Mitigating the Effects of Cellular Stress to Return to Optimal Function

TRUESCIENCE® LIQUID COLLAGEN AND PROTANDIM® NRF2 SYNERGIZER® STUDY

Principal Investigator:

LifeVantage Corp.

Objective:

To evaluate the benefits of TrueScience® Liquid Collagen alone and in combination with Protandim® Nrf2 Synergizer® on skin appearance and helping fibroblast cells recover from a constant stress cycle and get back to more optimal function.

Sponsor:

LifeVantage Corp.

Reference:

LifeVantage Corp, Lehi, Utah, USA

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 Protandim® Nrf2 Synergizer® and its positive and beneficial effects on the oxidative stress response in the body.

TrueScience® Liquid Collagen is a ready-to-drink shot that contains 10 different types of hydrolyzed fish collagen peptides together with a red quinoa grain and a blend of blueberry and young Ponkan citrus. Red quinoa and the blueberry/young Ponkan citrus blend were evaluated in vitro for their capabilities to boost collagen secretion with excellent results.

Red quinoa has also previously been shown to activate and upregulate skin-barrier genes as well as significantly increase the activation of the COL1A2 gene, which codes for a protein that is a key part of collagen production. Efficacy evaluation of red quinoa was also performed in subjects and showed significant results in skin moisturizing, skin brightening, skin texture, improving the appearance of crow's feet, and skin collagen density.

TrueScience® Liquid Collagen was distributed to subjects for a 30-day self-reported before-and-after test with pictures showing clear improvements in skin appearance. Other unexpected benefits on joint health, general well-being, quality of life, etc. were also reported.

Due to the compelling benefits of both products, gene expression analysis was performed to determine if synergy exists between the two products.

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a. Fibroblasts

Selecting the correct cell type was a key first step in developing a protocol for the gene expression study. After deliberation and research, fibroblasts were chosen. Fibroblasts are found throughout the body, and their primary role is to provide support to tissues, but this looks different for different locations within the body.

Fibroblasts' role specifically in skin is to produce and organize components of the skin that act as the scaffold or support for the skin through proteins, including collagens, elastin, and laminin. Fibroblasts not only produce collagen and other proteins that make up the extracellular matrix for the skin, they also perform the same function for other organs, such as the intestines, spleen, brain, lung, liver, kidney, and blood vessels. They provide support to the structure of capillaries. Cardiac fibroblasts are found in the walls of the heart, helping to support heartbeats. Muscles contain three layers of fibroblasts that support skeletal muscles. They are also important for tendons and bones as the collagen proteins produced by fibroblasts are a critical component for each.

As a result, fibroblasts have the functional versatility to allow for investigation of a wide array of possible effects when Protandim® Nrf2 Synergizer® and TrueScience® Liquid Collagen are combined. RNA sequencing was used to investigate these potential synergies.

a. What is RNA Sequencing?

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 is useful for studying cellular responses. It shows which genes are turned on (upregulated or expressed) or turned down/off (downregulated or suppressed) at different time points. Essentially, RNA sequencing allows us to look at the entire genome or complete set of genetic material of the fibroblast cell and see gene expression and, ultimately, which key cellular genes and pathways are upregulated or downregulated.

Methods

Global Gene Expression by RNA Sequencing

In the study, we tested TrueScience® Liquid Collagen alone, Protandim® Nrf2 Synergizer® alone, and the two products in combination. 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 fibroblasts were treated with the different product combinations. Then RNA was extracted from the cells and sequenced. Only the RNA sequencing methodology and results are presented.

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RNA-Sequence Methodology

Normal human fibroblasts were seeded into a 10-cm dish and allowed to attach overnight before treated at 75% confluency with Control, TrueScience® Liquid Collagen, Protandim® Nrf2 Synergizer®, or a combination of both products. Cells were lysed, and RNA was isolated at the 8-hour specified time point using a Zymo Quick RNA MiniaPrep System. RNA QC, Library construction, Library QC, sequencing, and trimming were performed 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.
- Following is the workflow of library construction: 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, reads containing N>10% (N represents a base that cannot be determined), and 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 read-files from the filtered sequencing were processed using Galaxy (useGalaxy.org). FASTQ files were aligned with HISAT2 (Galaxy Version 2.2.1) using human b38 hg38 canonical female (cell line was female). HISAT2 was performed with “Unstranded” and “Paired end” options selected. The resulting SAM file produced with HISAT2 was used to generate count tables for each sample using the program FeatureCounts (Galaxy Version 2.0.3). Count tables were then put into Limma Voom to perform differential gene expression (relative to DMSO control) (Limma, Galaxy Version 3.50.1). Limma settings included gene filtration set to require at least 2 samples having at least 1 count per million.

