Synergistic Activation Benefits of Using the Healthy Edge Stack

Objective: To evaluate the benefits of Healthy Edge (Protandim® Nrf2 Synergizer® and P84) in Caco-2 cells.

Sponsor: LifeVantage Corp.

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

Introduction

Healthy Edge is a dietary supplement system that consists of 3 products, Protandim® Nrf2 Synergizer® and P84 (which is a combination of two formulas, PhytoPower 1 and PhytoPower 2).

Protandim Nrf2 Synergizer is a dietary supplement comprising a synergistic, proprietary blend of 5 standardized extracts that includes milk thistle (*Silybum marianum*), ashwagandha (*Withania somnifera*), turmeric (*Curcuma longa*), *Bacopa monnieri*, and green tea (*Camelia sinensis*). The formula works through activation of the Nrf2 pathway, allowing the Antioxidant-Response-Element region (ARE) of the DNA binding site in the nucleus to become activated, resulting in increases of antioxidant enzymes such as hemeoxygenase-1, superoxide dismutase, catalase, and glutathione. Around 30 independent peer-reviewed studies have been published that mention Nrf2 Synergizer and its positive and beneficial effects on the oxidative stress response in the body. *

P84 is a combination of two products, PhytoPower 1 and PhytoPower 2, each with its own proprietary blend. PhytoPower 1 contains different phytonutrient-rich whole foods including fruits and vegetables with a wide color variety, as well as prebiotics, postbiotics, digestive enzymes, and 10 unique probiotic strains.

PhytoPower 1 Blend: Barley Grass powder (grass, *Hordeum vulgare* L.), Inulin (Jerusalem Artichoke, *Helianthus tuberosus*), Apple fruit powder (fruit, *Malus domestica*), Carrot powder (root, *Daucus carota*), Papaya fruit powder (fruit, *Carica papaya*), Pomegranate fruit powder (fruit, *Punica granatum* L.), Spinach powder (leaf, *Spinacia oleracea*), Kale powder (leaf, *Brassica oleracea* var. *acephala*), Rosehip fruit powder (fruit, *Rosa canina*), Prickly Pear Cactus powder (stem, *Opuntia ficus-indica*), Chlorella whole plant powder (whole plant, *Chlorella vulgaris*), Broccoli extract (aerial parts, *Brassica oleracea* L. var. *botrytis*), Sweet Potato powder (root, *Ipomoea batatas*), Mango fruit powder (fruit, *Mangifera indica* L.), Sea Buckthorn juice powder (fruit, *Hippophae rhamnoides* Linn.), Tomato powder (fruit, *Solanum lycopersicum*), Acerola Cherry powder (fruit, *Malpighia emarginata*), Blueberry extract (fruit, *Vaccinium corymbosum*), Baobab powder (fruit pulp, *Adansonia digitata*), Goldenberry powder (berry, *Physalis peruviana*), Probiotic Blend (*Bifidobacterium breve* BBr60, *Lactobacillus acidophilus* LA85, *Lactobacillus rhamnosus* LRa05, *Lactobacillus casei* LC89, *Bifidobacterium bifidum* BBi32, *Lactobacillus plantarum* Lp05, *Lactobacillus reuteri* LR08, *Bifidobacterium longum* subsp. *longum* BL21, *Lactobacillus helveticus* LH76, *Lactobacillus paracasei* LC86), Enzyme Blend (Papain, Amylase, Bromelain, Lactase, Cellulase), Watercress powder (leaf, *Nasturtium officinale*), Kelp powder (leaf, *Ascophyllum nodosum*), Asparagus powder (whole plant, *Asparagus officinalis* L.), Red Bell Pepper (fruit, *Capsicum annuum*), Black Currant powder (fruit, *Ribes nigrum*), Bilberry extract (fruit, *Vaccinium myrtillus*), Blackberry powder (fruit, *Rubus fruticosus*), Dulse Seaweed powder (leaf, *Palmaria palmata*), Purple Cone Flower powder (root, *Echinacea purpurea* L.), Leek powder (leaf, *Allium ampeloprasum*), Eggplant powder (fruit, *Solanum melongena*), Purple Grape powder (fruit, *Vitis vinifera* L.), Garlic powder (bulb, *Allium sativum* L.), Orange powder (peel, *Citrus sinensis*), Pumpkin fruit powder (fruit, *Cucurbita pepo*), Marigold Flower extract (flower, *Calendula officinalis* L.), Red Beet powder (root, *Beta vulgaris*), Purple Cabbage powder (leaf, *Brassica oleracea* var. *capitata*), Yellow Bell Pepper (fruit, *Capsicum annuum*), Cranberry fruit powder (fruit, *Vaccinium macrocarpon* L.), Raspberry fruit powder (fruit, *Rubus idaeus*), Ebony Carrot powder (root, *Daucus carota* subsp. *sativus* var. *atrorubens*).

