Scientific Validation of ASEA Redox Supplement

ASEA'S COMMITMENT TO RESEARCH

Since its inception, ASEA has put high priority on allocating resources for research into the efficacy of its products. It is important for us to confirm that these products make a difference in the lives of those who use and share them. ASEA will continue to create scientifically validated products, making continuous research paramount. The company has an ongoing commitment to show the safety and efficacy of their products. We certify them where possible and confirm the benefits of redox signaling supplementation.

THIRD-PARTY REDOX VALIDATION

To further validate our own internal testing, we have established partnerships with sophisticated and accredited chemical-analytical laboratories. These third-party labs offer a wide catalog of assay kits and electrochemical devices to help us measure multiple redox parameters. Their teams include chemists, biotechnologists, and biologists with industrial and postdoctoral experience, all who help monitor and analyze data markers such as total antioxidant capacity (TAC), enzymatic activity, phenolic compounds, and reactive oxygen species (ROS).

ANTIOXIDANT UP-REGULATION

Oxidative stress is an imbalance between the production of free radicals and the ability of the body to counteract or detoxify their harmful effects at a cellular level. ASEA commissioned Pacific Northwest National Laboratory (PNNL) to determine if exposure to ASEA Redox Supplement activated the cell nucleus to call for increased production of antioxidants, such as glutathione peroxidase (GPx).

Study Protocol

Researchers exposed human endothelial cells to either ASEA Redox Supplement or an inert phosphate buffer solution (PBS). Standard western blot analysis was used to determine if exposure to ASEA Redox Supplement activated cell nuclei to increase production of antioxidants such as GPx, an essential enzyme in cellular antioxidant defense systems. The concentration of messengers in the nucleus that activates increase of antioxidants was also measured in human endothelial cells and compared to cells not exposed to the redox supplement.

The movement of the agents into the nucleus can be seen with certain dyes under a microscope and offers a way to see the call for an increase of antioxidants.

Since the production of antioxidants can also increase by exposure of the cells to low levels of inflammatory toxins, tests were performed to ensure that ASEA Redox Supplement was not provoking the cells to undergo this low-level inflammatory or toxic response.

Results Summary

Verification by the western blot analysis showed clear responses in the increase of antioxidants upon exposure to the redox supplement in comparison to the saline control. This effect was temporary, lasting only about 120 minutes but was clearly visible.

Researchers indicate that the most impressive result of this evaluation is that exposure to ASEA Redox Supplement at any concentration did not invoke an inflammatory response but did invoke an antioxidant increase. Stimulating the production of antioxidants without stimulation of low-level inflammation is very rare.

INFLUENCE OF ASEA REDOX SUPPLEMENT INGESTION ON OXIDATIVE STRESS

Human Performance Laboratory
APPALACHIAN STATE UNIVERSITY

ASEA partnered with the North Carolina Research Campus Human Performance Laboratory to evaluate the effectiveness ASEA Redox Supplement in helping overweight/obese adult women improve disease risk factors such as arterial stiffness, cholesterol status, and oxidative stress.

Dr. David C. Nieman conducted clinical trials at the Human Performance Laboratory of Appalachian State University.

Study Protocol

A total of 106 overweight women (ages 20 to 73 years) ingested four fluid ounces of ASEA Redox Supplement or placebo (randomized groups) each day for 12 weeks under double-blind conditions.

Results Summary

Oxidative stress emerged as the disease risk factor most influenced by ASEA Redox Supplement ingestion during the 12-week study. Oxidized low-density lipoprotein (LDL) is the oxidized form of "bad cholesterol" and a major contributor to atherosclerosis or plaque that narrows blood vessels feeding the heart muscle. Oxidized LDL decreased 6.3% in the ASEA Redox Supplement group compared to a 0.9% increase in the placebo, a highly significant difference. Serum cholesterol also decreased 6.3% in the ASEA Redox Supplement group after 12 weeks compared to a 2.1% reduction in the placebo group.

ASEA Redox Supplement versus a placebo ingestion by healthy but overweight/obese females with multiple risk factors for heart disease had significant effects in lowering total cholesterol and oxidized LDL (a major contributor to atherosclerosis formation).

Post-study levels of a biomarker for cellular oxidative stress and other disease factors were lower in the ASEA Redox Supplement compared to placebo group, supporting the significant influence of daily ASEA Redox Supplement ingestion over a 12-week period in decreasing oxidative stress.

