ETERNUS:

PUTTING THE HEALTHY AGING PUZZLE TOGETHER

OPENING STORY

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Imagine back to a time period when you were younger. Did you have more energy? Were you able to bounce back more quickly from jet lag, exercise, lack of sleep, a late night, or an injury? Was it easier to build muscle and stay in shape? Were you more resilient, able to better adapt to stress, such as work pressure, changes in temperature, or disruptions to your schedule? Was your sleep better? Did you feel like you had more in reserve to pull from when you needed it? If so, you are not alone.

Some common themes in aging—themes that can start to show up even by our late-20's—are less energy, feeling more mentally and/or physically exhausted, not bouncing back as quickly, finding it more difficult to stay in shape, experiencing sleep disturbances, and having lower reserves to draw upon.

We all age. But, we don't all age at the same rate. You might know some people who have stayed healthy into old age … and others who seem to be old before their time. Some of this difference is because of genes (i.e., genotype) and some of it is a result of how genes express themselves on a cellular level as they interact with nutrition, lifestyle, and environment (i.e., phenotype).

Identical twins share the same genes, but the older they get, and especially if their nutrition, lifestyle, and/or environment substantially diverge, the way they express these same genes also diverges. The result is that their susceptibility to age-related diseases, the way they look as they age, and even their lifespans also diverge. The key thing to remember is that, while we can't change our genes, there's a great deal we can do to influence how they express themselves. While ageing isn't a choice, we can make choices which influence how gracefully we age, because our choices do influence how we express our genes.

As we age some genes become overexpressed, while others are underexpressed. This idea that our genes express themselves differently as we age is part of a model of aging that's sometimes referred to as "programmed aging." The aging process is also characterized by the accumulation of cellular and mitochondrial damage. This model is referred to as "aging as damage accumulation." Programmed aging and damage accumulation occur at the cellular level, with each amplifying the effects of the other (i.e., changes in gene expression accelerate damage accumulation, while damage accumulation affects a cell's ability to have healthy gene expression). Over enough time the result is a big difference between "young you" and "current you." One of these differences is that your cells were able to produce more energy when you were younger. They also communicated better with other cells, were better able to adapt to stress, and had less cellular damage. While many things go wrong as the human body ages, the roots of the problem are at the cellular level.

From a bottom-up point of view, we are a complex colony of tens of trillions of individual cells. Each of these cells has functional and structural units (such as mitochondria) and a network of interacting molecules that allow them to, among many things, produce cellular energy (in the form of ATP), repair damage, and respond to hormonal signals. Cells also sense and respond to nutrient status and stress, adjusting their behavior (i.e., gene expression) in ways that allow them to better respond to nutrient availability or to cope with the specific types of stress. As we age, cellular structure and function change in ways that leave some individual cells less able to perform all of these interrelated jobs. Simply put, cells don't have the same capacity to do their job or respond in the same way they did when we were younger. When enough individual cells are "aged," we tend to feel old and experience age-related health issues.

ETERNUS IS DESIGNED TO:

- ✓ Boost energy levels
- ✓ Promote healthy aging
- ✓ Enhance productivity
- ✓ Supports muscle performance

ETERNUS SUPPORTS LONG-TERM CELL AND MITOCHONDRIAL HEALTH:

- ✓ Elevates ATP
- ✓ Boosts NAD+
- ✓ Activates AMPK
- ✓ Upregulates sirtuins
- ✓ Supports insulin & thyroid signaling
- ✓ Promotes a fit mitochondrial network

> *Eternus was designed to give cells and mitochondria the nutrition they need to work better. When they perform at their best, we look, feel and perform at our best.*

Our cells are constantly being renewed. The lifetime of an average red blood cell is about 4 months. About half our fat cells will turnover every 8 years. We can donate blood periodically without having to worry about ever running out. This is because millions of new red blood cells are being formed in our body every minute. Except for the cells in the eye lenses and most neurons of our central nervous system, cells are constantly replacing themselves. But why, if new cells are constantly being born in most tissues, do we age? While the scientific answer is complicated, the simple answer is that specific types of cellular damage occur and accumulate as we age. Cellular damage is a normal part of metabolism. When we are younger, cells do a better job repairing this damage. As we age, some of the damage doesn't get repaired. When damaged cells replace themselves, the damage gets carried forward into the new cells. This accumulated damage affects how cells function, including their ability to repair themselves. If enough cells are damaged this reduces tissue performance, which, over time, leads to both visible and invisible aspects of unhealthy aging.

If you were interested in restoring the body, in terms of its structure, function, and capability to perform, back towards something more like it was during early adulthood, what should you look at? What needs to change at a cellular level? What molecules are important? What pathways and processes need to be influenced? How do they fit together? And, most importantly, what could you do about all of these interrelated things in order to promote a more youthful physiology?

These are the types of questions we asked ourselves. Our goal was to upregulate healthy function at a cellular level. But getting cells to work more like they did when they are young isn't about just one thing. It's about many things working well together. We believe this can't be done by just boosting one biomolecule. Instead, it requires influencing several important biomolecules, as well as multiple interrelated processes and cellular pathways. It also means supporting processes that repair cellular damage that takes place during aging.

Eternus was developed to comprehensively support and enhance the molecules, processes and pathways involved in health at the cellular level. A central goal was foundational support for cellular energy generation, because with more energy, individual cells can better do all the things they need to do. More energy also helps the network of interacting cells to work more effectively together. Working on a cellular level is taking a bottoms-up approach to supporting cellular fitness. Cellular fitness promotes tissue health and healthy tissues supports whole-body health.

While there is an increasing number of products in the cell energy space, their focus tends to be on some narrow aspect of cellular energy or metabolism. Our approach looks at the whole system required to support healthier cells. Aging isn't caused by just one type of cellular damage or too little/too much of one molecule and, as such, it can't be impacted by just focusing on one thing. To produce healthy function requires a complexity science approach, targeting the interacting network of molecules, pathways, and processes that allow cells to perform at their best.

Our answer to support healthy aging is Eternus. We think it is an important piece in the healthy aging puzzle.

COMPLEXITY SCIENCE AND AGING

The amount of time we live is called lifespan. The length of time that a person is healthy and functional—not just alive—is called healthspan. Two behaviors with known positive effects on lifespan and healthspan are:

1. **Calorie restriction (as well as related dietary strategies such as intermittent fasting and timed-restricted eating)**
2. **Exercise.**

Complex systems adapt to their environment, adjusting biology to better respond to the circumstances. If we go a day without eating, biology will change in response. But this is only the tip of the adaptation iceberg. Complex systems are in the business of anticipating the future. They learn from what we are doing now and adjust biology to better deal with things now and to prepare us for the next time something similar might occur.

Food scarcity is one of the most important environmental factors affecting survival. It causes dramatic changes in gene expression, metabolic monitoring, cellular signaling, mitochondrial function, energy metabolism, and circadian rhythms. These changes occur as part of a coordinated and integrated response. Certain molecules, pathways, and processes are upregulated; others are downregulated. The result is improved ability to deal with the present nutritional challenge ... and future challenges. The same is true for exercise. Muscles don't only get bigger because of lifting weights. They get bigger because complex adaptive intelligence anticipates we'll need to be able to lift weights again in the future. The cellular adaptations prepare us to perform better when we do. It's these coordinated cellular adaptations to exercise that positively influence healthspan.

While calorie restriction and exercise don't produce identical tissue-specific effects in all areas, the way organisms adapt to these two types of challenges has revealed a great deal about the fundamental mechanisms of healthy aging. These are the mechanisms we designed Eternus to support. In a sense, we focused on producing a formulation that is both a calorie restriction mimetic and exercise mimetic. Put another way, our goal was to put some of the functional cellular benefits of both calorie restriction and exercise into a dietary supplement. This doesn't mean you shouldn't exercise or eat a nutritionally sound diet: No pill can replace sound lifestyle habits. It does mean we designed our product to work along with other healthy habits to support more graceful aging.

