A NION Health™ White Paper #1 (Pre-Publication)

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– Parts 1 & 2 –

Proposed mechanism of Action for NCS-330, with a 330mV negative charge, to Enhance Mitochondrial Function, Biogenesis, and Mitophagy with Implications for Improved Cellular Function and Longer Human Health Span

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Overview

The notion that life is a force, connected in an intimate way to a mysterious, pulsating universe has inspired theories and discoveries for millennia. Some solidified around practical applications like John Maynard Keynes' unruly energy propelling economic growth that he called the "invisible hand." Keynes' "invisible hand" is a widely recognized economic theory. Similar theories like "earthing" and "quantum entanglement" are credible but were initially rejected as untethered to rationality or mere fantasies even when presented with scientific evidence.

A study published in 2021 opened such a door for NION Health™. That study documented physiological changes in college students exercising at different levels of intensity. The work was done at a human performance lab used by U.S. Olympic teams at a major university under strict double-blind, placebo-controlled protocols.

The data was startling, so the sponsor asked a National Institutes of Health (NIH) Research Fellow to review the data. The NIH Fellow concluded there was strong evidence that a 3-gram daily dose of a crystalline mineral holding a negative 330mV charge, improved mitochondrial function significantly.[1],[2]

The studies confirmed the findings of earlier pilot studies and were consistent with anecdotal evidence. However, a theory regarding the mechanism of action was illusive. This White Paper #1 examines a several theories for the mechanism of action and proposes further studies.

Improving mitochondrial function has significant implications. If simple, safe, and effective mechanisms can improve mitochondrial function, it is likely to improve cellular, tissue, organ, and even systemic health in humans. The result may be improved quality of life, stronger physical and mental resilience, and extended human health span.

Some Background: Mitochondria joined the evolutionary process about 1.8 billion years ago as a bacterium that joined with a single cell organism. That combination created an evolutionary competitive advantage that led us to the cell structure in virtually all human cells.

Cells evolved to make mitochondria the center of cell function and the center of life. They are known as "the powerhouse of the cell" but have many other functions that drive overall cellular health. Their "life force" has inspired

public awe, speculation, and even great stories. For more information about public fascination with the science of mitochondria, there is an Appendix A to this White Paper.

PART 1 – WHAT WE KNOW

Results from 2 double-blind, placebo-controlled studies of subjects taking NCS-330, a dietary ingredient carrying a negative 330mV change

NCS-330, manufactured by NION Health, holds a negative 330millivolts of electric potential in a dry, crystalline mineral matrix. The manufacturing process uses specially designed equipment in a Food & Drug Administration ("FDA") certified facility north of Seattle.

The FDA reviewed safety data and the proprietary production methods for NCS-330 and recognized the product as a new dietary ingredient (i.e. food) pursuant to the Dietary Supplement and Health Education Act (21CFR section §190.6).

All filing and necessary approvals are in place for NCS-330 to be sold with structure-function claims based on two clinical trials.

Independent laboratories test and certify the chemical composition of each NCS-330 lot for purity and potency. Samples of NCS-330 and a placebo were sent to Montana State University for clinical trials.

The double-blind, placebo-controlled trials were designed by university researchers to measure the physiological and cellular responses of college-age students undergoing physical stress with and without NCS-330 in students' diets. NCS-300 carries a highly negative 330mV electromotive charge.

Physiological Responses in Two Clinical Trials of NION

The Montana State University Human Performance Laboratory, led by Dan Heil, Ph.D., conducted two double-blind, placebo-controlled, cross-over studies that are informative. Both were published in peer-review journals.[3,4]

The two studies (Heil 1 and Heil 2) collected data on subjects (average age 20) exercising at various levels of intensity until they reached exhaustion. Some subjects received 3 grams of NCS-330. Others received a comparable placebo.

While the subjects were exercising, Dr. Heil's staff collected physiological and biomarker data that was later analyzed by a former Research Fellow from the National Institutes for Health (NIH) for insights on mitochondria functioning.