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PhytoPower 2 also contains a combination of whole fruits and vegetables, prebiotics, postbiotics, and 5 unique probiotic strains.

PhytoPower 2 Blend: Inulin (Chicory root, *Cichorium glandulosum Boiss.* et Huet or *Chicorium intybus* L.), Blueberry extract (fruit, *Vaccinium corymbosum*), Noni powder (leaf, *Morinda citrifolia*), Blackberry powder (fruit, *Rubus fruticosus*), Raspberry fruit powder (fruit, *Rubus idaeus*), Probiotic Blend (*Lactobacillus sakei* LSa79, *Bifidobacterium adolescentis* BAC30, *Saccharomyces boulardii* SB01, *Weizmannia coagulans* BC99, *Bifidobacterium lactis* BLa80), Grape extract (seed, *Vitis vinifera* L.), Acai juice powder (fruit, *Euterpe oleracea* Mart.), Red Beet powder (root, *Beta vulgaris*), Cranberry fruit powder (fruit, *Vaccinium macrocarpon* L.), Pomegranate fruit extract (fruit, *Punica granatum* L.), Spinach powder (leaf, *Spinacia oleracea*), Black Current powder (fruit, *Ribes nigrum*), Moringa powder (leaf, *Moringa oleifera*), Ebony Carrot powder (root, *Daucus carota* subsp. *sativus* var. *atrorubens*), Plum powder (fruit, *Prunus domestica*), Kombucha powder, Umeboshi Plum powder (fruit, *Prunus mime*), Turmeric powder (rhizome, *Curcuma longa* L.), Kale powder (leaf, *Brassica oleracea* var. *acephala*), Kelp powder (whole plant seaweed, *Ascophyllum nodosum*), Spirulina powder (whole algae, *Arthrospira platensis*), Chlorella whole plant powder (whole plant, *Chlorella vulgaris*).

A previous *in vitro* targeted gene expression study on P84 alone showed that it activated 14 key gut peptides involved in regulating, repairing and restoring gut function and microbial balance.* †††

Table 1. 14 Peptides/proteins involved in regulating, repairing, and restoring gut function and microbial balance activated by P84 * †††

Peptides/Proteins	Function
Vasoactive Intestinal Peptide (VIP)	Promotes and stimulates intestinal secretions and smooth muscle relaxation, helping to calm overreactive areas of the gut
Gastrin-Releasing Peptide (GRP)	Stimulates gastrin release and gastric acid secretion, promoting digestion and sending alerts to stimulate digestion
Neurotensin (NTS)	Regulates gut motility, secretion, and epithelial health
Oxyntomodulin (OXM)	Regulates appetite, slows gastric emptying, and coordinates nutrient absorption
Somatostatin (SST)	Inhibits excessive hormone (e.g., CCK, gastrin, insulin) secretion, helping to regulate key regulatory peptides
Cholecystokinin (CCK)	Stimulates bile and pancreatic enzyme secretion, regulating satiety and making sure fats and proteins are properly processed
Trefoil Factor 3 (TFF3)	Promotes mucosal and gut function repair and health
Zonulin (HP-2)	Gatekeeper of cell barriers, keeping the good in and the bad out. Elevated levels indicate a negative shift in balance and weaker barrier function
Claudin-1 (CLDN1)	Supports healthy gut permeability (paracellular permeability) and ensures gut barriers are watertight
E-Cadherin (CDH1)	Promotes cell adhesion and maintains epithelial barrier integrity
Occludin (OCLN)	Maintains a healthy gut barrier, strengthening and stabilizing tight junctions
Epiregulin (EREG)	Promotes epithelial cell proliferation and supports new tissue formation
Mucin 2 (MUC2)	Creates the mucous barrier that protects and keeps the gut environment stable
Glucagon-Like Peptide 2 (GLP-2)	Stimulates mucosal growth and reduces permeability to foster a balanced environment for microbes to grow and thrive

Following this research on P84 alone, the purpose of the new study was to understand the synergy between Protandim Nrf2 Synergizer and P84 based on their individual benefits and determine if any unique pathways would be activated.