A central tenet of complexity is called holism. Holism means that systems and their properties are not just a collection of independent parts. Everything is interconnected, influencing and being influenced. Scientific research has identified a number of molecules, pathways, and processes that influence lifespan and healthspan. Most of these are linked, interacting with and influencing each other. This means that cellular health isn't one isolated thing (i.e., a single molecule or pathway); it's a robust network of interacting things. For example, over the past few years there's been a tremendous interest in boosting a critical aspect of cellular health, the amount of a molecule called nicotinamide adenine dinucleotide (NAD+). In general, NAD+ decreases as we age and boosting it supports several important healthspan processes and pro-longevity pathways. An important molecule like NAD+ doesn't just do one thing; it does many things. And, importantly, many things influence it and its action, some of which are in turn influenced by NAD+. One of these is cellular energy in the form of ATP. We require NAD+ to make ATP, but we can't make NAD+ without ATP.

Networks allow for redundancy—having more than one way to do something. Focusing again on NAD+, there isn't just one way to make it in a cell; there's multiple ways. The result of this complexity is that the same healthy behavior, such as exercise, doesn't just do one thing, it produces an integrated and coordinated response that occurs in multiple molecules, across many interacting pathways and processes, in tissue-specific ways. Certain plant-derived compounds, many of which fall into a category called polyphenols, act in similar ways, triggering a wide variety of cellular adaptations.

Cellular adaptations do not occur in general (i.e., the same way in every cell in the body); adaptation occurs in tissue-specific ways. Continuing to use exercise as an example, going from a sedentary lifestyle to starting a new routine will cause many genes to be turned on and others off in muscle cells. It will also turn genes on and off in fat tissue. Some of what gets turned on and off will be the same in muscle cells and fat cells; some of it won't. But it gets even more specific. Depending on the type of exercise—endurance versus weight training—slightly different genes will be turned on and off. This specificity is a good thing. It allows the body to fine-tune its adaptations to better match behaviors or environmental challenges. It makes much more sense to turn on and off a portfolio of genes that promotes better endurance in someone who is running or bicycling. In someone working out with weights, it makes more sense to adjust gene expression to boost muscle size and strength (endurance isn't as important).

We focused on the known fundamental mechanisms of cellular aging. But we didn't just do this in a general way. We did this through a complexity science looking glass. We had tissue-specific goals in mind. And, we had performance-specific responses in mind. Aging is accompanied by a loss of muscle function and size. Fat cells don't age in the same way. While turning on/off some of the same healthspan pathways in both muscle and fat cells makes sense (such as sirtuins or inflammation pathways), others don't (such as mTOR). While it might make sense to factor only for endurance in a person wanting to improve their running endurance, or only for muscle size in a body builder, for healthspan it's important to factor for all facets of healthy muscle function.

Our goal was not to simply boost the amount of an isolated molecule or upregulate a pathway or process; the goal is to support the entire network of interacting molecules, pathways, and processes in ways that allow a full experience of healthspan. No matter what has caused a given unit of cellular dysfunction in the first place, by supporting the underlying cellular mechanisms of healthy aging, cells can be helped to help themselves.

Optimize NAD+ & NAD+ :NADH Ratio

- Nutritionally support the three linked NAD+-producing pathways (*De Novo* Synthesis, Preiss-Handler, Salvage)
- Upregulate gene expression and activity of pathway, especially rate-limiting enzymes
- Protect cells against health-damaging pathway intermediates
- Support Nrf2 --> NQO1 to shift NAD+ : NADH Ratio

Optimize Activity of NAD+ Consumption Pathways

- Upregulate Silent Information Regulators (Sirtuins)
- Slow CD38
- Balance PARPs for DNA repair

Elevate Cellular Energy Production (ATP)

- Supply cofactors and nutritional substrates for the four linked cellular energy pathways (e.g., sugar breakdown, beta-oxidation, Krebs cycle, and electron transport)
- Enhance enzyme upregulation and performance
- Support mild uncoupling
- Enhance mitochondrial ATP output

Build a Fitter Mitochondrial Network

- Enhance mitochondrial structure (e.g., cristae, membrane proteins)
- Support and protect mitochondrial DNA (mtDNA)
- Upregulate PGC-1α/PGC-1β (i..e, master regulators of mitochondrial fitness)
- Support mitochondrial biogenesis

Enhance Cellular and Mitochondrial Protection

- Build antioxidant defenses (e.g., glutathione, antioxidant enzymes)
- Upregulate Nrf2 and NQO1 (master regulators of cell defenses and detoxification)
- Protect against advanced glycation endproducts (AGE) load
- Support protein quality control (i.e., proteostasis)
- Downregulate inflammation

Upregulate AMPK to support healthy weight and metabolism

- Enhance muscle and fat cell AMPK response
- Downregulate hypothalamic AMPK response
- Provide nutritional support for LKB1 & CaMMK pathways of AMPK

Support Intercellular Communication (Endocrine Regulation of Longevity)

- Support insulin signaling
- Support thyroid signaling

Optimize Circadian System Performance

- Provide nutritional support for central and peripheral clock function
- Promote healthy clock gene expression and activity
- Support more rapid entrainment after body clock stress

Support Gut Microbiome

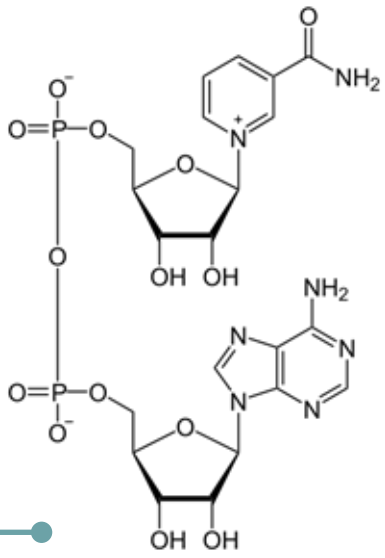
- Provide prebiotic plant compounds
- Enhance gut microbiome diversity and composition
- Enhance barrier function and gut immunity performance

THE HEALTHY AGING PUZZLE

Broadly speaking, there are two major theories of aging. One focuses on accumulated damage as the primary cause (i.e., aging as damage accumulation). The other focuses on gene expression, with aging caused by the "programs" our genes run, overexpressing some genes and underexpressing others as we get older (i.e., programmed aging). Both agree that cellular damage and dysfunction are at the heart of aging. They also agree that both damage accumulation and gene expression issues occur with aging. But they disagree about which is the root cause and should be addressed first. While there's arguments in favor of both camps, it's a bit of a chicken and egg, which came first problem. Dysfunction in gene expression can increase damage accumulation, while damage accumulation can impair gene expression. The cycle of dysfunction/damage begetting more dysfunction/damage is the bad news. The good news is that this cycle can be interrupted at many points, with functional support in one molecule, pathway, or process, enhancing others. The even better news is that we don't need to do just one thing in isolation; we can support many of them together, amplifying the benefits. That's the complex systems approach.

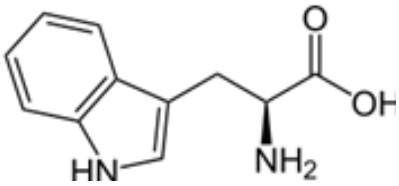
Nothing in a cell occurs in complete isolation from other ongoing processes. And, all individual cells are just a single node in a vast cellular network within the body. When we formulated Eternus, we focused on enhancing a number of cellular biomolecules, pathways, and processes. Complexity science is not about one thing in isolation; it's about interacting networks of things (including our cellular network with the gut microbiome), redundancy, self-regulation, and whole system responses. With that in mind, let's take a closer look at how some of the main puzzle pieces fit together, starting with the NAD molecule.

Over the past few years there's been a tremendous interest in boosting NAD+. In general, NAD+ decreases as we age, while boosting it supports several important healthspan processes and pro-longevity pathways. The attention NAD+ has received is well-deserved. Cells rely on NAD+ to carry out hundreds of metabolic functions, including a vast array of processes ranging from energy creation to maintaining healthy DNA. Increasing NAD+ is important to us, so it's something we want to support. It's a central piece in the puzzle.

