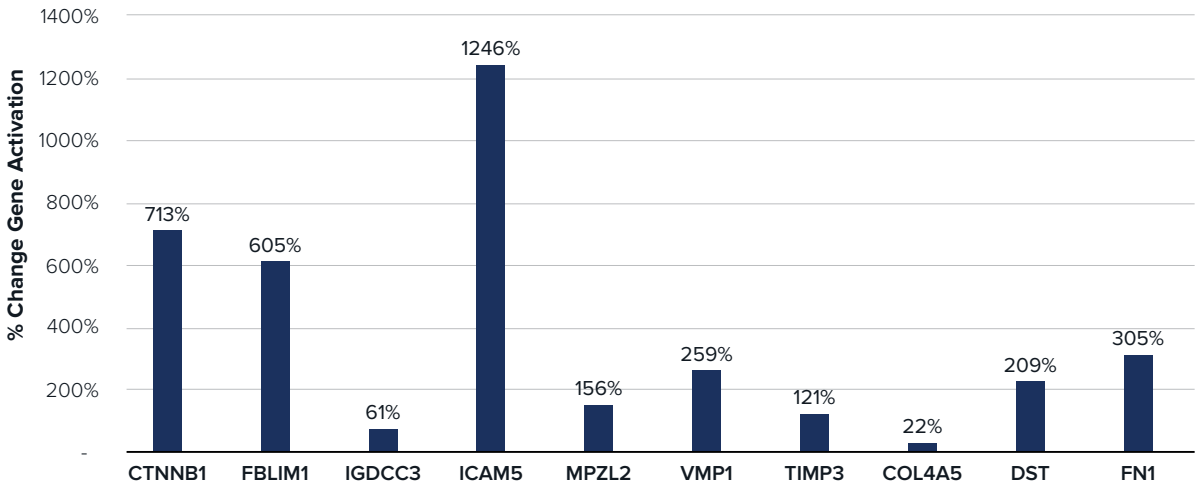

Figure 1. Significant ($p<0.05$) cellular adhesion genes upregulated in the 'Gene Cluster analysis – Cellular Adhesion' by the Healthy Weight Stack. None of the genes were significantly influenced by either Nrf2 Synergizer of MindBody GLP-1 System alone. Neuron integrity (IGDCC3, ICAM5, MPZL2, VMP1, DST), blood vessel integrity (TIMP3, COL4A5), and gut integrity (CTNNB1, FBLIM1, FN1) genes.

We know that the sphingolipid pathways play critical roles in regulating cell survival, proliferation, and apoptosis through the production of bioactive sphingolipids. These lipids serve as signaling molecules that influence various cellular processes, including pro-survival pathways that impact tissue repair and regeneration.

A closer look at the genes involved in the sphingolipid pathway showed significant upregulation in 6 pro-survival genes involved in neuronal functions, neuronal signaling, cellular metabolic processes, and endothelial cell membrane integrity, which were not seen with either Nrf2 Synergizer or MindBody GLP-1 System alone. (Figure 2) The upregulation of these genes allows for:

- Improved gut-brain axis (including immune and metabolic processes) *
- Maintained neuronal health and communication under stress *
- Maintained health of cardiovascular blood vessels and smooth muscle cells involved in contraction/relaxation of healthy heart function *

In general, sphingolipids are involved in membrane structures and serve as bioactive molecules in signaling pathways. They have both chemical and electrical signaling properties. In their chemical signaling role (i.e., signal transduction), they are often critical regulators of cell-to-cell signaling pathways, acting as secondary messengers or intermediaries. In their electrical signaling role, sphingolipids can influence the generation and propagation of signals by modulating the function of essential ion channels, receptors, and other membrane-associated proteins. This ensures organs, nerves, and tissues communicate clearly with each other to keep every system working in synchrony.

By activating these genes and their bioactive molecules, the Healthy Weight Stack improves chemical signaling and supports the proper flow of electrical impulses within and between organs, improving coordination, signaling, and impulses with tissue cells and nerves.

* These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure or prevent any disease.

