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(54) Title: RESETTING BIOLOGICAL PATHWAYS FOR DEFENDING AGAINST AND REPAIRING DETERIORATION
FROM HUMAN AGING

(57) Abstract: Compositions for addressing one or more of the effects of aging are described. The compositions comprise a first component comprising repair system activator(s) such as nicotinamide adenine dinucleotide (NAD⁺), nicotinamide mononucleotide (NMN), nicotinamide riboside (NR), nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), nicotinic acid riboside (NAR), 1-methylnicotinamide (MNM), cyclic adenosine monophosphate (cAMP) and combinations thereof; a second component comprising methyl donor(s) such as S-5'-adenosyl-L-methionine (SAM), methionine, betaine, choline, folate, vitamin B12, or combinations thereof; and a third component comprising antioxidant defense activators such as H₂O₂, N₂S, NaSH, N₂S₂, and several others, including combinations thereof. Methods of administering the disclosed compositions or separate formulations of repair system activator, methyl donors, and antioxidant defense activators are also disclosed.



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RESETTING BIOLOGICAL PATHWAYS FOR DEFENDING AGAINST AND REPAIRING DETERIORATION FROM HUMAN AGING

FIELD

The disclosed subject matter generally relates to compositions for defending against
5 and repairing the effects of aging.

BACKGROUND

Early human ancestors did not have enough energy to do everything they could
benefit from doing. Energy availability limitations during early human evolution led to
biological tradeoff mechanisms governing energy use, which limits energy use for defense
10 and repair of human cellular damage. Cellular damage has been proposed to be causal for
human biological aging (Olson C 1987, Holliday R 2004, Kirkwood T 2005, Gavrilov L
2001). Human biological aging has been proposed to be causal for the human "Diseases of
Aging" (Cutler R 2006).

Feedback loops are a part of energy tradeoff controls

15 Feedback loops in biochemical synthetic pathways turn off energy expenditures in
areas of non-use to increase efficiency of total organismic energy use. Use of energy and
nutrients in evolution needed to be balanced with the ability to obtain these calories and
nutrients from the environment, which was varied and limited. In part this was achieved by
what in medicine is known as "The use it or lose it principle," which is adaptive up-
20 regulation / down-regulation based on need. An example is that of the antioxidant enzymes
systems that are turned to lower settings if they do not receive, over time, new oxidative
pulses to keep the antioxidant enzymes on higher settings.

Cellular repair involves energy trade offs

Energy that is used for the repair of cellular damage is energy that is not available to
25 use for other functions that are beneficial for cellular function and life. Cellular repair
systems and the complex human immune systems represent two such competing
energetically demanding systems that compete for energy use. In their "Disposable Soma
Theory of Aging" Kirkwood and Rose (Kirkwood and Rose 1991) proposed that to
optimize energy use, biological systems may invest most of their energy in growth and
30 development and little in damage control and repair for non-germline (soma) cells.

Energy availability from diet effects energy tradeoffs

The second law of thermodynamics teaches that the state of entropy in a closed
system can only change in one direction over time. Animals need to eat food, thus

maintaining an open system, to improve, repair, or maintain their structure over time at the expense of the food they eat, which gains in entropy becoming fecal matter.

In evolution, food and its nutrients and energy were often limiting and only sporadically available. Evolution had to adapt for this. In calorie restricted times, energy pathways adapted for these limitations. These pathways have benefits. The beneficial effect of calorie restriction is seen by recent research to be directed by Sirtuin enzymes. Sirtuin enzymes are involved in human cellular repair. There are 7 known human Sirtuin enzymes. All 7 of these human Sirtuin enzymes use NAD⁺ (Imai S 2000). Nicotinamide is the end product of these Sirtuin reactions.

An example of a feedback loop in Sirtuin pathways is that the end product, nicotinamide, is able to bind to the Sirtuin enzymes and decrease their enzymatic properties. The feedback loop changes if the nicotinamide is methylated by the human nicotinamide-N-methyltransferase (NNMT) enzyme in the cell using S-5'Adenosyl -L- methionine (SAM). The new methylated nicotinamide is then unable to bind in the nicotinamide binding site because of steric hindrance of the physical size of the newly attached methyl group to the nicotinamide (Schmeisser K 2013). With this methylation change the Sirtuin enzymes are able to keep working instead of stopping their activity.

Defenses against pathogens conies with energy tradeoffs

People with disease, especially chronic disease, age faster. The innate immune system (example white blood cells) when throwing oxidants (example C1-) at pathogens to kill them creates background damage in its own cells that leads to faster aging for the organism. Pathogens have been a major killer of humans, so without energy for fighting these pathogens, individuals would be more quickly removed from evolution. Making this trade off of how much energy to expend in a pathogen attack, how much energy to use to repair the damage from a pathogen attack, and even the energy to use to heighten the immune system to be ready for an immune attack, are all important tradeoffs in evolution.