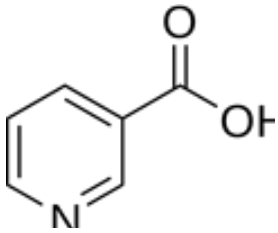


1. NAD+ Molecule

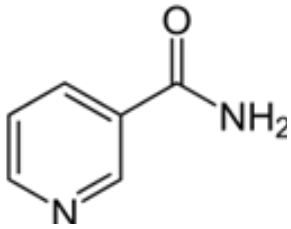
Biological complex adaptive systems, whether at a cellular or whole-body level, usually have more than one way to accomplish the same outcome. This redundancy is needed for surviving and adapting to real world challenges. Given the importance of NAD+, it should be no surprise that there's more than one way our bodies make it. NAD+ can be made via the "*De Novo* Pathway," starting from the essential amino acid L-tryptophan. It can be made by the "Preiss-Handler pathway" using nicotinic acid, a form of vitamin B3 usually called niacin, which is known for producing flushing when taken in high amounts. And, it can be produced in the "Salvage Pathway" from niacinamide (nicotinamide), the non-flushing form of vitamin B3. Different tissues use these three pathways to greater or lesser extents to meet changing NAD+ needs. In general, giving more choices allows for better adaptation. With that in mind it makes sense to provide support for all three pathways.



2. L-Tryptophan



3. Nicotinic Acid



4. Niacinamide

Nutrients like **L-tryptophan**, and both forms of **vitamin B3**, are important puzzle pieces because they are the building blocks (or substrates) used to make NAD+. Cellular pathways convert these building blocks into NAD+ using enzyme reactions. Many enzymes require "helper molecules" called cofactors, which are essential for the activity of the enzyme. There are several major types of cofactors: (1) minerals, (2) vitamins and their derivatives, and (3) non-vitamin molecules. Enzymes in the "De Novo Synthesis Pathway" require the nutritional cofactors **riboflavin** (vitamin B2), **pyridoxal 5'-phosphate** (vitamin B6), **magnesium**,and **trace minerals**. Supplying required cofactors is a piece of the puzzle.

5. *De Novo* Synthesis Pathway Cofactors

- Riboflavin (vitamin B2)
- Pyridoxal 5'-phosphate (vitamin B6)
- Magnesium (as magnesium glycinate)
- elevATP® (supplying 70 trace minerals)

Enzymes are products of genes. Their expression and activity can be turned up or down by nutrition, behaviors, and environmental challenges that influence the way genes express themselves. Expression and activity levels can also change with aging, as part of programmed aging. And they can be upregulated and downregulated by factors within cells (i.e., non-vitamin molecules such as ATP and AMPK) and outside the cell (such as circadian rhythms, hormones, and inflammation). Nutritional support can influence the expression and activity of some enzymes. This occurs with both the *De Novo* Synthesis & Preiss-Handler pathways, with the same nutrition supporting both. This extra nutritional support is the next puzzle piece.

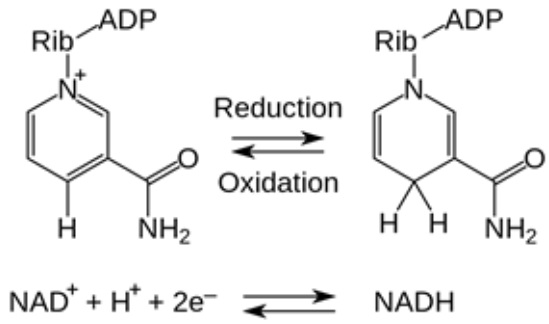
6. *De Novo* Synthesis & Preiss-Handler Enzyme Expression & Activity Support	• BioVin® French Red Grapes Extract (5% resveratrol) • Magnesium (as magnesium glycinate)

COMMENT: Supplying the building blocks & cofactors, nutritionally supporting gene expression, and enhancing factors inside & outside cells needed to upregulate a pathway is what we call full pathway support.

Most pathways create intermediate molecules on the journey between the starting substrate and the finished product. Intermediate molecules can play important roles in metabolism, but, in some cases, they can be health-damaging when the pathway backs up and the intermediate accumulates. *De Novo* Synthesis is an example of a pathway where this can occur, with an intermediate molecule—quinolinic acid—contributing to neuroinflammation if allowed to accumulate. One part of the solution is to prevent an intermediate from accumulating by upregulating the enzyme that moves it forward in the pathway. For quinolinic acid this is an enzyme abbreviated QPRT and the nutritional solution is the previous piece in the puzzle—BioVin® French Red Grapes Extract (5% resveratrol). The other part of the solution is giving the body, the brain in the case of quinolinic acid, nutrition that helps it help itself against the molecule.

7. Support Quinolinic Acid Neuroprotection	• BioVin® French Red Grapes Extract (5% resveratrol) • Magnesium (as magnesium glycinate) • L-carnitine • *Citrus grandis* Fruit Extract (98% apigenin)

NAD+ is a central piece in the healthy aging puzzle. Making more of it is important. But it is a means to an end. We don't care about NAD+ on its own; we care about it because of what it allows cells to do. One of these functions—the original one identified many decades ago—is acting as an electron carrier in cellular oxidation-reduction (redox) reactions. In these reactions, the NAD molecule switches back and forth between NAD+ (oxidized form) and NADH (reduced form). This switching back and forth is required for cellular energy production.



8. NAD+ : NADH Redox

One of the insights arising from the scientific studies of calorie restriction is that the ratio of NAD+ to NADH (NAD+ : NADH ratio) is important. This ratio declines with age (it's part of programmed aging) and might be more significant than the amount of cellular NAD+ in isolation. Calorie restriction decreases NADH. This decrease in NADH appears to be an important variable for enhancing lifespan, because, on its own, it increases activity of the NAD+-consuming enzymes that boost longevity processes (e.g., Sirtuins) and DNA repair (e.g. PARPs).

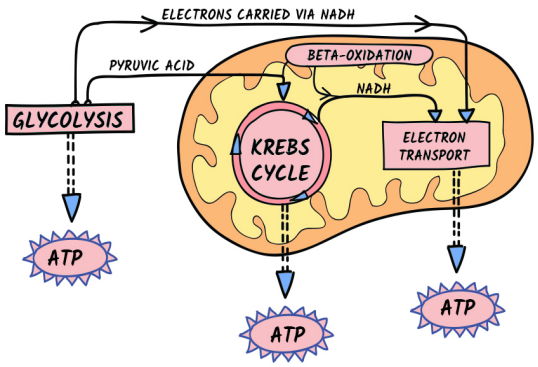
Note: NADH is an inhibitor of these enzymes, so lowering it releases the inhibition.

While boosting NAD+ is a useful strategy, it shouldn't be the only strategy. It's also important to increase the NAD+ : NADH ratio. Increasing the cellular consumption of NADH is an additional lever toward that end. Calorie restriction induces an enzyme called Nrf2, which is considered to be a master regulator of cellular defense; it's responsible for many cellular protection processes and involved in mitochondrial biogenesis (i.e., building new mitochondria). This enzyme then changes how other genes (and the enzymes the genes produce) express themselves, including a cellular detoxification enzyme called NQO1. In order to protect cells from potentially harmful agents, NQO1 uses NADH, resulting in lowering of cellular concentrations and an improved NAD+ : NADH ratio. Upregulating the Nrf2 --> NQO1 pathway is the next piece of the puzzle. It's part of addressing programmed aging.

9. Support Nrf2 and NQO1

- **BioVin® French Red Grapes Extract (5% resveratrol)**
- **R-Lipoic Acid sodium**
- ***Rosmarinus officinalis* Leaf Extract (50% ursolic acid)**
- ***Theobroma cacao* Seed Extract (10% theobromine)**
- ***Sophorae japonica* L. Flower Extract (95% rutin)**
- ***Citrus grandis* Fruit Extract (98% apigenin)**
- ***Citrus sinensis* L. Fruit Extract (40% nobiletin)**
- **Strawberry Seed Extract (0.5% trans-tiliroside)**

The NAD redox reaction isn't only important for cellular protection, it plays a central role in four linked cellular energy pathways. One of these, "Glycolysis," breaks down sugars. It starts inside cells and finishes in specialized organelles within cells called mitochondria. The other three linked pathways occur in mitochondria (i.e., cellular powerhouses). One is called "Beta-Oxidation." It breaks down fats for energy. Another is called the "Krebs Cycle" (also called tricarboxylic acid cycle or the citric acid cycle). Both of these occur inside mitochondria. The third takes place in the folds (called cristae) of the inner membranes of mitochondria and is called oxidative phosphorylation (OXPHOS or electron transport). These four linked pathways need to perform at their best to optimize the NAD+ : NADH ratio and cellular energy production (in the form of ATP).