A technique called mRNA sequencing (also known as RNA-Seq) was used, which is a genomic approach for the detection and quantitative analysis of messenger RNA molecules in a biological sample that is useful for studying cellular responses. It can be used to detect unique pathways and quantify gene expressions within those pathways.

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††† Results based on a cell culture study on P84.

Essentially, mRNA sequencing allowed evaluation of the complete cellular transcriptome (complete set of RNA molecules produced from the cell's genes at a given time), providing insight into gene expression changes and revealing which genes and cellular pathways were upregulated or downregulated.

METHODS

Global Gene Expression by RNA Sequencing

In the study, the following products were tested: Protandim Nrf2 Synergizer alone, P84 alone, and Protandim Nrf2 Synergizer and P84 combined (Healthy Edge). This study was conducted in three parts:

a) a dose-finding study
b) a gene expression study to verify dose timing and concentration
c) global gene expression study using RNA sequencing

After determining the proper dosage and time points for evaluation, the Caco-2 cells were treated with Protandim Nrf2 Synergizer, P84, or the combination of the two (Healthy Edge). RNA was then extracted from the cells and sequenced. Only the RNA sequencing methodology and results are presented.

RNA SEQUENCE METHODOLOGY

The RNA sequencing was performed by seeding Caco-2 gut cells into 10 cm dishes and allowed to attach overnight. Cells were then treated with Protandim Nrf2 Synergizer, P84, and/or Healthy Edge at 75% confluency with 50µL of Protandim Nrf2 Synergizer, P84, and Healthy Edge article (0.5% DMSO final concentration). Cells were lysed, and RNA was isolated at the 8-hour specified time point (Zymo Quick RNA MiniPrep system). RNA QC, library construction, library QC, sequencing, and trimming were performed by Novogene (Sacramento, CA) as follows:

- RNA samples were evaluated for RNA integrity using an Agilent 2100 Bioanalyzer.
- Messenger RNA (mRNA) was purified from total RNA using poly-T oligo-attached magnetic beads.
- After fragmentation, the first strand cDNA was synthesized using random hexamer primers followed by the second strand cDNA synthesis.
- The library was ready after end repair, A-tailing, adapter ligation, size selection, amplification, and purification.
- The workflow of the library construction was mRNA > Fragmentation > Reverse transcription > Second strand cDNA synthesis > End repair and A-tailing > Adapter ligation > Size selection > PCR amplification.
- The library was checked with Qubit and real-time PCR for quantification and bioanalyzer for size distribution detection. All samples passed quality control.
- Samples were sequenced on an Illumina NovaSeq instrument for paired end 150bp reads. 40–50 million reads were obtained for each sample.
- All resulting sequencing reads were filtered by removing adapters, removing reads containing N>10% (N represents a base that cannot be determined), and removing reads containing low quality (Qscore≤ 5) bases (removed when low quality bases represent over 50% of the total bases).

  5' adapter: 5'AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCATT-3'.
  3' Adapter: 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACGGATGACTATCTCGTATGCCGTCTTCTGCTTG-3'.
- Approximately 97% of all samples had clean reads that passed filtering

FASTQ (Fast Quality Score) read files from the filtered sequencing were processed using HISAT2 (Hierarchical Indexing for Spliced Alignment of Transcripts 2) aligning to the human hg38 canonical genome. HISAT2 was performed with "Unstranded" and "Paired end" options selected. The resulting SAM (Sequence Alignment/Map) file produced with HISAT2 was further processed to BAM files (Binary Alignment Map) using SAMtools. FeatureCounts (2.0.6) was used to count the reads numbers mapped to each gene then FPKM (Fragments Per Kilobase of transcript sequence per Millions base pairs sequenced) of each gene was calculated based on the length of the gene and reads count mapped to the gene. Count tables were then input into DESeq2 (R package 1.42.0) to perform differential gene expression (relative to control).

Differential expressions were ranked by adjusted P-value (Benjamini and Hochber's method), and gene enrichment analysis was performed using cluster Profiler (4.8.1).