An example of this tradeoff is seen in a study of 684 individuals over 100 and 536 individuals 85 to 99 years old in Japan (Arai Y 2015). Lower levels of inflammation (4 immune variable composite score) was the best predictor of who was going to continue to live (life-span) and be physically and cognitively healthy (health-span). Immune markers (a simple index of serum interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-alpha) which were two of the Arai 4 markers) were found to be the best predictor of mortality in 1,155 older adults in a 10 year all-cause mortality study after adjusting for variables already known to cause death (Varadhan R 2014). Just one immune marker, Serum IL-6, predicted

all-cause mortality, cancer, cardiovascular disease and liver disease in a 1843 person prospective cohort study (Lee JK 2012). These studies confirmed results in smaller prior studies (Derhovanessian E 2010, Reuben DB 2002, Taaffe DR 2000).

The biological cellular mechanism of this tradeoff may be due to when Nrf2 releases Keap1, it is available to capture IKK β thus inhibiting NF- κ B target genes. This interaction correlates the expression of antioxidant enzymes by Nrf2 and the turning on and off of the immune system by NF- κ B.

Sexual animals have energy use tradeoffs that asexual animals do not have

Asexual animals like sea anemones do not age. There is no apparent senescence in asexually reproducing Hydra, but there are signs of aging when Hydra reproduce sexually (Yoshida K 2006). Hydra share 6071 genes with humans (Wenger Y 2013) and at least 80% of known human aging genes are shared with Hydra (Tomczyk S 2014). Research has shown sexual animals, like humans, age faster in somatic cells after puberty and less if sexual hormones are lowered. A human example is eunuchs in India and Korea, with no testicles, live on average 9 to 13 years longer. In flat worms, a research model named *C. elegans*, the heat shock response (HSR), essential for proteostasis and cellular health, is repressed after sexual maturity in somatic (non-sexual) cells by germline (sexual) cells by triple methylation at stress gene loci. This competition between the interest of the germline and the soma cells (Kirkwood TL 2000) determines the rate of aging in sexually mature individuals (Labbadia J 2015). Research also shows trade-offs between ability to bear children and aging. An example is use of low dose RU-486, the abortion drug, gives lower fertility and longer life span on average (Landis G. 2015). Bearing children, and especially bearing children later in life (Sun F 2015, Perls TT 1997), has been linked to increases in the probability of a longer life in women, although cause and effect is still uncertain. Time of menopause has also been correlated to rate of aging.

Energy use tradeoffs seen with chronological age

In youth, humans have an excess or a reservoir of ability and energy in excess of what is needed by cells and organs on average but this decreases with age. In youth one has less knowledge and wisdom and smaller body size but evolution makes up this deficit with a higher metabolism allowing more energy expenditure (especially per body mass) thus one is able to live more per unit of time. Higher metabolism is correlated with faster aging generally across species, although humans are known to age faster after puberty showing this need not be a hard and fast rule. The "Rate of Living" theory (Pearl R 1928) was updated to "Livingness" (Sohal R 2012) to include temperature, hibernation, fecundity and

metabolic potential from initial oxygen-use observations (Rubner M 1908). Older individuals have more experience, knowledge and wisdom and are thus able to still maintain themselves in life with less energy consumption. This lower energy production may at least partially be due to the decline of the quantity and function of mitochondria to produce energy during one's life span.

Extra brain energy used in humans conies with energy tradeoffs

Animals are known to trade off larger brain size with smaller fat reserves and smaller musculature. Humans have done both in evolution for the increased energy needed (the human brain uses about 30% of the organism's energy) by our larger (and 3X more dense with cells) brain (per body size). This indicates energy was in short supply in evolution for human ancestors. Cooking food to make its energy more available helped with this energy equation as well.

Exercise conies with energy tradeoffs

Because of the "use it or lose it" principle of biological feedback loops, more exercise will continue the energy flow to tissue and biological systems like muscles and anti-oxidant defense systems when they are used in excess of the normal amount. When they are not used the body turns off energy flow to them to conserve energy. It has been known by medical science for a while that exercise in the long term is "good" for humans, but exercise in the short term is "bad" for humans.

The mechanism of this effect is seen as that the "bad" of exercise comes from the release of oxidants, including the oxidants from energy production in the mitochondria. This pulse of oxidation turns on defense and repair mechanism and then this in turn benefits the cells and body during the non-exercising hours of the day, this is called oxidative pre-conditioning.