ASEA REDOX SUPPLEMENT IN VITRO PRODUCT SAFETY STUDY

Pacific Northwest
NATIONAL LABORATORY

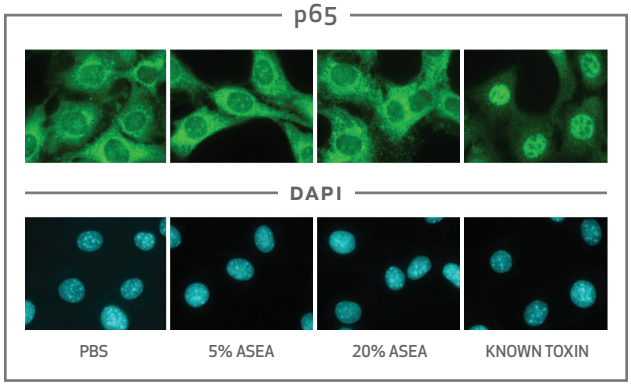
ASEA commissioned Pacific Northwest National Laboratory to study the toxicity response of eukaryotic cells when in contact with ASEA Redox Supplement.

Eukaryotic cells contain an array of cellular structures that play important roles in energy balance, metabolism, and gene expression. These cells, when stressed by a toxin, respond by sending transcription factors—proteins that control which genes are turned on or off—into the nucleus. Once inside the nucleus, these transcription factors activate the genes responsible for cellular defense and protection against toxins. The arrangement of the chromosomes (called translocation) of particular transcription factors into the nucleus can be seen under a fluorescent microscope when specific indicator dyes stain the cells.

If the cell undergoes a toxic response, the fluorescent dye is pulled into the nucleus along with the transcription factor. In this experiment, two transcription factors, the p65 subunit of NF-kappaB and P-Jun, were monitored. These two transcription factors are known to activate in all toxic responses.

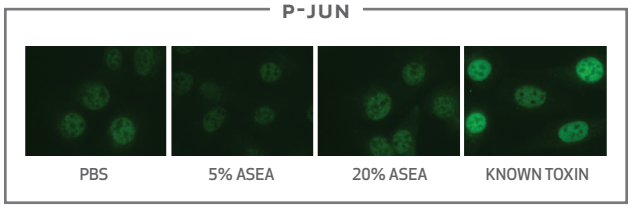
Study Protocol

In the photographs from the fluorescent microscopic images of the cells, a toxic response is registered if the green dye is seen to move into the nucleus.

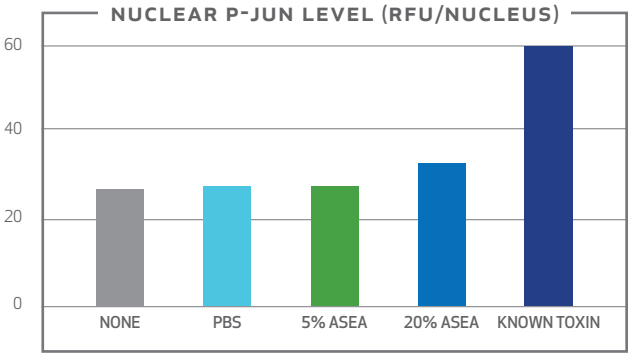


The target cells were cultured and exposed to:

1. Phosphate buffered saline (PBS)—the negative control where no toxic response was expected
2. 5% ASEA Redox Supplement—supplementing the equivalent to replacing 5% of a blood plasma solution with ASEA Redox Supplement
3. 20% ASEA Redox Supplement—Supplementing the equivalent to replacing 20% of a blood plasma solution with ASEA Redox Supplement
4. A known toxin—the positive control where a toxic response was expected



The reaction of the transcription factors (tp65 subunit of NF-kappaB and P-Jun), were photographed under a microscope after exposure to the four solutions. A DAPI stain was applied to the nuclei to help computer software to identify the cell nucleus in the pictures.



Results Summary

Visual evidence from the study manifested that direct exposure of cells to relatively high concentrations of ASEA Redox Supplement does not register a significant toxic response as measured by nuclear translocation. Based on these results, ASEA Redox Supplement, orally administered, does not manifest a toxic response or inflammation to exposed tissue.

DETERMINING THE ANTIOXIDANT EFFICACY OF ASEA REDOX SUPPLEMENT

Oxidative damage has been implicated in aging and age-dependent diseases, including cardiovascular disease, cancer, neurodegenerative disorders, and other chronic conditions. If the generation of free radicals exceeds the protective effects of antioxidants and some co-factors, this can cause oxidative damage.

Glutathione peroxidase (GPx) is an essential enzyme in cellular antioxidant defense systems, detoxifying peroxides and hydroperoxides. Superoxide dismutase (SOD) is an enzyme that helps break down potentially harmful oxygen molecules in cells, which might prevent damage to tissues.