Differential expression was ranked by adjusted P-value and gene enrichment analysis was performed (rank ordered by NES value (Normalized Enrichment Score). The gene sets C2 and C5 from https://www.gsea-msigdb.org/gsea/msigdb/human/collections.jsp#C2 were used in the analysis.

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Results and Discussion

1. Gene Expression Study

a. *Pathways*

A total of 13,708 genes were influenced in fibroblasts, which were distributed over 18,671 cellular pathways. Tables 1–3 represent the most important pathways that were significantly impacted by Nrf2 Synergizer, Liquid Collagen, and the combination of the two products.

First, we looked at the influence of Protandim® Nrf2 Synergizer® alone in fibroblasts. From previous research in liver, endothelial, kidney, and muscle cells, we know that Protandim® Nrf2 Synergizer® influences the Nrf2 pathway by 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.

Results for the data in fibroblasts with Protandim® Nrf2 Synergizer® alone were expected. The most significant pathways influenced by Protandim® Nrf2 Synergizer® were Nrf2-modulation pathways (**Table 1.**) Of the 18,671 total cellular pathways affected in the fibroblasts, 894 (4.8%) were significantly affected by Protandim® Nrf2 Synergizer® and the top pathways impacted were all Nrf2-associated pathways.

Table 1. Pathways modulated by Protandim® Nrf2 Synergizer®.

Pathways
WP_NRF2_PATHWAY
REACTOME_NUCLEAR_EVENTS_MEDIATED_BY_NFE2L2
REACTOME_KEAP1_NFE2L2_PATHWAY
WP_NRF2ARE_REGULATION

- NRF2_PATHWAY: This was the most significant pathway influenced. It is the overall Nrf2-regulatory pathway that includes transcription factors called nuclear receptors. It is activated under oxidative stress conditions and subsequently activates many associated genes. A total of 97 genes were influenced in this pathway.
- NUCLEAR_EVENTS_MEDIATED_BY_NFE2L2_Pathway: These are pathways that are directly activated in the nucleus by NFE2L2 (nuclear factor erythroid 2-related factor 2), which is the molecular name for Nrf2. It influenced 77 genes.
- KEAP1_NFE2L2_PATHWAY: This is an important modulator pathway that keeps the Nrf2 molecule in an active or inactive state in the cytoplasm. It also influenced 98 genes.
- WP_NRF2ARE_REGULATION: 22 genes are influenced in this pathway. They are involved in the ARE (Antioxidant Response Element) – Nrf2 interaction on the promoter region of the DNA.

This was further confirmation of what we have seen in study after study and is consistent with our knowledge of how the mechanism of activation for Protandim® Nrf2 Synergizer® works in the activation of antioxidant enzymes.

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With TrueScience® Liquid Collagen alone, the results were also expected, with the greatest impact on pathways that have a role in the structural and stabilization functionality of fibroblasts. We saw pathways associated with individual collagen-strand production (influenced 46 genes), strand assembly into fibers (influenced 17 genes), crosslinking of the collagen fibrils (influenced 14 genes), as well as organization in the cell (influenced 230 genes) of these components.

Of the 18,671 total cellular pathways affected in the fibroblasts 1,006 (5.4%) pathways were significantly affected by TrueScience® Liquid Collagen. Interestingly, several innate immune-system pathways that impact the cellular stress response were also significantly influenced (**Table 2.**)

Table 2. Pathways modulated by TrueScience® Liquid Collagen.