Sleep is an energy use tradeoff

All animals with neurons sleep. With sleep, one gets more time to repair cellular damage, and thus extend one's life quality and length at the expense of the things that cannot be accomplished during sleep hours.

Biological aging vs /chronological aging

The amount of human biological aging has been shown to vary during a chronological year. In a study (Belsky DW 2015) of 954 "young" humans in their third and fourth decade of life (at studies end, 38 chronologically years old and without signs of the diseases of aging), all born in a one year time period in the same New Zealand town, aged at rates (biologically years old) that varied from 1 biological year per chronological year to

nearly 3 biological years per chronological year as determined by a grouping of 10 diagnostic tests measured at 3 time points. 3 of the 954 even appeared to have reversed biological age in the time period. This variation in the amount of human biological aging in a chronological year indicates that the rate of biological aging in humans is not fixed and has the possibility to be changed.

A "Unified Theory of Aging"

Over the years, four major theories of aging have developed. These four general theories have arisen from numerous branches of scientific inquiry. The four major theories of aging are:

- The calorie restriction theory of aging, (McCay C 1935)
- The free radical theory of aging (now called Redox), (Harmon D 1956)
- The methylation theory of aging in 1967 (Vanyushin B 2005), and
- The somatic mutation theory of aging (Szilard L 1959).

Others aging theories include:

- Rate of Living Theory of Aging (Pearl R 1928, Rubner M 1908, Sohal R 2012)
- Disposable Soma Theory of Aging (Kirkwood and Rose 1991)
- Redox Stress Hypothesis of Aging (Sohal R 2012)
- Inflammaging (Franceschi C 2007, 2007, 2014) Para-inflammation (Medzhitov R 2008)
- "Metchnikoff's Hypothesis of Aging" (Metchnikoff E 1901)

There are connections and overlaps between all these nine theories of aging, and the compounds, compositions, formulations, and methods disclosed herein further support these theories and in fact provide for their unification.

Caloric restriction (CR) theory of aging

- In 1935, Clive McCay first discovered that caloric restriction (CR) increased life span in animals. CR is the practice of reducing caloric consumption without inducing malnutrition. This requires an organism receive adequate amounts of water, vitamins, minerals, and protein, but limits carbohydrate and fat calories (to less than the recommended dietary allowance (RDA) for humans). CR can be done safely without harmful health effects with total caloric restrictions in the range of 10-40% less than RDA recommendations. In 1986, Richard Weindruch showed that restricting calories to 2/3rds of

the normal amount in mice increased lifespan by 40%. To date, a large number of experiments in animal models have corroborated these results. Animal models of CR have also helped researchers discover the molecular biology pathways that account for the increase in life span and health span (Colman RJ 2014). A randomized controlled two year
 5 calorie restriction study in humans (Ravussin E 2015) showed feasibility and effects on predictors of health-span and longevity (life-span).

Sirtuins and Caloric Restriction

In the 1990s, a MIT research team led by Leonard Guarente discovered that a certain enzyme found in yeast was a "nutrient sensor" and could possibly be the molecular
 10 mechanism that would explain the effects of caloric restriction (Guarente L 2000). In yeast, caloric restriction increased the life span of yeast by 40%. When this enzyme, called Sirtuin, was "knocked out", the yeast did not live longer in response to caloric restriction.

Sirtuins, NAD+, and the solution to the rate-limiting step of NAD+ biosynthesis

All Sirtuin enzymes required a cofactor called nicotinamide adenine dinucleotide
 15 (NAD+) (Imai S 2000). This compound is naturally occurring, found in all cells and is one of the "energy currencies" of the cell; much like ATP. NAD+ is the "depleted energy form" of NADH, which is the actual "energy currency form" of the molecule. Thus NAD+ is a "signal" that the cell is out of energy and this "signal" activates and is used by the Sirtuin enzymes. This explains how caloric restriction, which is an "energy depletion state", can
 20 activate the cell to trigger cellular stress pathways to promote survival. All 7 of the Sirtuins found in humans appear to be triggered by cellular nutritional stress. NAD+ is the trigger for this response. NAD+ is produced from nicotinamide mononucleotide (NMN) and NMN can be made by an enzyme called NAMPT. NAD+ has a half-life of 3 to 5 hours in unstressed cells (Suave A lab: reported in Canto C 2013). Unfortunately, in humans there
 25 does not seem to be not enough NAD+ being made in the body due to energy-use-regulation. In 2011, it was shown that the regulatory stopping point in the synthesis of NAD+ is the enzyme NAMPT, which converts the precursor of NMN into the compound NMN. When NMN is given to mice, they create NAD+ out of the NMN in 15 minutes. Thus the solution for the "NAD+ synthesis restriction problem" is to bypass the rate-
 30 limiting step, which was the production of NMN. This was demonstrated in 2011 (Jun Yoshino and Kathryn Mills 2011).