10. Linked ATP Pathways

A good starting point for a discussion of these linked cellular energy pathways is the acetyl-CoA molecule. It is the gateway sugars and fats must use to enter the Krebs Cycle.

Cells break down sugars into pyruvate through an eleven enzyme pathway called glycolysis. Magnesium is the critical cofactor; it's used in five of the eleven enzyme steps. The net result of glycolysis is a gain of two molecules of cellular energy (ATP), but at a cost of two molecules of NAD+ being switched to NADH in redox reactions. An enzyme complex—pyruvate dehydrogenase—then converts the pyruvate to acetyl-CoA, with thiamine (or benfotiamine), riboflavin, lipoic acid, and magnesium as cofactors. This comes with a cost of one additional NAD+ being switched to NADH. Coenzyme A is a co-substrate, along with pyruvate, in this last reaction and requires pantothenic acid and cysteine: This costs one ATP. The process of breaking down sugars into acetyl-CoA produces a net gain of ATP, but an increase of NADH at the expense of NAD+. While this trade-off might not seem worthwhile (if we only focus on NAD+), (1) we can't survive without producing cellular energy, (2) this NADH is going to come in very handy soon enough, because it is carrying a large amount of "potential" cellular energy.

11. Glycolysis & Pyruvate Dehydrogenase Cofactor Support

- Magnesium (as magnesium glycinate)
- R-Lipoic Acid sodium
- Benfotiamine
- Thiamine (vitamin B1)
- Riboflavin (vitamin B2)
- Niacin and Niacinamide (vitamin B3)
- Pantothenic Acid (vitamin B5)
- N-Acetyl-L-cysteine

It's important to efficiently use sugars for energy as part of an overall strategy aimed at minimizing cellular damage. One of the cornerstone elements of "aging as damage accumulation" is cross-linked macromolecules. There are many "sugary" molecules floating around in our body. If these sugars aren't used for cellular energy, they can react (i.e., cross-link) with proteins and fats, forming a class of health-damaging compounds named AGEs (advanced glycation endproducts). AGEs accumulate inside and outside cells as we get older, interfering with cellular signaling and causing tissue damage, while contributing to unhealthy aging. The next piece of the puzzle is nutritional support to help the body help itself against AGE load.

12. AGEs Support

- Benfotiamine
- Thiamin (vitamin B1)
- Pyridoxal-5'-Phosphate (vitamin B6)
- R-Lipoic Acid sodium
- Coenzyme Q10 (ubiquinone)
- L-Carnitine
- N-Acetyl-L-cysteine
- BioVin® French Red Grapes Extract (5% resveratrol)
- Cinnulin PF® *Cinnamomum burmannii* Bark Extract
- Sirtmax® *Kaempferia parviflora* Root Extract (15% polymethoxy flavonoids)
- *Rosmarinus officinalis* Leaf Extract (50% ursolic acid)
- *Sophorae japonica* L. Flower Extract (95% rutin)
- *Citrus grandis* Fruit Extract (98% apigenin)
- Myo-Inositol
- *Ceratonia siliqua* Pod Extract (98% D-chiro-inositol)

Cells turn fats (or fatty acids) into acetyl-CoA by breaking them down using a process called beta-oxidation. Fatty acids exist as chains of linked carbon molecules of different lengths. Those with lengths of 14 or more (i.e., long-chain fatty acids) need help from the carnitine shuttle to get into the mitochondria, where they are broken down. While the specific enzymes involved can vary depending on chain length and whether a fatty acid is saturated or unsaturated, in general, shortening of fatty acid chains occurs through a series of repeated enzyme steps. Each cycle through the enzyme steps removes two links in the fatty acid chain, at a cost of one NAD+ switching to NADH. Longer chain fatty acids require more cycles, so are bigger producers of the potential energy-carrying NADH at the expense of NAD+. Most fatty acid chains have lengths ending in even numbers, but some can have an odd number, which requires one additional biotin-dependent enzyme. Supplementing enzyme cofactors and support for the carnitine shuttle ensures that beta-oxidation isn't going to be slowed down by poor nutrition: It's the next puzzle piece.

13. Beta-Oxidation Cofactor Support

- L-Carnitine
- Biotin
- Riboflavin (vitamin B2)
- Niacin and Niacinamide (vitamin B3)
- Pantothenic Acid (vitamin B5)

Beta-oxidation tends to struggle with aging, especially in the tissues that rely on fats as a primary source of fuel (e.g., heart, skeletal muscle). Programmed aging, and the resultant changes in gene expression, appear to contribute to this slowing of performance. Supplying enzyme cofactors (i.e., helper molecules) supports enzyme activity, but doesn't directly address expression issues. Because of this, the next piece of the puzzle is providing nutritional support that upregulates beta-oxidation performance.

14. Beta-Oxidation Performance Support

- ***Theobroma cacao*** **Seed Extract (10% theobromine)**
- **BioVin® French Red Grapes Extract (5% resveratrol)**
- ***Citrus grandis*** **Fruit Extract (98% apigenin)**
- **Pyrroloquinoline Quinone**

Now that we have plenty of acetyl-CoA (from breaking down sugars and fats), the next stop is the Krebs cycle. As the word "cycle" in the name suggests, this pathway is a circular loop consisting of ten steps. Each step starts with a substrate (e.g., citrate, malate), uses an enzyme, and produces a product. The product is then used as the substrate in the next step, driving the pathway forward through the cycle. Adding any of the intermediate substrates, either through metabolic reactions or nutritionally, spins the Krebs cycle wheel, so to speak, increasing all the other intermediates since they are interconverted one into the next. But for this to occur, there's an absolute requirement for the acetyl-CoA molecules we gained by breaking down fats or sugars: It provides the fuel, with each turn of the cycle consuming one molecule of acetyl-CoA. Each progression through the entire cycle (or turn of the wheel) produces one cellular energy molecule (ATP) and converts three NAD+ into three NADH molecules. In addition to the ATP and NADH formed, the intermediate products formed in this cycle are used to build many important molecules including proteins, DNA, and RNA. The Krebs cycle is at the center of building molecules and moving cell energy production forward, but, similar to beta-oxidation, activity of its enzymes declines with age as part of programmed aging. Three additional pieces of the puzzle enter with the Krebs cycle: (1) adding intermediates into the cycle, (2) providing cofactors needed for enzyme activity, and (3) supporting upregulation of enzyme performance.

15. Add Krebs Cycle Intermediates

- **Malic Acid (from in tricreatine malate)**
- **Citric Acid (from tripotassium citrate)**

16. Provide Krebs Cycle Cofactors

- Magnesium (as magnesium glycinate)
- Benfotiamine
- Thiamin (vitamin B1)
- Riboflavin (vitamin B2
- Niacin and Niacinamide (vitamin B3)
- Pantothenic Acid (vitamin B5)
- Vitamin B12 (as methylcobalamin and adenosylcobalamin)
- R-Lipoic Acid sodium
- Biotin

17. Support Krebs Cycle Enzyme Performance

- *Theobroma cacao* Seed Extract (10% theobromine)
- Sensoril® Ashwagandha *Withania somnifera* Root and Leaf Extract (10% withanolide glycoside conjugates)
- *Gynostemma pentaphyllum* Whole Herb Extract (50% gypenosides)
- Pyrroloquinoline Quinone

In the three linked cellular energy pathways we've discussed so far, we've gained a bit of cellular energy (ATP), broken sugars and fats down to produce acetyl-CoA for Krebs cycle fuel, and helped cells build lots of important molecules. This has come at the cost of switching a number of NAD+ molecules to NADH. This is where the NADH gets put to good use. Each NADH molecule carries the potential to produce three molecules of ATP when it's fed into oxidative phosphorylation (OXPHOS). The fourth linked cellular energy pathway, OXPHOS, liberates the stored energy from NADH to make most of the ATP for our cells. OXPHOS moves through five steps (usually referred to as complex I, complex II, ... complex V). Each complex uses an enzyme, with the final one—ATP synthase (Complex V)—producing ATP. During OXPHOS, NADH is converted back to NAD+, so not only do we get a large amount of cellular energy, when all is said and done, we get it at no NAD+ cost. The next puzzle piece is providing cofactors needed to ensure OXPHOS can function.