Pathways
GOCC_COLLAGEN_TRIMER
GOBP_EXTERNAL_ENCAPSULATING_STRUCTURE_ORGANIZATION
REACTOME_CROSSLINKING_OF_COLLAGEN_FIBRILS
GOCC_COMPLEX_OF_COLLAGEN_TRIMERS
REACTOME_CYTOKINE_SIGNALING_IN_IMMUNE_SYSTEM
GOBP_CYTOKINE_MEDIATED_SIGNALING_PATHWAY
KEGG_NOD_LIKE_RECEPTOR_SIGNALING_PATHWAY
WP_TOLLLIKE_RECEPTOR_SIGNALING_PATHWAY

- REACTOME_CYTOKINE_SIGNALING_IN_IMMUNE_SYSTEM: Cytokine pathways and their signaling in the immune system. It influenced 522 genes.
- GOBP_CYTOKINE_MEDIATED_SIGNALING_PATHWAY: Pathways that involve molecular signals initiated by the binding of a cytokine to receptors on the surface of a cell. It influenced 294 genes.
- KEGG_NOD_LIKE_RECEPTOR_SIGNALING_PATHWAY: This pathway initiates signaling cascades that eventually lead to upstream regulation of the NF- B transcription factor and its resulting cytokines. It influenced 47 genes.
- WP_TOLLLIKE_RECEPTOR_SIGNALING_PATHWAY: Another pathway regulated by toll-like receptors that play a crucial role in the innate immune system and cellular stress response. It influenced 67 genes.

In addition to confirming the pathways each product influenced individually, we also investigated the synergy of adding Protandim® Nrf2 Synergizer® to TrueScience® Liquid Collagen. The combination of Protandim® Nrf2 Synergizer® and TrueScience® Liquid Collagen showed an unexpected, positive synergistic effect on the activation of various pathways involved in the innate immune system and cellular stress response. Other unique pathways were also significantly influenced, which were involved in various cell-cycle processes and replication (**Table 3**). Of the 18,671 total cellular pathways affected in the fibroblasts 1,172 (6.3%) were significantly affected by the combination of Protandim® Nrf2 Synergizer® and TrueScience® Liquid Collagen.

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Table 3. Pathways modulated by the combination of Protandim® Nrf2 Synergizer® and TrueScience® Liquid Collagen

Pathways
WP_NRF2_PATHWAY
REACTOME_NUCLEAR_EVENTS_MEDIATED_BY_NFE2L2
REACTOME_KEAP1_NFE2L2_PATHWAY
WP_NRF2ARE_REGULATION
REACTOME_CYTOKINE_SIGNALING_IN_IMMUNE_SYSTEM
GOBP_CYTOKINE_MEDIATED_SIGNALING_PATHWAY
KEGG_NOD_LIKE_RECEPTOR_SIGNALING_PATHWAY
WP_TOLLLIKE_RECEPTOR_SIGNALING_PATHWAY
GOBP_IMMUNE_RESPONSE
REACTOME_INNATE_IMMUNE_SYSTEM
GOBP_REGULATION_OF_IMMUNE_SYSTEM_PROCESS
REACTOME_SIGNALING_BY_INTERLEUKINS

- NRF2_PATHWAY: This was the most significant pathway influenced. It is the overall Nrf2 regulatory pathway that includes transcription factors called nuclear receptors. It is activated under oxidative stress conditions and subsequently activates many genes. The combination influenced 97 genes in this pathway.
- NUCLEAR_EVENTS_MEDIATED_BY_NFE2L2_Pathway: These are pathways that are directly activated in the nucleus by NFE2L2 (nuclear factor erythroid 2-related factor 2), which is the molecular name for Nrf2. The combination influenced 77 genes.
- KEAP1_NFE2L2_Pathway: This is an important modulator pathway that keeps the Nrf2 molecule in an active or inactive state in the cytoplasm. The combination influenced 98 genes.
- WP_NRF2ARE_REGULATION: Genes in this pathway are involved in the ARE (Antioxidant Response Element) – Nrf2 interaction on the promoter region of the DNA. The combination influenced 22 genes
- REACTOME_CYTOKINE_SIGNALING_IN_IMMUNE_SYSTEM: Cytokine pathways and their signaling in the Immune system, impacting the cellular stress response. The combination influenced 522 genes.
- GOBP_CYTOKINE_MEDIATED_SIGNALING_PATHWAY: Pathways that involve molecular signals initiated by the binding of a cytokine to receptors on the surface of a cell. The combination influenced 294 genes.
- KEGG_NOD_LIKE_RECEPTOR_SIGNALING_PATHWAY: This pathway initiates signaling cascades that eventually lead to upstream regulation of the NF-κB transcription factor and its resulting cytokines. The combination influenced 47 genes.
- WP_TOLLLIKE_RECEPTOR_SIGNALING_PATHWAY: Another pathway regulated by toll-like receptors that play a crucial role in the innate immune system and cellular stress response. The combination influenced 67 genes.