RESULTS

Global Gene expression

Out of about 13,000 protein-encoding genes investigated, a total of 188 (0.07%), 1818 (13.9%), and 1795 (13.8%) were significantly influenced by Protandim Nrf2 Synergizer, P84, or Healthy Edge, respectively. Many encoding genes overlapped, but there were 110, 384, and 358 uniquely and only expressed by Protandim Nrf2 Synergizer, P84, or Healthy Edge, respectively.

Protandim Nrf2 Synergizer

Protandim Nrf2 Synergizer was specifically developed to activate cellular detoxification and protection pathways, so the observation that 110 unique genes were expressed in Caco-2 cells was not unexpected. These 110 genes were all associated with antioxidant production, cellular detoxification, and cellular protection. *

P84

P84 was specifically developed for activating key gut function repair, regulation, and restoration genes and pathways found within the gut (see **Table 1** above in introduction). A total of 384 genes were uniquely expressed by P84 alone that were associated with gut function repair, regulation of stress responses, and gut metabolism, supporting the prior research findings. * †††

Healthy Edge

When analyzing the results for Healthy Edge, significant results were seen.

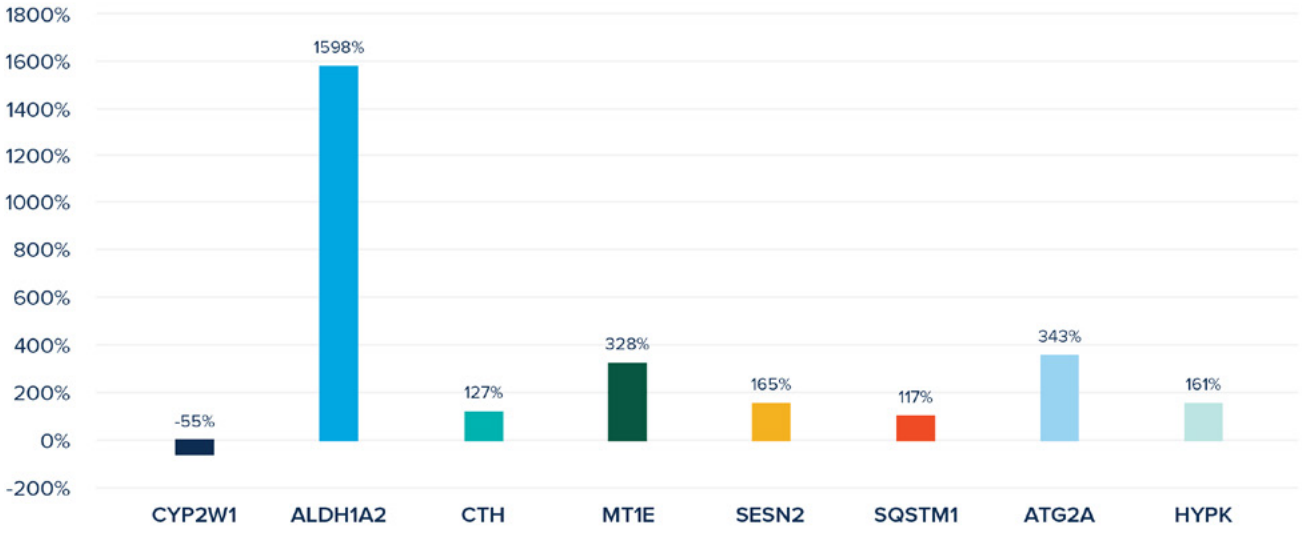
1. Enhanced benefits

a. When looking at the influence of Healthy Edge compared to the influence of Protandim Nrf2 alone, eight additional detoxification and cellular protection pathway genes were activated. *

Expression of antioxidant and detoxification genes *CTH, MT1E, SESN2, SQSTM1* increased by 127%, 328%, 165%, and 117% respectively. Expression of cellular protection genes *ALDH1A2, SESN2, ATG2A*, and *HYPK* increased by 1,598%, 165%, 343%, and 161% respectively. *CYP2W1* gene expression decreased by 55%, which is desirable as higher activity can increase oxidative stress burden. *

The result of this is enhanced and more robust protection against cellular oxidative stress both inside and outside the cell. **(Figure 1)** *

Figure 1. Detoxification and cellular protection genes uniquely activated with Healthy Edge