18. Provide OXPHOS Cofactors

- Coenzyme Q10 (ubiquinone)
- Riboflavin (vitamin B2)
- Niacin and Niacinamide (vitamin B3)
- Biotin
- Sulfur (from elevATP® and N-acetyl-L-cysteine)

Performance of OXPHOS can be slowed because of many factors. It can be inhibited by toxins. It generally suffers with increased age—it's part of programmed aging. Performance can also suffer at a younger age in persons with poor metabolic health or inflammation, as examples. The results of this underperformance include (1) decreased cellular energy production (i.e., reduced ATP), and (2) a lower than optimal NAD+ : NADH ratio. If cells don't have enough ATP, some of their work doesn't get done. And, if they can't maintain a high enough NAD+ : NADH ratio, many healthspan processes, including cleaning up damage and DNA repair, suffer. Calorie restriction, conversely, significantly upregulates OXPHOS performance, boosting ATP production, while shifting the NAD+ : NADH ratio in favor of NAD+ by lowering NADH. This OXPHOS performance increase is required for pro-longevity effects from calorie restriction. Exercise also boosts OXPHOS, because it triggers gene expression that produces a fitter mitochondrial network. The key point is that it's critical to have high performance of OXPHOS, so more ATP can be produced and a low amount of NADH can be maintained, but many circumstances can inhibit it. Nutritional support is focused on upregulating complex I-V enzyme activity and allowing OXPHOS to continue to function at a high level even when faced with challenging circumstances that would normally slow activity. This is a central piece of the puzzle.

19. Support OXPHOS Performance

- BioVin® French Red Grapes Extract (5% resveratrol)
- *Theobroma cacao* Seed Extract (10% theobromine)
- Sensoril® Ashwagandha *Withania somnifera* Root and Leaf Extract (10% withanolide glycoside conjugates)
- *Gynostemma pentaphyllum* Whole Herb Extract (50% gypenosides)
- *Citrus grandis* Fruit Extract (98% apigenin)
- *Citrus sinensis* L. Fruit Extract (40% nobiletin)
- Vitamin K2 (as menaquinone-7)
- R-Lipoic Acid sodium
- Pyrroloquinoline Quinone

One aspect of OXPHOS support is supplying cofactors needed for enzyme function in complex I-V. Another is providing nutrition to upregulate performance of the enzymes involved, helping them to perform at a high level. The third is more directly supporting ATP production capability, which, after all, is the primary mitochondrial function. It has been estimated that we make (and use) approximately our body weight of ATP every day. Without sufficient ATP, cellular life literally ceases. Supporting the number and performance of cristae (the folds of the inner membranes of mitochondria) is critical for ATP output, because this is where OXPHOS occurs and ATP is generated. Creatine is another critical component; it's used in the phosphocreatine circuit (also called the phosphagen system). This circuit is the fastest way to replenish ATP during times when it's being rapidly used, such as during intense exercise. elevATP®, a combination of trace minerals from ancient peat (including ionized magnesium) and apple polyphenols, has increased ATP output in clinical studies. Finally, ATP must be bound to a magnesium ion in order to be biologically active: What is called ATP in cells usually occurs as a magnesium-ATP complex.

20. Enhance ATP Output

- *Theobroma cacao* Seed Extract (10% theobromine)
- Tri-creatine Malate
- elevATP® Ancient Peat and Apple Fruit Extract
- Magnesium (as magnesium glycinate)

COMMENT: Having more ATP allows cells to do many things. One of these things is creating more NAD+. Availability of ATP affects performance of many enzymes. In these enzymes, ATP might be thought of as providing "energy" for the reaction. The four linked cellular energy pathways—sugar breakdown, fatty acid breakdown, Krebs cycle, and OXPHOS—all use NAD+ to produce ATP. And, the three NAD+ generating pathways all have enzymes that use ATP as part of the process of producing the NAD+ molecule. Cells need NAD+ to make ATP … and they need ATP to produce NAD+. In our body, molecules and pathways don't exist in isolation. Cellular health is a network of interrelated molecules and pathways, many of which co-regulate each other. In co-regulated processes, improving one molecule can have a positive effect on the other, but the system's designed to run more efficiently when both are upregulated.

Mitochondria play a central role in producing cellular energy and are an integral part of the healthspan response to calorie restriction and exercise. They are also among the first parts of the cell to become dysfunctional/damaged, especially in metabolically active tissues requiring high amounts of cellular energy like the brain, heart, and muscles. By old age, relatively few mitochondria are fully functional because of accumulated damage, with some of this damage being self-inflicted. OXPHOS produces ATP by oxidative reactions. NAD is switched back from its reduced form to its oxidized state (NAD+) as a byproduct. Another byproduct is reactive oxygen species (ROS), which produce some degree of oxidative stress. Left unchecked, ROS damage mitochondria and accelerate aging, so are sometimes simplistically thought of as "bad." A better way to think of ROS would be as an example of the "Goldilocks principle," something where we want a just right amount because too little or too much are a problem. Exercise is an example; it results in an increase in ROS. This increase is part of the stimulus that causes adaptation and results in improved performance. If it's prevented, we also prevent some of the benefits of exercise from occurring. In this example, exercise is acting as a positive stress, triggering healthy cellular and mitochondrial adaptations. The idea that a just right amount of stress is healthy is called hormesis. When applied to mitochondria, it's called mitohormesis.

Because of mitohormesis, the goal isn't to prevent mitochondria from producing ROS. Instead, it's to (1) allow them to produce a just right amount, and (2) help them respond to the ROS they are generating by upregulating their own antioxidant defenses. As we age, ROS tend to get produced in amounts greater than the just right amount. This contributes to signs and symptoms of aging, is considered a part of programmed aging, and directly causes some of the aging as damage accumulation (especially damage to cellular and mitochondrial DNA). Rather than trying to put out the ROS with large amounts of antioxidants (this strategy hasn't worked in scientific studies), a better approach is to help mitochondria help themselves. The key pieces of this support are (1) supplying substrates (e.g., N-acetyl-L-cysteine, glycine) that cells can use to produce more glutathione (a cellular antioxidant and detoxification molecule), and (2) supporting the upregulation of antioxidant enzymes (e.g., catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase) that mitochondria use to protect themselves against ROS. Collectively, we think of this strategy as promoting antioxidant defenses. This is the next piece of the puzzle.

21. Promote Antioxidant Defenses

- N-Acetyl-L-cysteine
- Glycine (as magnesium glycinate)
- R-Lipoic Acid sodium
- Pyrroloquinoline Quinone
- BioVin® French Red Grapes Extract (5% resveratrol)
- *Theobroma cacao* Seed Extract (10% theobromine)
- Sensoril® Ashwagandha *Withania somnifera* Root and Leaf Extract (10% withanolide glycoside conjugates)
- *Gynostemma pentaphyllum* Whole Herb Extract (50% gypenosides)
- *Rosmarinus officinalis* Leaf Extract (50% ursolic acid)
- Sirtmax® *Kaempferia parviflora* Root Extract (15% polymethoxy flavonoids)
- Cinnulin PF® *Cinnamomum burmannii* Bark Extract
- elevATP® Ancient Peat and Apple Fruit Extract
- Strawberry Seed Extract (0.5% trans-tiliroside)
- *Citrus grandis* Fruit Extract (98% apigenin)
- *Citrus sinensis L.* Fruit Extract (40% nobiletin)

In theory, each molecule of NADH fed into OXPHOS would produce three molecules of ATP. In practice mitochondria produce closer to 2.5. This is because mitochondrial membranes aren't perfectly efficient. It's also because it's healthy for them to have a small amount of "mild uncoupling" (i.e., some of the electrons carried by NADH are not used to drive ATP synthesis). Mild uncoupling allows cells to produce heat instead of cellular energy, which is an important part of cold adaptation. It also allows cells to burn more fat for fuel, since some is "wasted" as heat, which is protective against metabolic disorders. Mild uncoupling is thought to be part of how mitochondria protect themselves from ROS and oxidative damage. And, it's part of the healthspan portfolio of benefits triggered by calorie restriction and exercise. The next piece of the puzzle is supporting this mild uncoupling.