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- GOBP_IMMUNE_RESPONSE: Pathways regulating any immune system process in response to insult. The combination influenced 941 genes.
- REACTOME_INNATE_IMMUNE_SYSTEM: Pathways involved in the innate immune system. The combination influenced 717 genes.
- GOBP_REGULATION_OF_IMMUNE_SYSTEM_PROCESS: Pathways and processes that modulate the immune response. The combination influenced 909 genes.
- REACTOME_SIGNALING_BY_INTERLEUKINS: Signaling pathways for interleukins. The combination influenced 336 genes.

a. ***Individual Gene Expression***

Based on the surprising and exciting pathway results listed above, we looked at the individual genes involved in those pathways, especially for the Protandim® Nrf2 Synergizer® and TrueScience® Liquid Collagen combination.

Because the human body is constantly under stress and other insults, we looked at the effect of the combination of the two products primarily on the innate immune system and cellular stress response.

The human immune system is comprised of two systems:

1. The innate or non-specific immune system (immediate)
2. The adaptive-antigen immune system (long-term)

The innate immune system is composed of several pathways. Each is controlled by its own master switch complex or master key complex, depending on the insult. One of the main master switch complexes is the NF-κB complex (nuclear factor kappa-light-chain-enhancer of activated B cells). This NF-κB complex can be found in almost all cell types, even fibroblasts, and is involved in the cell response to stress and other insult. It belongs to the rapid-acting primary transcription factors because it is usually in an inactive state and does not require protein synthesis to become activated. It is thus able to be one of the first proteins to respond to an insult. There is a fine balance of activating and deactivating this complex, so cellular stress responses don't become overstimulated.

The active NF-κB complex consists of five protein members: RelA (p65), RelB, Rel, p50, and p52, which are encoded by genes RELA, RELB, REL, NFKB1, and NFKB2. Its end products are small specialized signaling proteins called cytokines. The NF-κB complex can be rendered inactive by the addition of the IκBα (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha) protein in the cytoplasm, which blocks the ability of NF-κB to translocate into the nucleus and bind to DNA at kappa B-binding motifs. The result is that the cell cannot produce cytokines. The IκBα protein is encoded by the NFKBIA or NFKBIB gene. A closer look at the genes (RELA, RELB, REL, NFKB1, NFKB2, NFKBIA, NFKBIB) involved in activating/creating and deactivating the NF-κB complex showed Protandim® Nrf2 Synergizer® had no significant influence by itself. TrueScience® Liquid Collagen showed some activity on the RELB, NFKB2, and NFKBIA genes, which we used as our baseline and control. When Protandim® Nrf2 Synergizer® was added to TrueScience® Liquid Collagen, an interesting new synergistic effect was observed in fibroblasts.

A significant downregulation of NFKB1, NFKB2, and RELB was seen when using the Protandim® Nrf2 Synergizer® and TrueScience® Liquid Collagen together as compared to baseline (**Figure 1**). All three genes were significantly downregulated by 33%, 30%, and 20%, respectively. This suggests that there are fewer protein components available to create the master switch complex NF-$_{\kappa}$B and, as a result, a lowered cellular stress response.