The data from the Heil study published in 2021 is summarized below:

Bio-Markers w/ Significant[1] Differences

After Seven Days Taking Product...	NCS-330 v. Placebo
Pre-Exercise Stress Biomarkers indicated:	
Systolic blood pressure	NCS-330 lower
Diastolic blood pressure	NCS-330 lower
Moderate Intensity Stress Biomarkers indicated:	
Heart Rate	NCS-330 lower
Perceived Exertion	NCS-330 lower
Blood Lactate Levels	NCS-330 lower
High Intensity Stress Exercise indicated:	
Heart Rate	NCS-330 lower
Perceived Exertion	NCS-330 lower
Blood Lactate Levels	NCS-330 lower
Maximum Effort Exercise Biomarkers indicated:	
VO_2max	NCS-330 higher
Heart Rate	NCS-330 lower
CO_2/O_2 Respiratory Exchange	NCS-330 higher
Blood Lactate Levels	NCS-330 higher
Time to Exhaustion	NCS-330 longer
Blood Lactate Levels at Exhaustion	same

[1] A p-value of 0.05 or less is considered statistically significant.

Time to Exhaustion ("TTE") is a surrogate measure for a limit of adenosine triphosphate (ATP) production by the mitochondria. Longer TTE indicates a more robust mitochondrial function in the subjects taking NCS-330.

Presumably, the powerhouses of the cells were not able to maintain ATP production to keep the muscle cells contracting after the 20-year-old subjects had worked to exhaustion.

The implications are that a simple, seven-day regimen of 3gm of NCS-330 can significantly improve mitochondrial ability to generate ATP and improve general mitochondrial function. It is also noteworthy that this result is significant even in a 20-year-old cohort – people who were at the peak of mitochondrial function.

It is generally recognized that mitochondrial function declines at about age 30 and is the first stages of age-related decline.[5]

Other takeaways from the 2021 Heil study are:

1. NCS-330 improves efficient use of oxygen as indicated by higher VO2max and higher respiratory exchange ratio.

2. NCS-330 lowers general physiological stress on the body as measured by heart rate even when more work had been performed.

3. NCS-330 improves blood lactate clearance which is a measure of mitochondrial efficiency.

The conclusion is that nutritional intake of 3 grams of NCS-330 improves mitochondrial function even for subjects whose age would suggest they were at the prime of their mitochondrial efficiency. Earlier pilot studies had shown dramatic improvements in mitochondrial function for older athletes that were so significant they defied logical explanation.

NIH Research Fellow Conclusions About Mitochondrial Function

Lisa Price[2], a former NIH Research Fellow, analyzed the data from Heil 1 and Heil 2. Her conclusions were:

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[2] Dr. Lisa Price is a licensed Naturopathic Physician with expertise in complimentary cancer care and culinary nutrition curing cancer treatment. She is an adjunct faculty

1. Subjects taking NCS-330 for seven (7) days showed consistent improved muscle output as evidenced by decreased muscle fatigue, increased time to exhaustion, increased total work performed to exhaustion and lower perceived exertion throughout the trial.

2. Improved muscle output is a direct indication of higher adenosine triphosphate (ATP) production in muscle tissue and indicates increased mitochondrial functionality in subjects taking NCS-330.

3. Consistent with improved ATP production is improved cardiovascular function as noted with lower blood pressure in subjects taking NCS-330 but those indications may not be directly associated with improved mitochondrial function.

4. Subjects taking NCS-330 for seven (7) days also showed improved lactate clearance which is highly correlated with improved mitochondrial function.

5. Higher blood plasma pH and higher urine pH levels were associated with taking NCS -330mV for seven (7) days but this is not a direct indication of improved mitochondrial function.

6. The most significant biomarkers indicating improved metabolic and mitochondrial function for subjects taking NCS-330 are:
    a. Increased VO_2max at maximum exercise levels;
    b. LowerVO_2 consumption at a set exercise level;
    c. Increased CO_2 clearance at all exercise levels; and
    d. Lower ventilation rates with higher muscle output.

Dr. Price concluded the biomarkers and physiological improvements support a hypothesis of improved mitochondrial function with consumption of NCS-330 for seven (7) days and warrants further study.

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member at Bastyr University in Kenmore, Washington. She is the Chairperson of Research for the Institute of Naturopathic Medicine serves on its Board. Dr. Price served as a Research Fellow for the National Institute of Health (NIH) National Center for Complementary and Alternative Medicine from 2005 through 2010 and holds a certificate in Biomedical Regulatory Affairs from the University of Washington.