22. Support Mild Uncoupling Puzzle Pieces

- *Theobroma cacao* Seed Extract (10% theobromine)
- Sirtmax® *Kaempferia parviflora* Root Extract (15% polymethoxy flavonoids)
- *Rosmarinus officinalis* Leaf Extract (50% ursolic acid)
- Cinnulin PF® *Cinnamomum burmannii* Bark Extract
- R-Lipoic Acid sodium

We inherit DNA from our parents (i.e., the human genome) that gives cells instructions on how to make gene products (mostly proteins). This is called nuclear DNA, because it's located in the nucleus of a cell. Mitochondria don't share our inherited nuclear DNA; they have their own called mitochondrial DNA (mtDNA). The number of mtDNA decreases with age. This occurs because mtDNA are susceptible to damage by both external and internal factors, including the ROS mitochondria produce as part of generating ATP via OXPHOS. Several nutrients support a healthier level of mtDNA. This nutritional support is the next piece in the puzzle. Improving antioxidant defenses (a previous puzzle piece) indirectly supports mtDNA by preventing oxidative damage

23. Support for mtDNA

- R-Lipoic Acid sodium
- Coenzyme Q10 (ubiquinone)
- Pyrroloquinoline Quinone
- N-Acetyl-L-cysteine
- L-Carnitine
- Tri-creatine Malate
- Folate (as folic acid, calcium L-5'-methyltetrahydrofolate, and calcium folinate)
- BioVin® French Red Grapes Extract (5% resveratrol)
- *Sophorae japonica L.* Flower Extract (95% rutin)

Although a mitochondrion is commonly depicted as a single oval-shaped structure, mitochondria are in reality dynamic networks within a cell (i.e., individual cells can contain hundreds of mitochondria). When faced with certain types of challenges that stress ATP capabilities (such as exercise), healthy cells adapt by increasing the size and number of the mitochondria. This allows them to produce more cellular energy in the future. This process is called mitochondrial biogenesis. It results in a more capable and resilient (i.e., fitter) mitochondrial network. Mitochondrial biogenesis decreases with age, but increases with calorie restriction or exercise, so is an important part of the adaptations that produce enhanced healthspan.

Mitochondrial biogenesis requires a coordinated action of many interacting processes and genes. As an example, building the mitochondrial network requires copying mtDNA. It also requires transporting products of nuclear DNA (i.e., proteins made by our inherited cellular DNA) through the mitochondrial membrane. The nuclear DNA in the human genome allow us to make ~19,000-20,000 protein-coding genes. mtDNA only produce 37 genes. Mitochondria can't fully function (and biogenesis can't occur) with just the products of its own genes, so they have evolved to "borrow" what's made by our cells. PGC-1α and PGC-1β are the master regulators of mitochondrial biogenesis, turning on more than 150 genes (including the Nrf2 puzzle piece mentioned earlier) and coordinating gene expression, enzyme activity, and signaling pathways to increase the size and number of mitochondria. Upregulating PGC-1α/PGC-1β activity is the next piece of the puzzle; it helps support a healthy and fit mitochondrial population within cells.

24. Upregulate PGC-1α / PGC-1β

- R-Lipoic Acid sodium
- Pyrroloquinoline Quinone
- Niacin and Niacinamide (vitamin B3)
- L-Carnitine
- Tri-creatine Malate
- Benfotiamine
- *Rosmarinus officinalis* Leaf Extract (50% ursolic acid)
- BioVin® French Red Grapes Extract (5% resveratrol)
- Sirtmax® *Kaempferia parviflora* Root Extract (15% polymethoxy flavonoids)
- *Theobroma cacao* Seed Extract (10% theobromine)
- elevATP® Ancient Peat and Apple Fruit Extract
- *Citrus grandis* Fruit Extract (98% apigenin)
- *Sophorae japonica L*. Flower Extract (95% rutin)

Cells have integrated biological processes that control the biogenesis (i.e., creation), folding, trafficking, and degradation of proteins. Collectively this is called cellular proteostasis (i.e., protein homeostasis) or protein quality control (PQC). These processes are especially important for mitochondrial function, because mitochondria can't function with only the proteins made by their own mtDNA. Instead they fold (and unfold) externally produced proteins as they traffic them through the mitochondrial membrane. During normal metabolic function some proteins can become misfolded or damaged. To prevent these proteins from accumulating cells and mitochondria have redundant processes that allow them to degrade dysfunctional proteins. A core aspect of "aging as damage accumulation" is the inability of PQC processes to keep pace with the load, leading to a growing burden of dysfunctional proteins (many of which tend to clump together or aggregate). Supporting PQC is the next piece of the healthspan puzzle. It's a necessary component of maintaining healthy cellular and mitochondrial populations.

25. Support PQC	• BioVin® French Red Grapes Extract (5% resveratrol) • *Sophorae japonica L.* Flower Extract (95% rutin) • *Rosmarinus officinalis* Leaf Extract (50% ursolic acid) • N-Acetyl-L-cysteine • Coenzyme Q10 (ubiquinone) • Magnesium (as magnesium glycinate)

We mentioned that we care about NAD+ because of what it allows cells to do. Scientific research, much of it arising from efforts put into understanding calorie restriction effects on healthspan and lifespan, has revealed non-redox NAD+-dependent reactions. These reactions are often described as "NAD+-consuming," because they break apart (i.e., consume) the NAD+ molecule. There are three main NAD+-consuming pathways. One involves sirtuins that turn on and off many genes involved in healthspan and lifespan extension. Another is a group of enzymes called PARPs involved with cellular DNA repair. The third is a cellular second messenger involved in calcium (Ca2+) signaling called CD38. When these reactions consume NAD+, niacinamide (NAM; nicotinamide) is leftover.

The leftover niacinamide can be "salvaged" and used to regenerate NAD+. This salvage process is essential for replenishing the NAD+ pool in human metabolism, because most of the NAD+ used by our cells isn't built from the vitamin B3 we get from food or supplements, it's rebuilt from recycled niacinamide.

The first step in salvaging niacinamide uses an enzyme called Nampt, which is rate-limiting for the pathway. Nampt activity decreases with age. This is thought to be a primary cause of the age-related decrease in tissue levels of NAD+ (i.e., programmed aging). Conversely, Nampt activity and/or gene expression is upregulated by both calorie restriction and exercise. A great deal of emphasis has been placed on boosting NAD+ by supplying extra niacin (recently in the form of nicotinamide riboside), but upregulating Nampt, without supplying any extra niacin, boosts NAD+. So, a better strategy is to boost the availability of niacinamide (or other salvageable vitamin B3) substrates and upregulate the Nampt salvage enzyme. Magnesium is part of the nutritional support because ATP-magnesium complex is a Nampt cofactor.

COMMENT: No matter how NAD+ was made in the first place—whether from a newer niacin (NR or NMN), an older niacin (NA or NAM), or from tryptophan—nicotinamide is constantly being produced in cells because the NAD+ is continuously being consumed. It must be salvaged via Nampt if we want to optimize cellular NAD+ levels.