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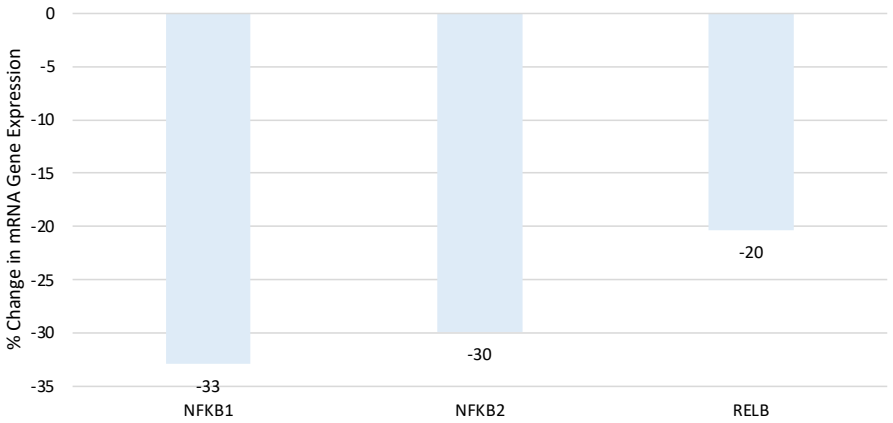
Figure 1. % Change in mRNA expression of genes that code for some of the proteins in the key master switch complex NF-κB as compared to TrueScience® Liquid Collagen alone when adding Protandim® Nrf2 Synergizer®. Genes that code for proteins in this complex (RELB, NFKB1, NFKB2) are all significantly downregulated, suggesting fewer proteins available to make NF-κB.



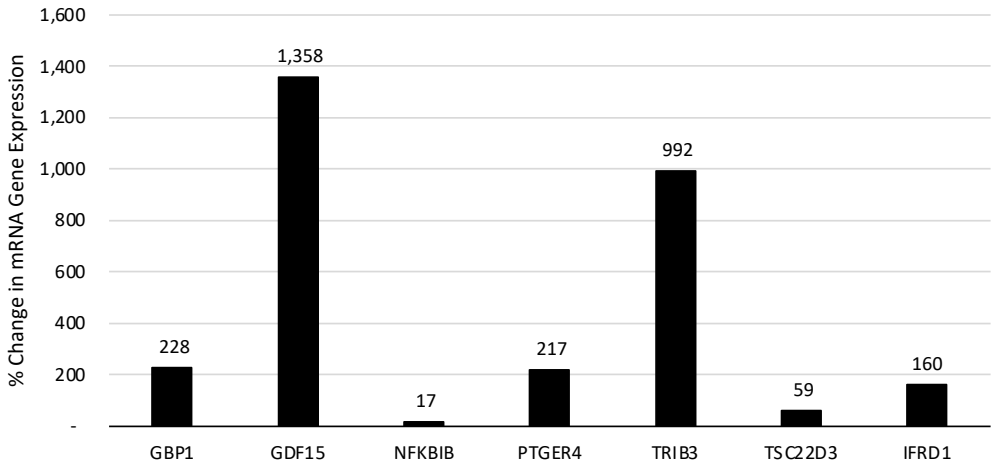
a. *Individual Gene Expression*

As mentioned above, the NF-κB complex can be rendered inactive by the addition of the IκBα protein (coded by genes such as NFKBIA or NFKBIB) in the cytoplasm, which blocks the ability of NF-κB to translocate into the nucleus and activate around 150 genes. When Protandim® Nrf2 Synergizer® and TrueScience® Liquid Collagen are used together, there is a 17% increase in the NFKBIB mRNA gene expression (**Figure 2**). This indicates there are more components available to create the IκBα protein to interfere with the assembly of the NF-κB proteins by complexing with it and trapping it in the cells' cytoplasm.

At the same time, there are many additional genes upregulated that either interfere with the assembly of the NF-κB complex or that are transcriptional regulators that interfere with NF-κB activity. Some of these are GBP1, GDF15, PTGER4, TRIB3, TSC22D3, and IFRD1 genes.

An interesting observation was that both Protandim® Nrf2 Synergizer® and TrueScience® Liquid Collagen both upregulated these genes to some degree. But with the combination of the two products, this upregulation became higher and statistically significant, showing the synergy between Protandim® Nrf2 Synergizer® and TrueScience® Liquid Collagen. The amount of % change in mRNA expression of the TRIB3 and GDF15 genes showed a 1,358% and 992% change, respectively.