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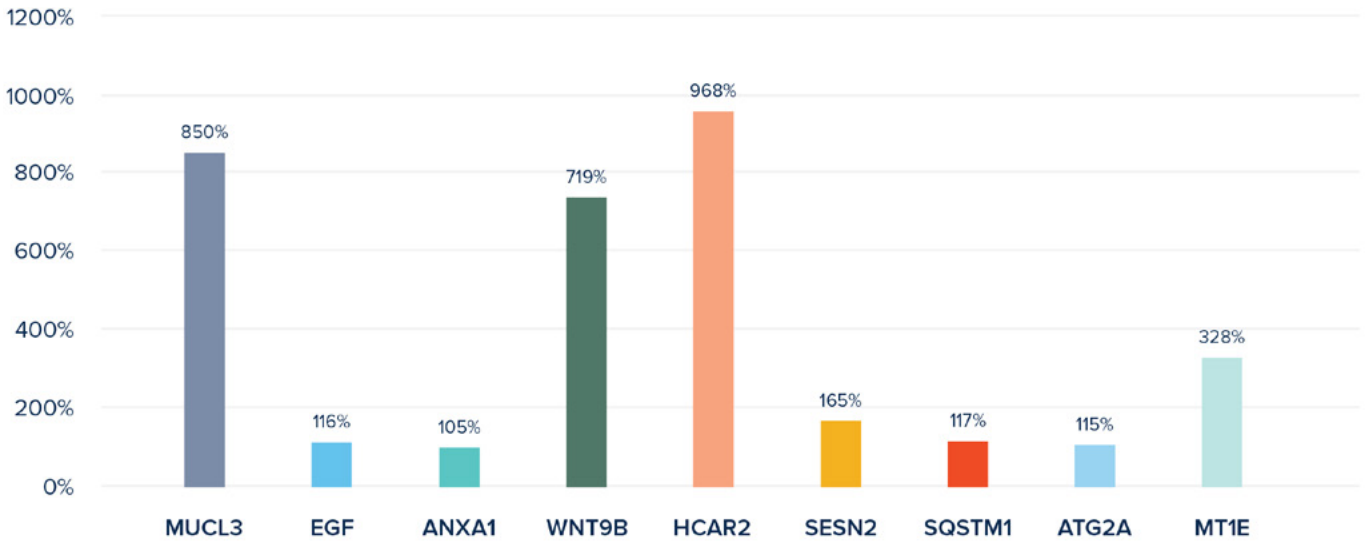
b. When looking at the influence of Healthy Edge compared to P84 alone, it was observed that the gut repair and regulatory pathways activated by P84 alone were enhanced by activation of an additional nine gut regulation/repair genes specific to these pathways. *

Expression of repair genes *MUCL3, EGF, WNT9B, SESN2, MT1E*, and *SQSTM1* increased by 850%, 116%, 719%, 165%, 328%, and 117%, respectively. *

Expression of regulatory genes *ANXA1, HCAR2*, and *ATG2A* increased by 105%, 968%, and 115%, respectively. *

Healthy Edge therefore enhanced a more robust gut repair mechanism to support gut health and, consequently, better nutrient uptake. **(Figure 2)** *

Figure 2. Gut regulation and repair genes uniquely activated with Healthy Edge



2. New benefits

The most unexpected results, however, were seen when looking for new benefits. We found that when treating the cells with Healthy Edge, two pathways stood out that involved:

- Circadian rhythm, which supports cellular processes that allow cells to respond at the right time necessary for optimal cell function. *
- Cellular adaptation, where cells react, adjust, and respond better to stress. *

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RESULTS CONTINUED

a. Circadian Rhythm

When the body's circadian rhythm is out of sync or misaligned, its internal clock no longer matches the external 24-hour day-night cycle, disrupting the coordination between the brain, hormones, metabolism, immune function, gut repair, microbiome interaction, and intestinal timing. It can lead to altered microbiome composition, detox and nutrient absorption become erratic, gut barrier weakens and becomes reactive, stress signals are in constant 'on' position, and the entire gut tissue can become fragile instead of adaptable. Your brain may not fully restore, which can lead to challenges with focus, memory, and emotional regulation. Hormones such as cortisol, melatonin, insulin, growth hormones, etc. can also be disrupted, affecting the organs they control. These are only a few systems, but almost every biological system will be impacted and may stop working in synchrony.