26. Support Nampt Enzyme Expression & Activity	• BioVin® French Red Grapes Extract (5% resveratrol) • Magnesium (as magnesium glycinate)

AMPK is part of the cellular adaptive response. It's considered the master cellular energy sensor and overlaps with many of the other puzzle pieces we've discussed. AMPK acts a bit like a thermostat, monitoring cellular ATP levels. When levels get lower than desired, AMPK coordinates the response, turning on and off a host of genes. The changes in gene expression impact each of the four linked cellular energy pathways, increasing the use of sugars and fats as fuel, while upregulating the Krebs cycle and OXPHOS. The short-term result is a restoration of cellular ATP. A more long-term result of AMPK activation is an enhanced capacity for cells to make ATP in the future. AMPK is a co-regulator in several nutrient sensing pathways that are responsible for the healthspan and lifespan responses to calorie restriction. In this role it enhances (1) sirtuin function, (2) mitochondrial biogenesis (AMPK-Sirtuin-PGC-1α pathway), (3) antioxidant defenses, (4) cellular protection (e.g., Nrf2 cellular defenses and detoxification), and (5) degradation processes in protein quality control (e.g., autophagy and mitophagy). It also downregulates the NF-κB "inflammaging" pathway. In general, the AMPK response to nutrient or exercise stress declines with age (i.e., programmed aging), while enhancing AMPK addresses aspects of both (1) programmed aging, influencing signaling pathways, and, (2) aging as accumulated damage by upregulating damage repair processes. We've mentioned that factors within cells, including other molecules like ATP, can influence the performance of some enzymes. Nampt is one of these enzymes: It uses an ATP-magnesium complex as a cofactor. AMPK is another one of the cellular factors affecting Nampt. When AMPK is activated Nampt gets upregulated. So, a strategy aimed at NAD+ optimization should also include AMPK enhancement. Supporting AMPK is the next piece of the puzzle.

27. AMPK Support

- BioVin® French Red Grapes Extract (5% resveratrol)
- *Theobroma cacao* Seed Extract (10% theobromine)
- *Rosmarinus officinalis* Leaf Extract (50% ursolic acid)
- *Citrus sinensis* L. Fruit Extract (40% nobiletin)
- Sirtmax® *Kaempferia parviflora* Root Extract (15% polymethoxy flavonoids)
- Cinnulin PF® *Cinnamomum burmannii* Bark Extract
- *Sophorae japonica L.* Flower Extract (95% rutin)
- *Gynostemma pentaphyllum* Whole Herb Extract (50% gypenosides)
- Strawberry Seed Extract (0.5% trans-tiliroside)
- *Ceratonia siliqua* Pod Extract (98% D-chiro-inositol)
- *Citrus grandis* Fruit Extract (98% apigenin)
- R-Lipoic Acid sodium
- Coenzyme Q10 (ubiquinone)
- Tri-creatine Malate
- Calcium B-Hydroxy-B-Methylbutyrate
- Folate (as folic acid, calcium L-5'-methyltetrahydrofolate, and calcium folinate)
- Biotin

Aging is characterized by having too little NAD+ available in cells to feed all of the pathways that use it. This occurs because old cells do not efficiently salvage niacinamide to rebuild the NAD+ molecule. In a sense there's a supply problem, but it isn't the only problem. The NAD+ produced gets used differently during aging. The three NAD+-consuming pathways—sirtuins, CD38, and PARPs—typically respond to aging by decreasing, increasing, and slightly increasing (at the wrong body clock time), respectively. Since these three pathways compete for the same finite pool of NAD+, excess demand by one leaves less available for others. This means that in addition to the NAD+ supply problem, aging is characterized by a distribution problem, with sirtuins suffering the most.

Sirtuins play an important role in coordinating an organism's adaptive response to nutritional status (including calorie restriction or fasting), exercise, stress, toxicity, and other environmental challenges. In general, sirtuins activate cellular gene expression programs that allow the organism to better respond to circumstances, by adjusting metabolic function, cellular energy, mitochondrial networks, insulin signaling, circadian clock regulation, inflammation, and prolongevity pathways. Like AMPK, sirtuins turn on and off many genes, including PGC-1α and, indirectly, AMPK. Sirtuins are a key element in the lifespan extension response to calorie restriction and the healthspan response to exercise, improving cellular signaling (i.e., reversing programmed aging) and damage repair (i.e., countering aging as accumulated damage). Because of their many roles in coordinating cellular adaptive responses, sirtuins are sometimes referred to as "master switches of metabolism." Part of upregulating sirtuin function is making sure more NAD+ is available. Part is also upregulating sirtuin gene expression and enzyme activity. This is the next puzzle piece.

28. Sirtuin Support

- **BioVin® French Red Grapes Extract (5% resveratrol)**
- ***Theobroma cacao* Seed Extract (10% theobromine)**
- ***Rosmarinus officinalis* Leaf Extract (50% ursolic acid)**
- **Sirtmax® *Kaempferia parviflora* Root Extract (15% polymethoxy flavonoids)**
- **Cinnulin PF® *Cinnamomum burmannii* Bark Extract**
- ***Sophorae japonica L.* Flower Extract (95% rutin)**
- **Strawberry Seed Extract (0.5% trans-tiliroside)**
- ***Gynostemma pentaphyllum* Whole Herb Extract (50% gypenosides)**
- **Sensoril® Ashwagandha *Withania somnifera* Root and Leaf Extract (10% withanolide glycoside conjugates)**
- **R-Lipoic Acid sodium**
- **Pyrroloquinoline Quinone**

COMMENT: AMPK and Sirtuins are another co-regulatory loop. AMPK upregulates Nampt activity, which boosts tissue levels of NAD+. The extra NAD+ can be used by sirtuins to feed their activity. Sirtuins then turn on and off genes that allow cells to better respond to environmental challenges. One of these genes—LKB-1—activates AMPK. So, AMPK is involved in feeding sirtuins, while sirtuins activate AMPK in a co-regulatory feedback loop. Supporting both together is a better strategy than focusing on only one in isolation.

CD38 is one of the main NAD+-degrading enzymes. The NAD+ pool is finite. If CD38 consumes too much, less is available for sirtuins and PARPs … the important jobs they do suffer. This occurs with aging, with expression and activity of CD38 gradually increasing over the aging process—by older age it can be several fold higher. The good news is that CD38 can be slowed, which boosts NAD+ (less is being consumed by CD38) and upregulates sirtuins and PARPs (more NAD+ is available for them), improving programmed aging and enhancing cellular and mitochondrial processes that counter damage accumulation. Nudging CD38 in ways that downregulate its activity is complementary to strategies aimed at boosting NAD+ more directly. It is the next piece of the puzzle.

29. Support Downregulation of CD38	• *Citrus grandis* Fruit Extract (98% apigenin) • BioVin® French Red Grapes Extract (5% resveratrol)

PARPs are involved in DNA repair, so contribute to countering aging as accumulated damage. In general, repair and remodeling processes tend to be nighttime functions. This doesn't mean they only occur at night; it does mean they are subject to body clock (i.e., circadian system) regulation, with relative increases at night and decreases during the day. Body clock function tends to worsen with aging. One of the consequences is that the big day-night differences in many processes seen in younger cells diminishes. PARPs are an example. With aging their daytime activity tends to increase, while nighttime activity decreases. The result is less difference between the two. Even if we have the same overall amount of PARP activity over a 24-hour period, this decrease in the day-night difference affects performance. Signaling doesn't work because of the amounts of something; it works because of the changes in amounts. This is a general law of physiology. To replicate younger physiology, it's important to dampen PARP activity during the daytime (so sirtuins are free to consume more of the NAD+ pool), while allowing activity to shift back into the nighttime. We focused on ingredients that have a dual effect on boosting sirtuins, while slightly slowing PARPs. This is the next puzzle piece.

30. Slow PARPs Daytime Activity	• BioVin® French Red Grapes Extract (5% resveratrol) • *Theobroma cacao* Seed Extract (10% theobromine) • *Rosmarinus officinalis* Leaf Extract (50% ursolic acid) • *Sophorae japonica* L. Flower Extract (95% rutin) • R-Lipoic Acid sodium

What cells do is obviously important, When cells do it is also important, with "when" being dependent on body clock function (i.e., the circadian system). Regulation of many cellular process is timed, with predictable increases and decreases in activity (i.e., oscillation or rhythm) over a 24-hour day. This timing component is an important feature in complex adaptive systems. It allows cells to work effectively, scheduling processes so they don't interfere with each other, while allocating finite resources most efficiently. It also allows our internal processes to synchronize with the outside world, enabling coordination of cellular processes and physiological functions in anticipation of behaviors like feeding and sleep-wake cycles.