In this study, scientists attempted to determine if direct contact of ASEA Redox Supplement to cells affects the antioxidant efficacy of GPx and SOD.

Study Protocol

Cultures of standard epidermal cells were exposed to various small concentrations of ASEA Redox Supplement (less than 1%) and a phosphate buffered saline (PBS) solution for 24 hours. The decrease of oxidants due to GPx enzymatic activity was monitored over an eleven-minute period after a chemical agent (cumene hydroperoxide) initiated the reaction. The reduction of oxidants is an indication of antioxidant efficacy. Three replications of oxidant residual in the samples were read every two minutes to determine GPx efficacy at various concentrations of PBS or ASEA Redox Supplement.

Results Summary

An 800% increase in GPx antioxidant efficacy was seen after 24 hours of exposure from low concentration ASEA

Redox Supplement. A transitory increase of up to 500% was observed in SOD antioxidant efficacy between 30 and 90 minutes after exposure to low-concentration ASEA Redox Supplement (< 1%).

Exposure to high concentration ASEA Redox Supplement, in comparison, elicited only a small relative increase in GPx antioxidant efficacy that was not concentration dependent. An increase in SOD efficacy was not seen for either high concentration ASEA Redox Supplement or after long exposures (24 hours).

ASEA REDOX SUPPLEMENT ANTIOXIDANT EFFICIENCY (IN VITRO ANTIOXIDANT ENHANCEMENT)

Researchers at the Pacific Northwest National Laboratory (PNNL) performed in vitro tests to determine the antioxidant efficiency of the body's most powerful natural antioxidant enzymes glutathione peroxidase (GPx) and superoxide dismutase (SOD). Also, they looked at the increase in the natural production of these antioxidants inside epithelial and endothelial cells.

Study Protocol

Using an Assay Design® Stressgen® kit, the scientists measured antioxidant activity and the ability of the antioxidants to reduce oxidant activity. Additionally, several preliminary experiments were done to examine the accuracy of the results based on known standards of antioxidant activity.

Results Summary

The results showed significant, well-defined effects. The cell extracts exposed to ASEA Redox Supplement exhibited eight (8) times the antioxidant efficiency for GPx than those exposed to the saline solution. The SOD antioxidant efficiency was slightly less, with about five (5) times enhancements in efficiency. This efficiency was evident especially at low-level concentrations of ASEA Redox Supplement, tested down to 2.5% of full strength.

The scientists saw from these in vitro tests at least a 500% improvement in the overall antioxidant efficiency due to ASEA Redox Supplement exposure.

DETERMINING THE TRANSLOCATION OF ANTIOXIDANT ACTIVATING TRANSCRIPTION FACTORS

Translocation is a subcellular process in which activated proteins are transported into the cell nucleus as part of a signal pathway to modify cell function in response to a signaling event or condition. Transcription factors are proteins involved in the process of converting, or transcribing, DNA into RNA. NRF2 is one type of transcription factor that regulates the expression of antioxidant proteins that protect against oxidative damage triggered by injury and inflammation.

In this study, scientists attempted to determine if varying concentrations of ASEA Redox Supplement placed in physical contact with living cells activates translocation of transcription factors (NRF2) associated with increased expression of antioxidants in living human endothelial cells. Additional analysis was performed to verify the appearance of the transcription factors by western blot analysis.

Study Protocol

Human lung microvascular endothelial cell (HMVEC-L) cultures were exposed to a high concentration (5 – 20%) and a low concentration (1%) of ASEA Redox Supplement and analyzed in conjunction with cultures exposed to a phosphate buffered saline solution (PBS) as a negative control. At time points of 30, 60, 90, and 120 minutes, cell samples from each of the cultures were placed under a fluorescent microscope. The cultures were stained with a fluorescent dye designed to tag the NRF2 transcription factor along with the fluorescent nuclear stain that aids the computer software to find the nuclei.

Automated imaging was used to determine the degree of nuclear accumulation of NRF2 via fluorescent analysis over several cells. NRF2 regulates the transcription of some phase II antioxidant defense enzymes and raises the possibility that additional antioxidant defense enzymes, such as glutathione transferase, may be expressed through exposure to ASEA Redox Supplement.

The accumulation of NRF2 into the nucleus, as seen visually in the microscope images, is an indicator of increased antioxidant expression in the cells.

Results Summary

Results of the examination suggest that ASEA Redox Supplement at a lower concentration induces a 20 – 30% increase in the nuclear translocation of the NRF2 transcription factor in the cells over a short-lived period of 30 – 60 minutes.