Of particular note is the increased VO_2max with consumption of NCS-330.. Improvements in VO_2max are often noted by leading researchers (such as Peter Attia) as the most powerful biomarker of longevity.[3]

PART 2 – A CLOSER LOOK AT MITOCHONDRIA & THE CELL

An essential first step will be to underline the absolute importance of healthy mitochondria in sustaining life and overall vitality. Mitochondria are referred to as the source of cellular energy, the "powerhouse of the cell" or the "battery packs" of cellular life. They create adenosine triphosphate (ATP). ATP serves as fuel for a wide range of cellular functions throughout the body.

But speaking of mitochondria in terms of batteries and energy alludes to their electrical nature but it only touches the surface of the role mitochondria play in making life possible. Perhaps no one has captured the critical importance of mitochondria (or mitochondrion, using the singular spelling) as succinctly and eloquently, as Dr. Michael R. Duchen, Mitochondrial Biology Group, Department of Physiology, University College London, who wrote:[6]

> ***"The mitochondrion lies at the heart of cell life and cell death. That we must breathe oxygen to stay alive is simply the consequence of the demand of our mitochondria for oxygen. About 98% of inhaled oxygen is consumed by mitochondria, and without mitochondria, we would have no need of the oxygen transfer machinery of the lungs, red cells, hemoglobin, or even the circulatory system that delivers oxygen to the tissues. Similarly, the organization of food intake, digestion, and processing is designed primarily to supply substrates destined for mitochondrial oxidation."***

With mitochondria consuming 98% of our oxygen and serving as virtually the sole beneficiary of everything we eat, they are figuratively as well as literally

[3] Attia, Peter MD. Outlive: The Science & Art of Longevity. Penguin Random House. 2023.

at the very core of our life. There metabolic process produces water necessary to maintain hydration and normal cellular function. So, it isn't surprising that mitochondrial dysfunction is at the core of nearly all our degenerative diseases.[7, 8, 9]

An Organelle with its Own DNA and RNA

A mitochondrion is an organelle—a virtual cell within a cell. As an organelle, a mitochondrion is subdivided into compartments to support a variety of functions. While all cells contain a complete copy of DNA within their nucleus, a mitochondrion has its own genetic material—a subset of DNA and RNA used to create essential proteins, including proteins required for reduction–oxidation (redox), ATP synthesis, and related functions.

While absent from red blood cells, mitochondria are found in nearly all other cells of the body, in varying quantities, according to a cell's energy needs. Using their own DNA subset, mitochondria divide to increase their population to meet cellular demands. Mitochondria occupy up to 40% of the cytoplasmic space of heart muscle cells, for example, while filling about 20% of a liver cell's cytoplasm.[10]

Mitochondrial dysfunction is recognized as a hallmark of aging and illustrates the importance of mitochondria function for youthful cellular, tissue, organ, and general health.[11]

It also emphasizes that mitochondria are more than the powerhouse of the cell. They also play a key role in cell signaling and are a potent driver of biological aging and are linked with:

- Genomic instability.
- Epigenetic alterations.
- Loss of proteostasis.
- Deregulated nutrient sensing.
- Cellular senescence.
- Stem cell exhaustion.
- Altered intracellular communications.

What We Know About the Electromotive Gradient at the Mitochondrial Membrane

Healthy Mitochondrial Membranes are Essential for Human Health

Mitochondria have an outer membrane, which is semi-porous, and an inner membrane, which plays a critically important role in the production of ATP. Both membranes are composed of phospholipid bilayers and proteins.[12]

The mitochondrial membrane potential (ΔΨm) is the result of oxygen serving as the terminal receptor of electrons through the electron transport chain, thus creating an electric charge gradient. It is a form of energy storage which is used by ATP synthase to make ATP.

These transformations generate not only an electric potential (because of charge separation) but also a proton gradient, and together they form the transmembrane potential of hydrogen ions.[13] Normally, cells maintain stable levels of intracellular ATP and mitochondrial membrane potential, and this stability is thought to be a requisite for normal cell functioning.[14],[15]

It suggests that although these parameters can change with physiological activity, the changes should be transient, but a prolonged perturbation of one factor may compromise the viability of the cells and pathological consequences.[16]

According to recent findings, ΔΨm can be used for ATP synthesis and is a factor determining viability of mitochondria and staging cell senescence.

It is also a driving force for transport of charged compounds, some of which are essential for mitochondrial viability. The non-energy-producing functions are often treated superficially in literature but most likely have profound cell signaling functions. Altered intracellular communication is another hallmark of aging.