Figure 2. % Change in mRNA gene expression of genes coding for proteins that either interfere with the assembly of the NF-κB complex or that are transcriptional regulators that interfere with NF-κB activity (NFKBIB, TRIB3, IFRD1, TSC22D3) as compared to TrueScience® Liquid Collagen alone when adding Protandim® Nrf2 Synergizer®. Other genes also code for proteins that restrain cellular proliferation in innate immune/cellular stress pathways or code for beneficial cytokine proteins (GBP1, PTGER4, GDF15)



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a. ***Individual Gene Expression (continued)***

When NF-κB complex is activated, downstream proteins are also produced, such as cytokines that balance the cellular stress response, helper proteins that allow cells to proliferate or stick to one another, or proteins that allow cells to secrete unwanted cell modulators.

Some cytokines are beneficial, such as those coded by the GBP1, PTGER4, and GDF15 genes (Figure 2), whereas others are unwanted, such as interleukin-1β (gene IL1B), interleukin-6 (IL6), interleukin-8 (CXCL8), and the CXCL1 and NLRP10 genes. Other unwanted proteins are encoded by tumor necrosis factor-alpha genes (TNFAIP2 and TNFAIP3), the innate immune modulator genes BIRC3 and PTX3, and a stickiness gene ICAM1.

Protandim® Nrf2 Synergizer® alone did not influence any significant changes in the expression of the cytokine genes mentioned in the previous paragraph. TrueScience® Liquid Collagen had a minimal effect, which we treated as the baseline. Once again, when using both Protandim® Nrf2 Synergizer® and TrueScience® Liquid Collagen together, we saw a significant downregulation of all 10 mRNA gene expressions coding for proteins acting as unwanted cell modulators, including BIRC3 by 80%, CXCL1 by 91%, CXCL8 by 38%, ICAM1 by 69%, ILB1 by 97%, IL6 by 88%, NLRP10 by 94%, PTX3 by 70%, TNFAIP2 by 41%, and TNFAIP3 by 61% (**Figure 3**).