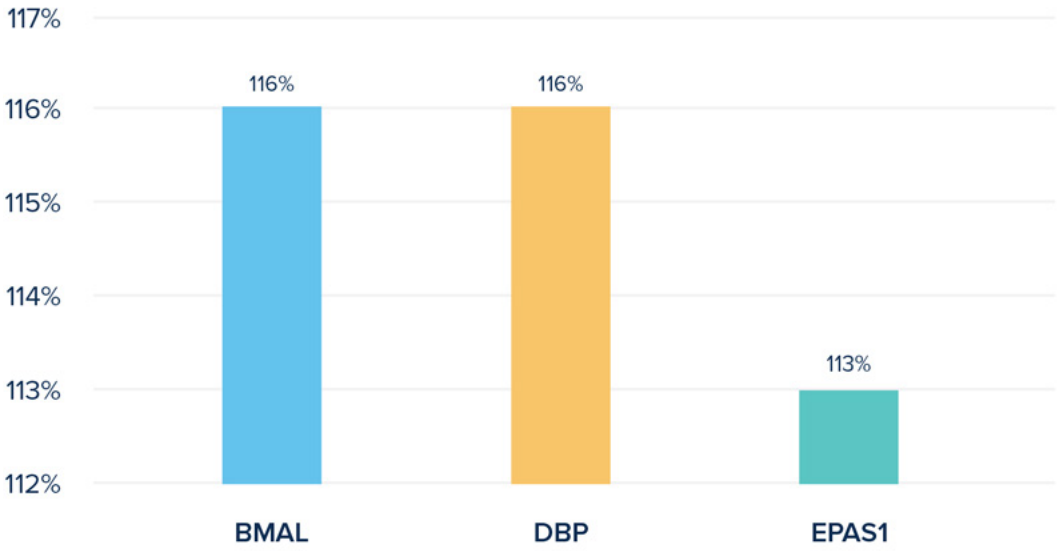
It was found that with the addition of Healthy Edge, Caco-2 cells uniquely expressed three genes involved in the 24-hour cycle that are key to the circadian rhythm.

BMAL1 – essential for maintaining daily physiological rhythms and setting the gut's healing rhythm, while also regulating gut motility, digestion, and microbiome interaction. Gene expression significantly increased by 116%. *

DBP – coordinates daily gut epithelial repair mechanisms and detoxification and was significantly increased by 116%. *

EPAS1 – interprets stress and oxygen demand in the gut. Activation of this gene significantly increased by 113%. **(Figure 3)** *

Figure 3. Key circadian rhythm regulation genes uniquely activated with Healthy Edge.



Increased activation of these genes influences the gut towards a more balanced circadian rhythm to synchronize cell renewal and function. There is a more balanced coordination between detoxification processes, nutrient absorption, and repair processes, while stress responses to oxygen demands become clearer.