The new nerve health benefits as seen in Table 1 showed a total of 6 new genes significantly upregulated by Healthy Weight Stack as compared to either Nrf2 Synergizer or MindBody GLP-1 System alone. (Figure 3) Healthy nerve function is required for proper communication between the various organs, in the form of chemical and electrical signaling. The nerve health benefits are seen through:

Neuroplasticity *
Maintenance of neural function and protection of nervous tissue from stress induced damage *
Maintenance of proper barrier function and tissue organization in the brain *
Brain homeostasis and healthy brain function *

Figure 2

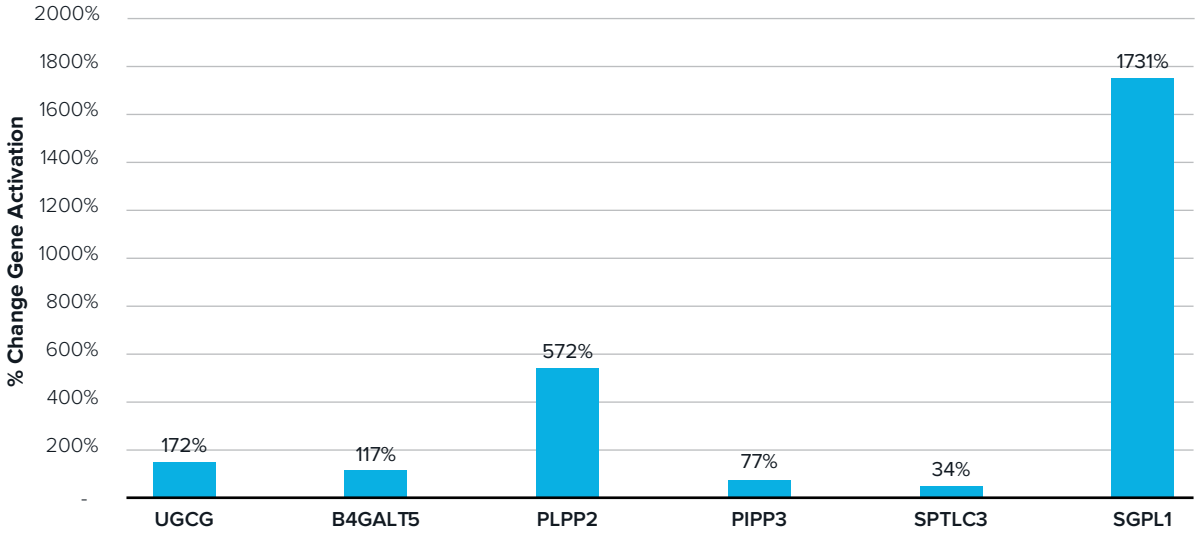

Figure 1. Significant genes upregulated in the sphingolipid metabolism pathway by the Healthy Weight Stack. None of the genes were significantly influenced by either Nrf2 Synergizer of MindBody GLP-1 System alone. Neuronal function and signaling (UGCG, B4GALT5, SGPL1), cellular metabolic processes (PIPP3, PLPP2), endothelial cell membrane integrity (SPTLC3).