Sitting between the outer membrane and the inner membrane is an area much like the cytoplasm of a cell, called the intermembrane space. The intermembrane contains cytochrome c and other proteins essential to the electron transport chain that assists in the creation of ATP.

Inside the Electron Transport Chain and the Mitochondrial Matrix

The electron transport chain operates between the mitochondrial outer and inner membrane.

The heavily folded inner membrane serves as a production platform for ATP. Unlike the semi-porous outer membrane, the inner membrane is highly impermeable to molecules. An electron transport chain is required to import or export materials across the inner membrane to the interior region known as the mitochondrial matrix.

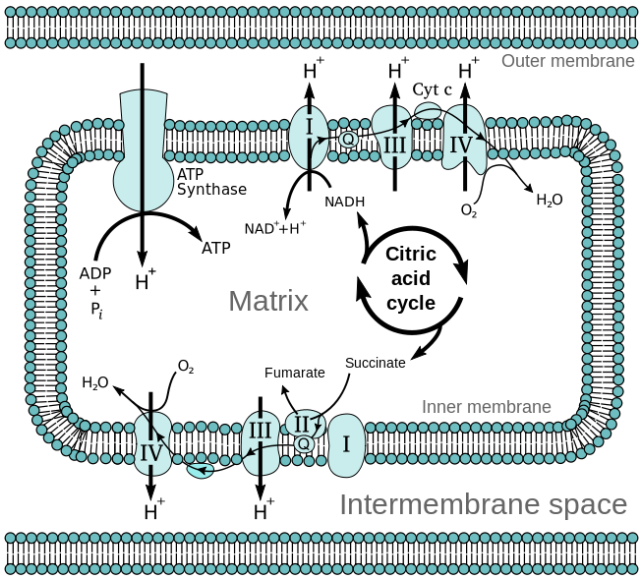
The mitochondrial membrane electric potential is typically around -160mV.

A shortage of electrons or an overabundance of protons in cellular cytoplasm can impact protonmotive forces (PMF) and change levels of intracellular ATP and ΔΨm.[17] This instability can alter normal cell function, reduce or enhance production of ATP, and impact cell signaling mechanisms.

Description of the Mitochondrial Electromotive Gradient, the PMF Protonmotive Force

As described earlier, electrochemical gradients power the mitochondria. The protonmotive force (PMF) is a proton gradient across the mitochondrial inner membrane (IM) and is analogous to voltage, an electric charge that powers a circuit.[18]

Also described earlier, the mitochondria remove electrons from cellular metabolites



Electron transport chain within mitochondria.
Credit: Fvasconcellos, 2007. Vector version by TimVickers, content unchanged. Public Domain. Wikimedia Commons. https://commons.wikimedia.org/w/index.php?curid=2716553

and shuttle them through an electron transport chain located in the IM. As electrons pass through oxidation-reduction reactions, some energy from each transfer is used to pump protons across the IM from the matrix to the intermembrane space. If the flow of electrons stop, the PMF stops.

Proton translocation by the electron transport chain establishes the PMF needed to make ATP. Through this process, mitochondria regulate calcium signaling, apoptosis, redox homeostasis, and stress resistance signaling.[19]

Several lines of evidence converge to suggest that regulation of PMF plays an important role in mitochondrial function during aging.[20] Decreased PMF is a well-known feature of cells from old animals and cells used as models for diseases of aging.[21]

That is to say, lower electromotive potential at the mitochondrial inner membrane is associated with aging. Presumably, any environmental factors that would decrease PMF will accelerate aging and, at least, reduce the efficiency of the mitochondria.

Environmental factors play a significant role in PMF and $\Delta\Psi m$. That subject is well-researched and deserves extensive documentation. It is too extensive for this White Paper #1 but is briefly discussed in Appendix B.

About NION Health™

This research was funded by NION Health, a subsidiary of Galahad Life Sciences.

For more than a decade, Galahad has been researching, developing, and testing intellectual property, patents, and manufacturing technology to make proprietary mineral-based, crystalline compositions like the NCS -330mV that can safely deliver powerful electric potential in human and other biological systems.

Galahad believes human health span can exceed 200 years. We want to empower humans to enjoy maximum vitality and health; spend less time and money fighting chronic diseases and more time loving & living life.