Human Sirtuins 1, 2, 3, 4, 5, 6, 7

Sirtuin 1 (Sirt1)

Sirtuin 1 (Sirt1) is localized in the nucleus and the cytoplasm. It is extremely sensitive to **H2O2** oxidation inhibition. Extracellular concentrations as low as 1 μ M of **H2O2** inhibit Sirt1 by oxidizing critical cysteine residues in the Sirtuin active center (Jung S-B 2013). In addition, the RNA-binding protein HUR binds to the 3' untranslated region of the mRNA encoding Sirt1, leading to its stabilization and increased levels. **H2O2** triggers the dissociation of HUR from the HUR-Sirt1 mRNA complex, promoting Sirt1 mRNA decay, reducing Sirt1 abundance, a process that seems to be regulated by Chk2 kinase (Abdelmohsen K 2007). Redox Factor-1 (REF-1) was found to chemically reduce Sirt1 cysteine residues, stimulating its activities (Jung S-B 2013). REF-1, which maintains sulfhydryl (thiol) groups of cysteine residues in Sirt1 in reduced form protecting Sirt1 from **H2O2** oxidation, has also been called APE1 (Apurinic/Apyrimidinic endonuclease)-1 because in a separate active site on the enzyme it is the rate limiting enzyme in mammalian base excision repair pathway. Sirtuin 1 is the most studied human sirtuin to date.

Sirtuin 2 (Sirt2)

Sirt2 is mainly in the cytoplasm (Yudoh K 2015, Gomes P 2015). Sirt2 is important in regulation of the cell cycle (Nie H 2014). It has been shown to be a histone deacetylase (Moscardo A 2015). It has been shown to maintain faithful chromosome division and replication (Kim HS 2011). A reported mechanism for this is the Sirt2 deacetylation of ATR-interacting protein (ATRIP) at lysine 32 in response to replication stress. BubR1, a mitotic checkpoint kinase, is a deacetylation target of Sirt2. By deacetylation of lysine 668, Sirt2 stabilizes BubR1 and keeps it from ubiquitination and degradation. This leads to a striking 58% (122% for male) median life span increase and 21% maximal life span increase in mice (North BJ 2014).

Sirt2 activity has been correlated to a decrease in depression (in a rat model system that created depression through stress) possibly by increasing neurogenesis (Liu R 2015).

Sirtuin 3 (Sirt3)

Sirtuin 3 is localized in the mitochondrial inner membrane and is an important regulator of cellular energy homeostasis (Nogueiras R 2012). A specific Sirt3 allele activated enhanced activity level and has been shown to be necessary for a life span over 90 in humans (Rose G 2003, Bellizzi D 2005, Halaschek-Wiener J 2009). Sirt 3 is the dominant mitochondrial deacetylase activity (Lombard DB 2007). Sirt3 expression, in the liver, increases after fasting (Hirschey MD 2010). Sirt3 expression in the muscle increases after exercise (Hokari F 2010), fasting, and caloric restriction and decreases with chronic high fat eating (Palacios OM 2009). Overall these studies indicate Sirt3 acts as a master

switch that is adaptive to energy shortage (Cho E-H 2014) to maintain ATP production, including the metabolic switch known as the Warburg effect (Guarente L 2014). Sirt3 deacetylates at lysine 926 and 931 to activate OPA1, a mitochondrial fusion protein, elevating its GTPase activity. About 20% of mitochondrial proteins can be acetylated.

5 Protein acetylation / deacetylation is thought to be a major regulatory mechanism in the mitochondria (Kim SC 2006). The role of Sirt3 in regulating mitochondrial biogenesis via activation of the PGC- α /ERR- α complex has been demonstrated (Giralt A 2012, Hirschey MD 2011, Kong X 2010).

10 Sirt3-dependent pathways are a putative molecular link between sleep-loss and neurodegeneration (Fifel K 2014, Zhang J 2014). Sirt3 mediates reduction of oxidative damage and prevention of age-related hearing loss (Someya S 2010) with OPA1 (Leruez S 2013). Sirt3 has also been implicated in Alzheimer's disease, Huntington's disease, Parkinson's disease, amyotrophic lateral sclerosis (Kincaid B 2013) and Non-alcoholic fatty liver disease (Cho E-H 2014).