Researchers also observed that ASEA Redox Supplement induced a parallel decrease in the phosphorylation of an extra-nuclear protein whose phosphorylation status increases in response to hydrogen peroxide treatment, consistent with an antioxidant mode of action. Serum-starving the HMVEC-L cells significantly increased the nuclear NRF2 signal induced by ASEA Redox Supplement.

NRF2 directly and dramatically amplifies the innate ability to produce antioxidant protection by signaling DNA. This study suggests that molecules from the NRF2 activating ASEA Redox Supplement may trigger the production of antioxidant molecules, providing protection against the effects of free radicals compared to standard antioxidant supplements.

DETERMINING CELL PROLIFERATION AND VIABILITY

Researchers evaluated ASEA Redox Supplement on proliferation cell counts of human cells and associated markers for cell viability and health.

Study Protocol

Human lung microvascular endothelial cells (HMVEC-L) received treatment of a 5 – 20% concentration of ASEA Redox Supplement for 72 hours. A control group received a 20% phosphate buffered saline solution treatment. Researchers used a Coulter counter—an apparatus for counting and sizing particles suspended in electrolytes—to determine cell count. Serum LDH levels measured cell culture viability at 0 – 20% ASEA Redox Supplement serum concentrations. Similar experiments were performed for murine (JB6) epidermal cells.

Results Summary

High concentrations (5 – 20% volume/volume percent) of ASEA Redox Supplement exposure inhibited cellular proliferation for both HMVEC-L and JB6 cell types (determined from cell counts). The HMVEC-L inhibition was clearly concentration dependent, with a 20% loss of cell count at 20% ASEA Redox Supplement concentration. In contrast to decreased proliferation, serum LDH levels significantly decreased with ASEA Redox Supplement concentration between 5 – 20%, indicating increased cell membrane integrity. The results seem to suggest that cellular proliferation decreases while membrane viability increases at high levels.

ACTION OF ASEA REDOX SUPPLEMENT ON STRESSED CELLS

In this examination scientists evaluated the effects of ASEA Redox Supplement on cells that were stressed with cytokines (cachexin), radiation and serum starvation. Cytokines are cell signaling molecules that aid cell-to-cell communication in immune responses and stimulate the movement of cells towards sites of inflammation, infection, and trauma.

Study Protocol

Cell cultures with normal random cell cycles and cultures approaching confluence received increasing concentrations of cachexin stressor (a group of proteins that can cause cell death). These cultures received either a pretreatment of 10% phosphate buffered saline solution control or 5 – 10% concentration of ASEA Redox Supplement for 24 hours. Researchers measured two indicators of cell viability: Serum LDH levels as an indication of membrane integrity and neutral red dye as a demonstration of lysosomal integrity.

As cell membranes fail, LDH releases into the serum medium. Lower quantities of LDH indicate higher cell viability. The integrity of lysosomes, necessary for viable cell function, are measured by absorption of Neutral Red dye stain. Higher quantities of Neutral Red absorbance indicate higher cell viability.

Results Summary

The response of the cells, when stressed with cachexin, depends on upon cell phase. Normal random cycling cells exhibited a typical decrease in cell viability accompanied by cell death. Confluent end-of-life-cycle and borderline cells were less sensitive to cachexin insult, exhibiting less pronounced decreases in cell viability and less cell death.

Exposure to ASEA Redox Supplement caused no significant change in the response of the normal random cycling cells to cachexin (showing a similar loss of cell viability and cell death). However, cultures approaching confluence exposed to ASEA Redox Supplement exhibited increased sensitivity to cachexin, restoring behavior comparable to that of normal cells. Borderline cells, exhibiting a relatively small cachexin response and confluent cells that are usually insensitive to cachexin insult exhibited a much stronger response to cachexin when exposed to ASEA Redox Supplement, both in a decrease in viability and increased cell death.

It appears that exposure to ASEA Redox Supplement causes increased rates of confluent cell death, enhancing the natural reception of cachexin in end-of-life-cycle cells. Exposure to ASEA Redox Supplement is not expected to cause any change in normal cell viability.

Cachexia is usually secreted to instigate cell death in damaged or dysfunctional tissues, allowing surrounding healthy cells to divide and fill in voids. Thus, increasing the sensitivity to cachexin in dysfunctional cells may help accelerate such a process and is not always considered harmful.

Acceleration of cell death in tissues stressed was seen with radiation and serum-starvation associated with exposure to ASEA Redox Supplement.