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RESULTS CONTINUED

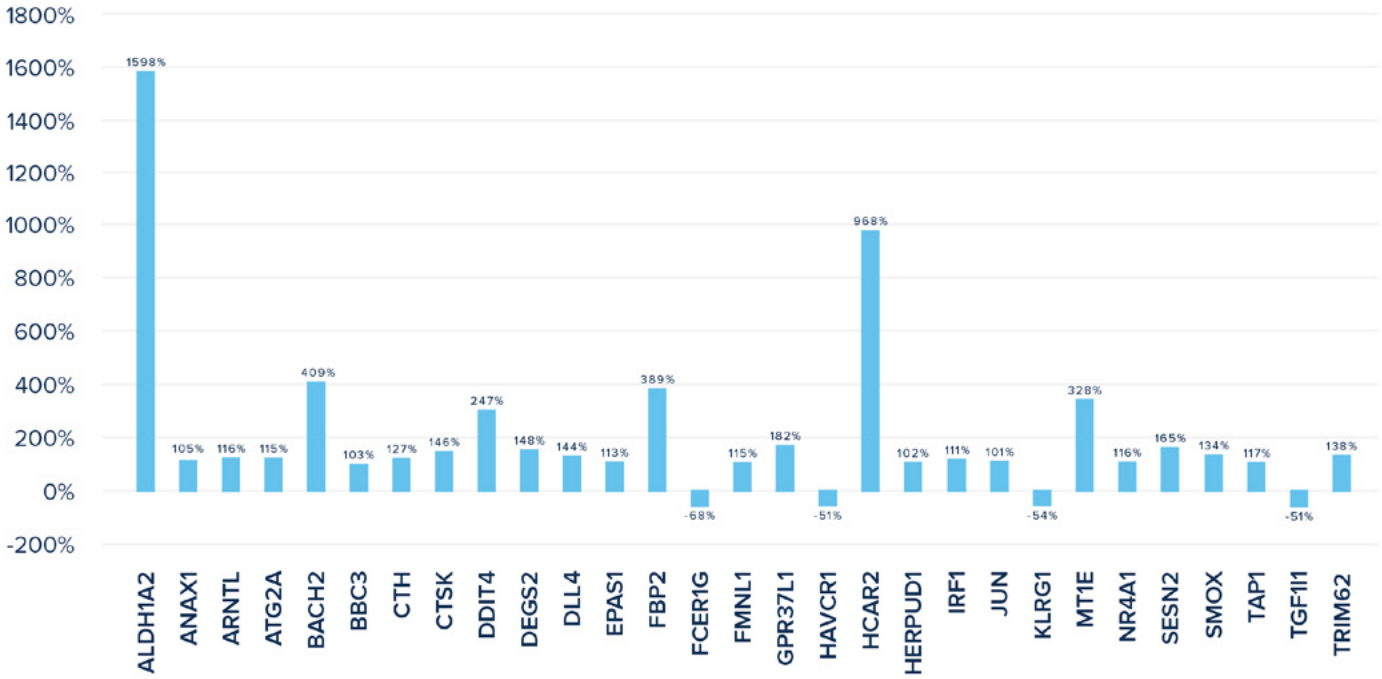
b. Cellular Adaptation

The human body is an integrated, self-regulating system designed to sense stress and challenges, activate targeted protective responses, repair damaged cells, and adapt for future responses that are more efficient and precise. Every stimulus triggers communication between cells through hormones, neurotransmitters, immune messengers, and metabolic cues. When this process functions optimally, these pathways are activated with accuracy and proportionality, and they remain engaged as long as necessary to recover, strengthen, and refine how they interpret similar signals in the future.

However, when this process is disrupted or prolonged due to age, stress, diet, lifestyle, etc., cellular communication can become less refined, persist longer than required, lose signal clarity, or become confused and less precise. Background noise is created within the cellular environment, and, over time, cells find it harder to adapt and self-correct, becoming reactive rather than adaptive.

When investigating the effect of Healthy Edge on pathways in Caco-2 cells, 29 unique genes associated with increased cell adaptability pathways were activated. **(Figure 4)** *

Figure 4. Cellular adaptation genes uniquely activated with Healthy Edge.



The function of each cell is detailed in **Table 2** below.

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Table 2. Function and significance of the 29 genes activated with Healthy Edge treatment.