15 **Sirtuin 4 (Sirt4)**

Sirtuin 4 is localized in the mitochondria. It is a cellular lipoamidase (or delipoylase), removing lipoyl modifications from lysine residues of substrates. Sirt4 delipoylates and modulates the activity of pyruvate dehydrogenase complex (PHD), which in turn inhibits the production of acetyl-CoA (Mathias RA 2014). It deacetylates malonyl-CoA decarboxylase (MCD) to regulate lipid catabolism (Laurent G 2013). It also performs ADP-ribosylation on glutamate dehydrogenase (GLUD1) (Haigis MC 2006).

Sirtuin 5 (Sirt5)

25 Sirtuin 5 is localized in the mitochondria. Sirt5 desuccinylates, demalonylates, and deglutarylates protein substrates such as carbamoyl phosphate synthase 1 (CPS1) to regulate the urea cycle (Du J 2011, Peng C 2011, Tan M 2014). Sirt5's deacetylating activity is weak (Du J 2011, Tan M 2014). Sirt5 has been proposed to regulate ammonia production and ammonia-induced autophagy and mitophagy by regulating glutamine metabolism (Polletta L 2015).

Sirtuin 6 (Sirt6)

30 Sirtuin 6 is localized in the nucleus and is a chromatin associated histone deacetylase (Kugel S 2014). It can deacetylate histone H3 lysine 9 (H3K9) thus participating in regulation of telomeric chromatin and cellular senescence (Michishita E 2008). When it deacetylates histone H3 lysine 56 (H3K56) it decreases the chromatin accessibility for transcription factors such as NF- κ B, Foxo3, and HIF1- α to their target

promoters thereby inhibiting the expression of their target genes (Kugel S 2014). Sirt6 deacetylates histone H4K16 which regulates the meiotic apparatus in the oocyte (Han L 2015). Sirt6 has been linked to the regulation of life-span and health-span (Kanfi Y 2012, Cardus A 2013, Shen J 2013, Liu R 2014, Sharma A 2013). Activation of Sirt6 is postulated to reduce atherosclerotic vascular diseases. Sirt6 expression suppresses cellular senescence and NF-kB mediated inflammatory responses, like TNF-alpha, in the human knee which leads to osteoarthritis development (Wu Y 2015). Increasing Sirt6 activity has also been implicated as a therapy in idiopathic pulmonary fibrosis (IPF) (Minagawa S 2011).

Sirtuin 7 (Sirt7)

Sirtuin 7 is localized in the nucleolus. Sirt7 has been functionally linked to transcriptional regulation. It positively controls ribosome production through direct interaction with the Poll machinery (Ford E 2006, Grob A 2009, Chen S 2013). Conversely Sirt7 negatively regulates transcription of genes outside rDNA repeats via histone H3K18 deacetylation (Barber MF 2012). Sirt7 targets acetylated lysine in the N-terminal tail of histone H3 (H3K18Ac). Sirt7 is downstream of Sirt1 and Sirt6 in the DNA damage signaling cascade. Sirt7 recruitment to DNA damage sites is dependent of PARP1 activity. There it can deacetylate H3K18Ac. H3K18Ac affects recruitment of damage response factor 53BP1 to double stranded breaks in DNA leading to their endjoining and genome stability.

Cyclic Adenosine Monophosphate (cAMP)

CAMP discovery as a second messenger led to a 1971 Nobel Prize. Calorie restriction increases cAMP. CAMP decreases with age. Higher levels of cAMP has now been correlated to longer life. CAMP performs a variety of metabolic-related hormone signaling processes. NAD⁺ contains an AMP moiety. CAMP interacts with the Sirtuin NAD⁺ binding pocket. This binding increases the hydrolysis of NAD⁺ into NAM and 2'-O-acetyl-ADP-ribose. Thus cAMP is a promoter of the enzymatic activity of Sirtuins (Wang Z 2015) acting as a reinforcement to the energy depletion signal of NAD⁺.

Phosphorylation

Sirt1 can be phosphorylated at the highly conserved Serine 434 location which is in the Sirtuin catalytic site. Phosphorylation at S434 increases the Sirt1 deacetylase activity. Protein Kinase A (PKA) or a kinase downstream of PKA is thought to phosphorylate Sirt1. This phosphorylation regulation is thought to regulate the Sirtuin activity on a shorter time frame (5 to 15 minutes) than normal measures that increase NAD⁺ levels. The shorter time frame allows the cAMP/ PKA induction for short term fatty acid utilization (Gerhart-Hines

Z 2011). In addition, Sirt1 transcriptional levels are regulated by the competition for promoter site binding by cyclic AMP response-element-binding protein (CREB) and carbohydrate response-element-binding protein (ChREBP). CREB itself can be phosphorylated which leads to its nuclear import leading to it being a better competitor for the promoter site on Sirt1 and Sirt1 transcription. This mRNA reaches a maximum in 12 to 18 hours and returns to basal levels at 24 hours (Noriega LG 2011) pointing to the desirability of not eating for 12 hours each day (Chaix A 2014).