Figure 3

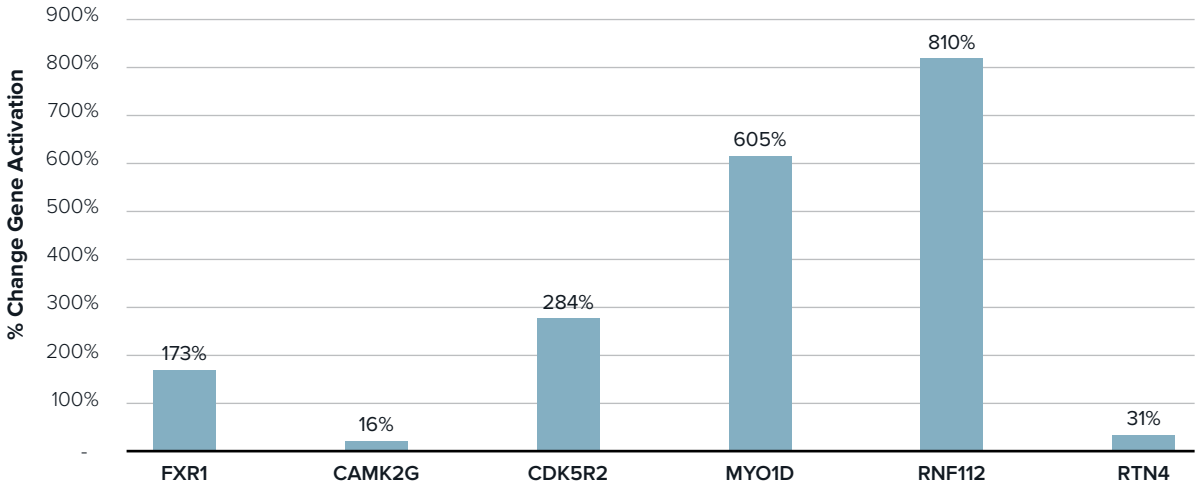

Figure 3. Significant($p<0.05$) neuronal health genes upregulated by the Healthy Weight Stack. None of the genes were significantly influenced by either Nrf2 Synergizer of MindBody GLP-1 System alone. Neuroplasticity (FXR1, CAMK2G, CDK5), neuronal cell health and function (MYO1D, RNF112, RTN4).

* These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure or prevent any disease.

Conclusion

We know that Nrf2 Synergizer, a blend of 5 botanical extracts, triggers the body's internal oxidative stress response by activating various antioxidant mRNA genes, such as hemoxygenase-1 (HMOX1), superoxide dismutase (SOD2), catalase (CAT), and many more. Around 30 peer-reviewed studies have shown the benefits of this product on various aspects of life and general health.*

We also know from our 12-week human clinical study, that the MindBody GLP-1 System increases the body's own GLP-1 hormone production by an average of 140%, with up to a 27% reduction in visceral fat. There are many peer-reviewed studies linking the benefits of a balanced GLP-1 response to healthy weight management.Ω‡

With this study we wanted to investigate what additional benefits the combination of the Nrf2 Synergizer and MindBody GLP-1 System would show.

When taken together these products amplified each other's unique benefits.

1. Amplify the oxidative stress protection benefits of Nrf2 Synergizer by activating an additional 4 antioxidant pathways and allowing the cells to be better at defending against and repairing the aging effects of oxidative stress.*
2. Amplify the fat metabolism benefits of Mind Body GLP-1 System by activating an additional 9 fat/fatty acid metabolism pathway and thus allowing more fat to be burned for energy.*‡

We also saw new and surprising activation and significant upregulation of 22 genes that address several key causes of age-related organ decline to support structural integrity, repair and regeneration, and communication.*

1. Key cell adhesion genes were upregulated. These genes are important in maintaining cellular adhesion in your organs, to support structure and strength, allowing them to better perform their functions.*
2. Pro-survival pathways that influence both nerve and cardiovascular health were activated, which supports cell survival, growth, and regeneration.*
3. Chemical and electrical cell signaling between cells and tissues, and especially neurons, was reinforced by upregulating the sphingolipid/ceramide metabolism pathway and key genes that impact neuronal cell health.*

In conclusion, the Healthy Weight Stack reinforces your body's foundation by enhancing the structural integrity of your organs and improving their ability to communicate effectively. This comprehensive support proactively addresses key factors contributing to organ decline, promoting healthier aging. It helps optimize organ function for increased energy and vitality while maintaining healthy brain and neuronal activity to support clear, rapid communication and sharp cognitive function. Additionally, it supports cardiovascular and respiratory health to maintain physical performance, a healthy gut barrier for proper digestive function, and efficient communication within the nervous and musculoskeletal systems to maintain balance and overall coordination. Collectively, these effects provide measurable support for overall physiological resilience and functionality.*

‡ Results may vary. Typical weight loss using this product in a 12-week weight management program is 1–2 pounds per week. This product should be used in conjunction with a healthy diet and regular exercise. Consult with a healthcare provider before starting any weight loss program.

* These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure or prevent any disease.