Gene	Role in Stress / Adaptation
Aldehyde Dehydrogenase 1 Family Member A2 – *ALDH1A2* (+1598%)	Retinoic acid synthesis, regulates differentiation, immune tone, and oxidative stress
Annexin A1 – *ANAX1* (+105%)	Pro-resolving mediator; helps turn off stress responses and promote tissue repair
Aryl Hydrocarbon Receptor Nuclear Translocator Like – *ARNTL* (+116%)	Core circadian clock gene; controls daily rhythms of metabolism, immunity, tissue repair, and stress response
Autophagy Related 2A – *ATG2A* (+115%)	Essential for autophagosome formation and cellular recycling under stress
BTB Domain and CNC Homolog 2 – *BACH2* (+409%)	Immune transcriptional regulator, promotes immune tolerance and oxidative stress response
BCL2 Binding Component 3 – *BBC3* (+103%)	Pro-apoptotic protein, removes severely damages cells
Cystathionine γ-Lyase – *CTH* (+127%)	Produces hydrogen sulfide; regulates redox balance, stress signaling, and gut barrier function
Cathepsin K – *CTSK* (+146%)	Lysosomal protease involved in extracellular matrix remodeling and immune cell function
DNA Damage Inducible Transcript 4 – *DDIT4* (+247%)	Inhibits mTOR signaling during hypoxia or nutrient deprivation, and promotes autophagy and stress adaptation
Delta 4-Desaturase, Sphingolipid 2 – *DEGS2* (+148%)	Sphingolipid metabolism; involved in membrane and stress signaling
Delta Like Canonical Notch Ligand 4 – *DLL4* (+144%)	Important in vascular remodeling under hypoxia stress
Endothelial PAS Domain Protein 1 – *EPAS1* (+113%)	Oxygen-sensing transcription factor; regulates angiogenesis, gut epithelial integrity, and iron metabolism
Fructose-Bisphosphatase 2 – *FBP2* (+389%)	Regulates glucose metabolism, especially during fasting states
Fc Fragment of IgE Receptor Ig – *FCER1G* (-68%)	Immune signaling adaptor for innate immune activation
Formin Like 1 – *FMNL1* (+115%)	Regulates actin dynamics, immune cell migration, and cytoskeletal remodeling
G Protein-Coupled Receptor 37 Like 1 – *GPR37L1* (+182%)	Astrocytic GPCR involved in neuroimmune and stress signaling and resilience
Hepatitis A Virus Cellular Receptor 1 – *HAVCR1* (-51%)	Injury marker in kidney/epithelium
Hydroxycarboxylic Acid Receptor 2 – *HCAR2* (+968%)	Butyrate and niacin receptor; promotes balanced stress signaling and gut immune tolerance
Homocysteine Inducible ER Protein w/ Ubiquitin Like Domain 1 – *HERPUD1* (+102%)	Endoplasmic reticulum stress response and protein quality control
Interferon Regulatory Factor 1 – *IRF1* (+111%)	Regulates innate and adaptive immune responses
Jun Proto-Oncogene, AP-1 Transcription Factor Subunit – *JUN* (+101%)	Transcription factor controlling stress responses and cell proliferation
Killer Cell Lectin Like Receptor G1 – *KLRG1* (-54%)	Marker of T-cell/NK cell senescence/exhaustion
Metallothionein 1E – *MT1E* (+328%)	Metal ion binding, detoxification, and protection against oxidative stress
Nuclear Receptor Subfamily 4 Group A Member 1 – *NR4A1* (+116%)	Immediate-early transcription factor; circadian linked stress, metabolic and immune adaptation
Sestrin 2 – *SESN2* (+165%)	Protects against oxidative stress; regulates mTOR and autophagy
Spermine Oxidase – *SMOX* (+134%)	Polyamine metabolism and regulation of oxidative stress
Transporter 1, ATP Binding Cassette Subfamily B Member – *TAP1* (+117%)	Transports peptides for MHC I antigen presentation and surveillance
Transforming Growth Factor Beta 1 Induced Transcript 1 – *TGF1I1* (-51%)	Mediates TGF-β signaling, cytoskeletal remodeling, and fibrotic responses
Transforming Growth Factor Beta 1 Induced Transcript 1 – *TRIM62* (+138%)	E3 ubiquitin ligase involved in immune regulation and stress signaling

CONCLUSION

In an earlier study, P84, a two-product system, activated regulatory, repair, and restoration peptides/proteins that guide gastrointestinal tract processes to support the GI tract in a holistic way. Previous studies have also shown Protandim Nrf2 Synergizer activates antioxidant and detoxification pathways in the body. * †††

An mRNA-sequencing method was used to investigate additional and unexpected beneficial effects on the synergies of Protandim Nrf2 Synergizer and P84, known as Healthy Edge.

The results showed that Healthy Edge enhanced the known pathways of Protandim Nrf2 Synergizer and P84 by activating an additional eight genes (*CTH, MT1E, SESN2, SQSTM, ALDH1A2, ATG2A, HYPK, CYP2W1*) associated with detoxification and cellular protection pathways and another nine genes (*MUCL3, EGF, WNT9B, SESN2, MT1E, SQSTM1, ANXA1, HCAR2, ATG2A*) associated with gut regulation/repair pathways. *

Two new benefits were discovered with Healthy Edge, and it showed significant activation of genes involved in the circadian rhythm and cellular stress adaptation pathways. *

Three genes (*BMAL1, DBP*, and *EPAS1*) involved in the 24-hour cycle were activated. These genes support a more balanced circadian rhythm, allowing cells to switch to a more adaptive, resilient state and synchronize cell renewal and function. Another 29 genes (Table 2) associated with adaptability to cellular stress were influenced, allowing cells to sense stress, respond in an intelligent manner, and rebuild into something stronger. *

As a result, Healthy Edge supports cellular adaptation through coordinated sensing, signaling, repair, and learning mechanisms that allow them to survive stress, improve efficiency, and function better over time. Adaptability supports stable energy production, efficient repair and regeneration, proper biological timing, and long-term tissue resilience. This adaptability is fundamental to building a strong, resilient body and overall health. *

When adaptation is efficient, cells don't just cope with change. They use it to learn, self-correct, and become more efficient, resilient, and capable over time.

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