The Sirt1 protein has several other phosphorylation sites on serine amino acid side chains. Ser27 is one of the sites that gets phosphorylated indirectly by JNK2 activation. When the Ser27 site on Sirt1 is phosphorylated, the Sirt1 protein becomes much more resistant to proteasome-mediated degradation. Thus it increases the half-life of the Sirt1 protein from less than 2 hours to greater than 9 hours. This is a very important part of maintaining Sirt1 protein levels within the cell (Ford J 2008).

Keap1 serves as a negative regulator of Nrf2, described later, which activates anti-oxidant enzymes. Keap1 degradation in response to antioxidants is controlled by tyrosine phosphorylation (Kasper JW 2011).

Nicotinamide Mononucleotide (NMN) as an anti-aging compound

Only NAD⁺ activates all 7 Sirtuins. In 2008 it was demonstrated that NMN, a precursor of NAD⁺, produced age-reversal effects in mice (Ramsey K and Mills K 2008). Then in 2009 it was shown that NMN had a powerful effect on reversing the effects of obesity-induced diabetes (Imai S 2009) as well as age-induced diabetes. In 2013 it was shown that high dose NMN reversed muscle aging with one week of NMN administration (Gomes A 2013).

CD 38

CD38 is a NADase as well as a NADPase. CD38 can be extracellular (a type II plasma membrane enzyme) and intracellular. CD38 transforms NAD⁺ into nicotinamide and cADPR. cADPR is a second messenger involved with cell function. Nicotinamide, as previously noted, feeds back to inhibit both the sirtuin enzymes as well as the PARP enzymes discussed in the next section. CD38 is found in many cell populations. CD38 is associated with the innate immune system as well as the adaptive immune system. CD38 is highly expressed in inflammatory cells and the loss of CD38 is associated with impaired immune responses. CD38 and CD157 are thought to enable energy recovery of energetically costly products which would otherwise be wasted. Two CD38 alleles are known in the Caucasoid population (Malavasi F 2007). CD157 is a second member of this

family, although CD157's catalytic efficiency is several hundredfold lower than that of CD38 (Hussain AM 1998). CD38 and CD157 can be in monomeric or dimeric forms. CD38 is a master Ca^{++} regulator that catalyzes the formation of endogenous Ca^{++} (Lee S 2015).

Ca^{++} releases can stimulate the production of IL-6 (Adebanjo OA 1999, Sun L 2003). IL-6

5 was shown to be lowered in "Example" section herein.

NAD⁺ is known to decline with age. CD38's protein levels, mRNA levels and enzymatic activity all increase (in all tissues tested: liver, white adipose tissue, spleen, skeletal muscle, ilium, jejunum, and kidney) with increase age. This increase in CD38 is required for age related NAD decline. Other proteins that use NAD do not appear to be the

10 cause of NAD⁺ decrease with age; examples include PARP1 and Sirt1 both of these decrease with age. An excellent inverse correlation coefficient was observed between CD38 activity and NAD⁺ decline in aging. CD38 is also able to degrade NAD⁺ precursor nicotinamide mononucleotide (NMN) (Grozio A 2013). The kcat for NMN⁺ was 5-fold higher than that of NAD⁺ and has the greatest reported kcat of any substrate for CD38

15 (Sauve AA 1998). When CD38 lowered NAD⁺ in cells, this led to the loss of mitochondrial function without changes in levels of Sirt3.

CD38 is induced by:

- i. Oxidation is associated with CD38 activation (Zhang AY 2004, Kumasaka S 1999, Wilson HL 2001, Dipp M 2001, Okabe E 2000, Ge Y 2010)
- 20 This is the opposite of Sirt1, where reduction is needed for Sirt1 to stay on, and oxidation turns it off. Oxidation also activates PARP-1 (Bai P 2011).
- ii. TNF-alpha is a potent inducer of CD38 expression in cells (Barata H 2004, Lee CU 2012).

Note: The triple therapy in "Example" herein demonstrates the reduction of both

25 TNF-alpha and IL-6.

a) CD38 has a TNF receptor (Prasad GS 1996);

b) TNF-alpha also induces a two-fold activation of the CD38 promoter.

So TNF controls both the (transcriptional regulation) RNA levels and the protein activity. The mechanism of this regulation is that TNF-alpha increase binding to the NF-kB site and

30 to some of the AP-1 binding sites (Tirumurugaan K 2008).