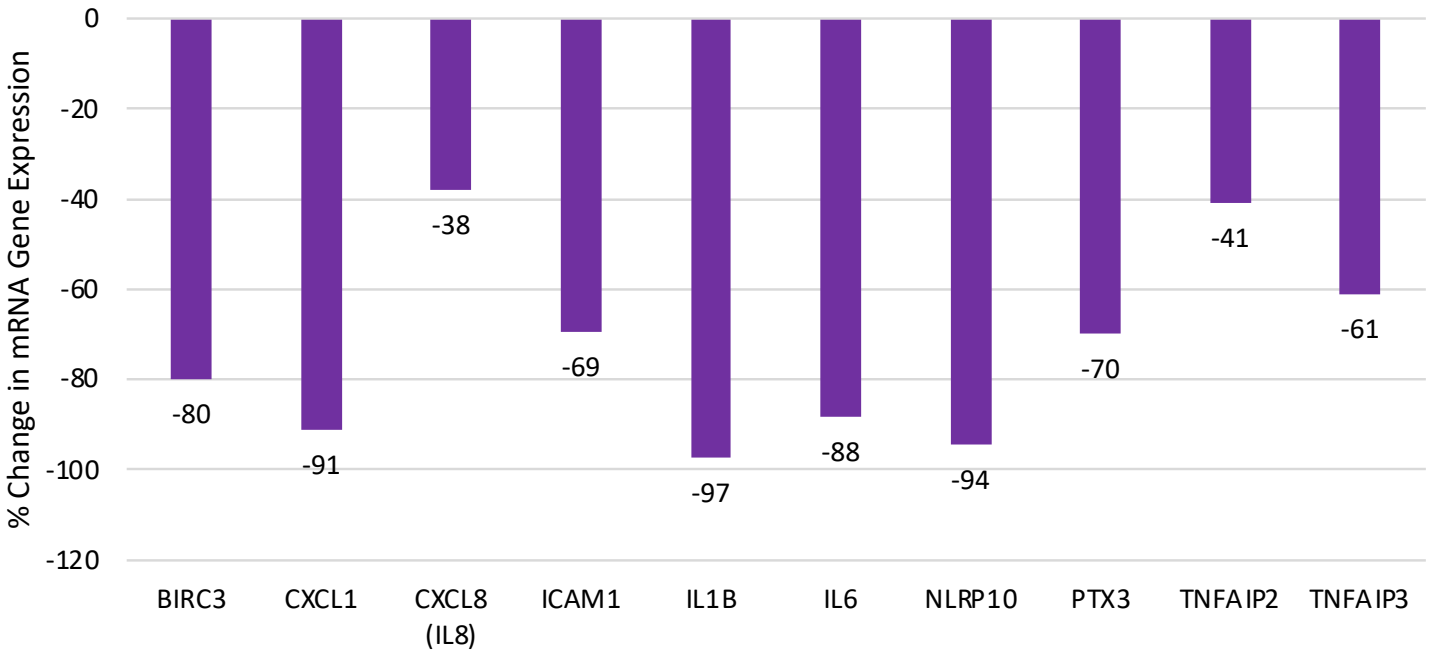

Figure 3. % Change in mRNA expression of genes coding for unwanted proteins in the innate immune response compared to TrueScience® Liquid Collagen alone when adding Protandim® Nrf2 Synergizer®. All genes are downregulated, suggesting the creation of a more balanced environment for healthy immune responses to occur.

These results are impressive results when compared to the individual products alone and show a true synergy in gene responses between Protandim® Nrf2 Synergizer® and TrueScience® Liquid Collagen. A healthy balance is essential to keep the body in harmony to create a more beneficial environment for stress responses to occur, even when the fibroblasts are constantly bombarded with insults.

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Conclusion

Fibroblasts are an integral part of our structural organs, such as intestines, spleen, brain, skin, joints, lung, liver, kidney, and blood vessels. They provide the backbone to the vascular system and skeletal muscles, bones, and tendons. Fibroblasts also play a central role in the body's immune response to stress.

We know that Protandim® 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.

TrueScience® Liquid Collagen contains a blend of collagen peptides and various unique botanicals that have shown to activate collagen production as well as genes involved in barrier function in the skin and downregulating the innate immune marker gene MMP9. From a consumer study, subjective testimonials came back that TrueScience® Liquid Collagen also had beneficial effects on skin appearance, joint health, and general well-being.

Because of their importance throughout the body, we chose fibroblasts to investigate the effect of Protandim® Nrf2 Synergizer®, TrueScience® Liquid Collagen, and the combination of Protandim® Nrf2 Synergizer® and TrueScience® Liquid Collagen on the total genome expression of the cell.

From the gene expression study, we saw that 13,195 genes distributed over 18,671 cellular pathways were influenced in the fibroblasts. Around 21% (2,786 genes) of them were significantly up, or downregulated either by Protandim® Nrf2 Synergizer®, TrueScience® Liquid Collagen, or a combination of the two.

Of the 2,786 genes measured, the combination of the two products synergistically influenced 2,439 genes ($p<0.05$), of which 418 genes (17%) were significantly up- (195 genes) or downregulated (223 genes).

Protandim® Nrf2 Synergizer® primarily affected the Nrf2/antioxidant pathways in the fibroblasts (**Table 1**), as it is designed to do. Whereas TrueScience® Liquid Collagen affected mostly immune-modulatory pathways as well as pathways associated with collagen-strand production, assembly, cross-linking, and organization of these components (**Table 2**). However, the effects on the immune modulatory pathways were not as large as seen with the combination of the two products.

Meaningful results were seen with the combination of Protandim® Nrf2 Synergizer® and TrueScience® Liquid Collagen. Together, they exert a synergistic effect in activating/de-activating cellular stress response pathways. These stress response pathways are all part of the innate immune response. We saw these changes through the synergistic activation or de-activation of key genes associated with these pathways.

Protandim® Nrf2 Synergizer® by itself did not significantly influence any of the innate immune and stress response pathways. It was only through adding it to TrueScience® Liquid Collagen that the synergistic effect on these pathways was shown.

This is an exciting discovery that shows amazing results when combining Protandim® Nrf2 Synergizer® and TrueScience® Liquid Collagen. Using both products together can deliver not only skin benefits but also have an impact on whole-body connective tissue health. This could support various functions and systems throughout the body, such as joint health, cardiovascular function, gut health, and general well-being.

Their synergistic impact on fibroblasts demonstrates that Protandim® Nrf2 Synergizer® and TrueScience® Liquid Collagen could make a significant impact on your health—on how you feel on the inside as well as what you can see on the outside.

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