Many of the puzzle pieces we've discussed are subject to timing. Sirtuins compete with PARPs for a finite pool of NAD+. The circadian system allocates the finite NAD+ resource, with sirtuins getting preference during daylight hours when dealing with stress is more important, while PARPs get preference during darkness, when repair and recovery processes tend to dominate. Nampt activity and expression are regulated by the circadian system: It's higher during daytime hours. Not surprisingly, since AMPK upregulates Nampt, AMPK activity is also higher during the daytime. Mitochondrial biogenesis and mitophagy are both needed for maintaining a healthy mitochondrial population, but are opposing processes: Coordination and regulation is a body clock function. Antioxidant defenses and inflammation are dependent on the circadian system. These are just a few of the many examples where cellular function relies on timing.

The human brain contains a light-sensitive master body clock. We also have clock functions in cells outside the brain (i.e., peripheral clocks). Metabolic and cellular health are characterized by robust oscillation (i.e., high amplitude rhythms), crisp timing, synchronization of the master clock with peripheral clocks (i.e., entrainment), and rapid re-entrainment after something disrupts timing (e.g., jet lag, changes in sleep schedule, disruption in meal patterns). These tend to suffer as we get older, with studies suggesting this occurs largely due to changes in expression of clock genes. The result is accelerated aging. Since healthy cellular function relies on timing, improving clock functions tends to make everything work just that much better. This is the next piece of the puzzle.

31. Support Body Clock Function

- ***Citrus sinensis L.*** **Fruit Extract (40% nobiletin)**
- **BioVin® French Red Grapes Extract (5% resveratrol)**
- **R-Lipoic Acid sodium**
- **L-Tryptophan**
- **Vitamin B12 (as methylcobalamin and adenosylcobalamin)**
- **L-Carnitine**

The total number of gut microorganisms is thought to about the same as the total number of human body cells (somewhere in the ballpark of 3–4 × 10^{13}). The network of interacting gut microorganisms is called the "gut microbiota." The complete set of genes or genetic material associated with the microbiota is the "gut microbiome." The gut microorganism network protects us from germs (approximately 70% of the human immune system is in the gut) and many toxins. It's involved in digesting food, producing vitamins, and making molecules used by our cells. In addition to these tasks, the gut microorganism network communicates with and influences our cellular signaling processes and gene expression, significantly affecting overall metabolic health. It also produces signaling compounds that can be carried through the bloodstream and decoded by the brain (i.e., the gut-brain axis) impacting cognitive performance and mood. Gut microbiota are influenced by behaviors, including diet, exercise, and sleep. In general, assuming these behaviors don't change substantially, the microbiome tends to be fairly stable after early childhood until older age, when it shifts in ways associated with unhealthy aging and frailty. These changes are thought to contribute to age-related declines in immune responses (immunosenescence) and low-grade chronic inflammation (inflammaging). The two agents most well-known for extending lifespan in model organisms both involve altering gut microbiota as part of their actions. The gut microorganism network has been linked to lifespan extension benefits from calorie restriction. Our cellular and mitochondrial networks are not isolated entities; they interact with the world around them including the gut microbiome. In a sense, they are both part of the the same complex system. The goal is to support the gut microorganism network and gut function (e.g.,barrier function, immunity) along with our cells and mitochondria. This is the next puzzle piece, with a subset of the full stack of ingredients supporting both. In general, many of the polyphenol-containing ingredients have shown some degree of prebiotic-like activity.

32. Gut Microbiome Support

- **BioVin® French Red Grapes Extract (5% resveratrol)**
- ***Theobroma cacao* Seed Extract (10% theobromine)**
- **elevATP® Ancient Peat and Apple Fruit Extract**
- ***Citrus sinensis L.* Fruit Extract (40% nobiletin)**
- ***Gynostemma pentaphyllum* Whole Herb Extract (50% gypenosides)**
- ***Citrus grandis* Fruit Extract (98% apigenin)**
- **Cinnulin PF® *Cinnamomum burmannii* Bark Extract**
- **Myo-Inositol**
- **N-Acetyl-L-cysteine**
- **Pyrroloquinoline Quinone**
- **Magnesium (as magnesium glycinate)**

Cell networks form larger functional units called tissues, such as adipose (i.e., body fat), heart, liver, and skeletal muscle. Getting individual cells spread across networks in different tissues to respond in coordinated ways is a critical part of adaptation and whole system response. Some of this coordination is provided by hormones. Hormonal signals are released into the blood and give instructions to remote cells throughout the body. While individual cells will be exposed to the same hormones, they respond in tissue-specific ways to the instructions. Insulin is an example. It's considered to be an anabolic hormone (i.e., growth producing). It supports growth in part by increasing a cell's ability to take in sugar molecules. In general, exercise increases muscle at the expense of body fat. But how do we get this response if insulin is causing growth … wouldn't both muscle and fat cells get bigger?

While this is an oversimplification, in general, when we are sedentary muscle cells are more insulin resistant, while fat cells are more insulin sensitive. This makes it easier for sugars to get into fat cells and fuel their growth. When we start exercising, the landscape shifts: Muscle cells become more insulin sensitive, while fat cells become

more resistant, allowing sugars to enter muscles more easily, fueling their growth. This tissue-specific shifting of sensitivity-resistance allows the same signal—insulin—to produce different adaptive responses in muscle and fat cells depending on the circumstances.

Hormones don't get inside cells. They communicate instructions. Cells listen for these instructions using specialized receptors, which act a bit like the ears of the cell. When receptors detect the hormone signal, a cascade of cellular signaling events is initiated (i.e., the cell follows the instructions). The way the cell responds to these instructions will be determined by local gene expression. Our focus is on the response to insulin and thyroid signaling, since they are critical for cellular energy production and metabolic function. The next pieces of the puzzle are about supporting an exercise mimetic cellular hormonal signaling response: We want muscle and fat cells to respond to these hormones in the same way they do when we are physically active; not the way they do when we are sedentary.

33. Support Insulin Signaling	• Myo-Inositol • *Ceratonia siliqua* Pod Extract (98% D-chiro-inositol) • Cinnulin PF® *Cinnamomum burmannii* Bark Extract • *Theobroma cacao* Seed Extract (10% theobromine) • *Rosmarinus officinalis* Leaf Extract (50% ursolic acid) • *Gynostemma pentaphyllum* Whole Herb Extract (50% gypenosides) • BioVin® French Red Grapes Extract (5% resveratrol) • Sensoril® Ashwagandha *Withania somnifera* Root and Leaf Extract (10% withanolide glycoside conjugates) • *Citrus grandis* Fruit Extract (98% apigenin) • Sirtmax® *Kaempferia parviflora* Root Extract (15% polymethoxy flavonoids) • *Citrus sinensis L.* Fruit Extract (40% nobiletin) • Vitamin K2 (as menaquinone-7) • R-Lipoic Acid sodium • Pyrroloquinoline Quinone • Coenzyme Q10 (ubiquinone) • L-Carnitine • Magnesium (as magnesium glycinate)
34. Support Thyroid Signaling	• Myo-Inositol • *Ceratonia siliqua* Pod Extract (98% D-chiro-inositol) • Sensoril® Ashwagandha *Withania somnifera* Root and Leaf Extract (10% withanolide glycoside conjugates)

We started building the healthspan puzzle with a single molecule, NAD+. We care about NAD+ because of what it allows cells and mitochondria to do better. But these are means to an end. What we really care about is what a network of healthier cells allows the body to do better. These are subjective experiences including improved muscle performance and recovery, better sleep, enhanced resilience to stress, improved cognitive performance, increased energy, and an overall upregulation of health. This is the big picture and there's many pieces to this puzzle.

For more about Eternus and the work we do at Neurohacker please visit neurohacker.com