CD38 appears not to be effected by an end product feedback loop:

- i. Nicotinamide rescues CD38 from inhibition of synthetic inhibitors (Sauve AA 2002, Sauve AA 1998). Nicotinamide inhibits Sirt1 and PARP (other users of NAD⁺). When Nicotinamide is methylated then this does not feedback to Sirtuins and PARPs and does not turn off these NAD⁺ using enzymes (due to steric hindrance).
- 5 CD38 is inhibited by:
 - ii. There are nicotinamide mononucleotide look-alike-molecules for example flavonoids luteolinidin, kuromanin, and luteolin (Kellenberger E 2011) these inhibit CD38, but these would probably also inhibit other reactions involving the three enzymes that make NAD⁺ from NMN as well as the other enzymes that use NAD⁺ like SIRT and PARP.
 - 10 iii. Methylation of CD38's gene may be a part of its regulation (Ferrero E 1999). This in addition to the effect of methylation of nicotinamide to methyl-nicotinamide altering the feedback loop of the Sirtuin and PARP enzymes. This gene methylation (epigenetics) may well be why CD38 increase with age.
 - 15 iv. Apigenin inhibits CD38. It also turns on Nrf2. Apigenin effectively reversed the hypermethylated status of the 15 CpG sites in the Nrf2 promoter in a dose-dependent manner. Apigenin enhanced the nuclear translocation of Nrf2 and increased the mRNA and protein expression of Nrf2 and Nrf2 downstream target gene NQO1. Apigenin restored NRF2 from the silenced status by CpG demethylation (Parededs-Gonzalez X 2014).
 - 20 v. Reduction turns off CD38. Reduction of Cys 118-Cys 201 disulphide in CD38 leads to inactivation (Prasad GS 1996). A disulphide is involved with the bifunctional activity at hinge region of the enzyme and the three dimensional structure dependent on the 10 cysteine residues (Lin Q 2005, Prasad GS 1996). By contrast Sirt1 is kept on by reduction.
 - 25 vi. There is a potential binding site upstream from the CD38 transcription start site for trans-interactions with IL-6 (Note, triple therapy in "Example" reduces IL-6).

Poly (ADP-ribose)polymerase (PARPs)

- DNA breakage repair takes a lot of energy and energy devoted to this is allocated.
- 30 DNA breakage increases with age on average, in part, because energy is not allocated to its repair, even though the DNA damage can be repaired. Poly (ADP-ribose) polymerases (PARPs) are NAD⁺ dependent enzymes that repair DNA (an ancient and evolutionarily conserved biochemical reaction Otto H 2005) and are responsible for other biological

functions as well. Nicotinamide is released as an end product of these PARP reactions. Thus not only do the Sirtuin enzymes compete for available NAD⁺ with the PARPs and CD38, but the Sirtuins are also inhibited by the end product of the use of NAD⁺, which is the nicotinamide from Sirtuin use of NAD⁺, the PARP use of NAD⁺, the CD38 use of NAD⁺ as well as other uses of NAD⁺. As stated before, NAD⁺ has been seen to decrease with aging (Braidy N 2011 and Massudi H 2012). There are 17 PARP enzymes in humans (Ame J 2004). PARP-1, 2, and 3 (and tankyrases) are all involved with DNA repair. Sirt1 and Sirt6 have been shown to be involved with DNA repair as well. The majority of DNA - induced PARP activity is covered by PARP-1 (85-90%) while PARP-2 is considered to be responsible for the rest (Szanto M 2012). PARP-1 is regulated by a) nicotinamide feedback, b) redox balance, H₂O₂ oxidation activates PARP-1, c) reversible methylation and d) PARP-1 is turned off by Sirt1 deacetylation. Prolonged PARP activation can deplete cellular NAD⁺ pools, thus the large drop in NAD⁺ with excess DNA damage. PARP-1 displays high catalytic turnover of NAD⁺ compared to Sirt1 (Bai P 2012). The K_m (20-60 μM - Ame J 1999) is 5-fold lower and PARP-1 has a much stronger V_{max} than Sirtuin-1 (Houtkooper R 2010). The affinity of PARP-2 with NAD⁺ and its degradation is about the same as with Sirt1. PARP-2 binds to DNA in the proximal region of the Sirt1 promoter. The K_m of most Sirtuins for NAD⁺ are in the range of 100-300 μM and fluctuations of NAD⁺ are reported at 200 to 500 μM. NAD⁺ levels generally fluctuate within a two-fold range (Chen D 2008). In addition NAD⁺ fluctuates in a circadian fashion (Ramsey K 2009). Although these measurements have several shortcomings it does appear that the activity of Sirtuins can be rate limited by NAD⁺ availability.