For more information visit our website at: www.NIONHealth.com

Sources & Bibliography

[1] Heil D., Fritz E, Hilpert J, Miller R, Robinson W, Shu W, *An alkalizing nutritional supplement that positively influences measures of health and aerobic performance.* International Journal of Applied Exercise Physiology. 2021;10(2):23-36.
[2] Dr. Lisa Price is a licensed Naturopathic Physician with expertise in complimentary cancer care and culinary nutrition curing cancer treatment. She is an adjunct faculty member at Bastyr University in Kenmore, Washington. She is the Chairperson of Research for the Institute of Naturopathic Medicine serves on its Board. Dr. Price served as a Research Fellow for the National Institute of Health (NIH) National Center for Complementary and Alternative Medicine from 2005 through 2010 and holds a certificate in Biomedical Regulatory Affairs from the University of Washington.
[3] Heil D, Fritz, E, Hilpert J, Miller R, Robinson W, Shu W, *An alkalizing nutritional supplement that positively influences measures of health and aerobic performance.* International Journal of Applied Exercise Physiology. 2021;10(2):23-36.
[4] Heil D, Jacobson E, Howe S. *Influence of an alkalizing supplement on markers of endurance performance using a double-blind placebo-controlled design* Journal of the International Society of Sports Nutrition 2012.
[5] Chistiakov DA, Sobenin IA, Revin VV, Orekhov AN, Bobryshev YV. *Mitochondrial aging and age-related dysfunction of mitochondria.* Biomed Res Int. 2014;2014:238463. doi: 10.1155/2014/238463. Epub 2014 Apr 10. PMID: 24818134; PMCID: PMC4003832.
[6] Duchen MR (2004) *Roles of mitochondria in health and disease*. Diabetes 2004 Feb; 53(suppl 1): S96-S102.
[7] Swerdlow RH. *Brain aging, Alzheimer's disease, and mitochondria*. Biochim Biophys Acta. 2011;1812(12):1630–1639
[8] Reddy PH. *Mitochondrial medicine for aging and neurodegenerative diseases.* Neuromolecular Med. 2008;10(4):291–315.
[9] Karbowski M, Neutzner A. *Neurodegeneration as a consequence of failed mitochondrial maintenance*. Acta Neuropathol. 2012;123(2):157–171.
[10] http://bscb.org/learning-resources/softcell-e-learning/mitochondrion-much-more-than-an-energy-converter/
[11] López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. *The hallmarks of aging.* Cell. 2013 Jun 6;153(6):1194-217.
[12] Alberts, Bruce; Alexander Johnson; Julian Lewis; Martin Raff; Keith Roberts; Peter Walter (1994). *Molecular Biology of the Cell*. New York: Garland Publishing Inc. ISBN 0-8153-3218-1.
[13] Mitchell P. *Chemiosmotic coupling in oxidative and photosynthetic phosphorylation*. Biol. Rev. Camb. Philosophical Soc. 1966;41:445–502.
[14] Zamzami N, Marchetti P, Castedo M, Decaudin D, Macho A, Hirsch T, Susin SA, Petit PX, Mignotte B, Kroemer G. *Sequential reduction of mitochondrial transmembrane*

potential and generation of reactive oxygen species in early programmed cell death. J. Exp. Med. 1995;182:367–377.

[15] Zorov DB, Juhaszova M, Sollott SJ. *Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release*. Physiol. Rev. 2014;94:909–950.

[16] Izyumov DS, Avetisyan AV, Pletjushkina OY, Sakharov DV, Wirtz KW, Chernyak BV, Skulachev VP. *"Wages of fear": transient threefold decrease in intracellular ATP level imposes apoptosis*. Biochimica Biophysica Acta. 2004;1658:141–147.

[17] Berry BJ, Kaeberlein M. *An energetics perspective on geroscience: mitochondrial protonmotive force and aging*. Geroscience. 2021 Aug;43(4):1591-1604.

[18] Berry BJ, Kaeberlein M. *An energetics perspective on geroscience: mitochondrial protonmotive force and aging*. Geroscience. 2021 Aug;43(4):1591-1604.

[19] Olson RK, Cornelius N, Gregersen N. *Redox signalling and mitochondrial stress responses; lessons from inborn errors of metabolism. J Inherit Metab Dis*. 2015;38:703-719.

[20] Hughes AL, Gottschling DE. *An early age increase in vacuolar pH limits mitochondrial function and lifespan in yeast. Nature.* 2012; 492:261-265.

[21] Sugrue MM, Tatton WG. *Mitochondrial membrane potential in aging cells*. *Biol Signals Recept*. 2001; 10:176-188.