Free Radical Theory of Aging

In 1956, Denham Harmon, studying the effects of X-ray radiation, proposed that the cause of aging was due to reactive oxygen species called "free radicals," and today is known as the "Free Radical Theory of Aging" (Harman D 2009). From his observations of the effects of X-ray radiation on animals, Dr. Harmon proposed that just like in X-radiation induced free radical production, normal aging generated free radicals and had similar effects on the organism. At that time, the source of these "free radicals" with normal aging was unknown. Subsequent efforts have confirmed that cells produce their own reactive oxygen species (i.e., free radicals). In fact, free radicals are produced in every cell from before birth until death. Many cellular biochemical reactions create reactive oxygen species within the cell. Aging is not due to the presence of these free radicals, per se, but rather due to the damaging excess of free radicals, because of the lack of free radical scavenging by the many

enzymes that quench these reactive oxygen species, cause to the cells. Control of Reactive Oxygen Species (ROS) is modified in muscles of old animals and release of ROS (superoxide) is reduced in old muscles. (Jackson M. 2011). This Free Radical field is now called the field of Redox Biology (Nathan C 2013) and there are now a growing number of reports detailing advantageous biological effects of free radicals involved in the modulation of cell signaling pathways (Powers and Jackson 2008). The "Redox Stress Hypothesis of Aging" conceptually shifts the importance of redox to signal transduction and gene regulation with a pro-oxidizing shift in the redox state of cells with age that leads to the over-oxidation of redox-sensitive protein thiols and the consequent disruption of the redox-regulated signaling mechanisms. Support for this theory comes from a) observations that oxidative byproducts increase from 25 to 100% from puberty to adulthood b) protein carbonyls increase with age and decrease with calorie restriction and c) average lifespan is proportional to protein carbonyls (Sohal R 2012).

Oxidation-Sensitive Protein Thiol Groups

Changing the redox potential of oxidation-sensitive protein thiols can allow the switch between distinct catabolic and anabolic processes as well as activate survival pathways. Protein methionine and cysteine residues are particularly sensitive to oxidative modification. Methionine is the step prior to SAM synthesis in the methylation pathway. Thus methionine is connected to the methylation pathway and regulated by redox balance. The percentage of cysteine residues increases with organism complexity but their prevalence are still significantly lower than occurrence simply based on codon usage. Cysteines that occur in clusters are highly conserved in evolution and usually are structurally or functionally important. pKa values for the thiol groups are influenced by their local environment. Oxidation states can range from the fully reduced thiol / thiolate anion to the fully oxidized sulfonic acid (Cremers CM 2013). The reaction rate of protein thiols with oxidants such as hydrogen peroxide (H₂O₂) spans over 7 orders of magnitude without any detectable correlation to the acidity of the respective active site thiol (Ferrer-Sueta G 2011).

There are reversible and irreversible cysteine modifications. Oxidation of cysteine thiol (RSH/RS⁻) by ROS, RNS, or RCS leads to the formation of highly reactive sulfenic acid (RSOH), which can react either with another thiol to form a disulfide bond (RSSR) or with GSH to become S-glutathionylated (RSSG). These oxidative modifications are reversible, and reduction is catalyzed by the Trx and/or Grx system. Further oxidation of sulfenic acid to sulfinic acid (RSO₂H) and sulfonic acid (RSO₃H) is thought to be generally

irreversible in vivo. Many of the thiol redox regulated proteins act as transcriptional regulators (e.g., OxyR, Yap1p) which rapidly induce expression of genes involved with antioxidant defenses (Zheng M 1998, Tachibana T 2009), others are involved with signal transduction cascades (Gopalakrishna R 2000 and Dinkova-Kostova AT 2005). (See

5 Supplement 1 from Cremer CM 2013 for more examples.)

An example of an enzyme with thiols in the active site is GAPDH which plays a crucial role in glycolysis. Oxidation of the GAPDH thiols blocks glycolysis and contributes to the generation of NADPH instead of NADH (Shenton D 2003). Another example is the oxidation of thiols in active sites that inactivate the phosphatase activity of SHP1/2, PTEN,
 10 Cdc25 enhancing signaling intensity achieved by substrate phosphorylation, this leads to activation of signaling pathways like NF- κ B-inducing kinase/I κ B, causing expression of genes involved in antioxidant defense (Jung KJ 2009). A third example is the Sirtuin thiol groups in Sirtuin active sites that are very sensitive to oxidation, which inhibit Sirtuin activity when oxidized. Human Sirtuin-1 has 3 (Cys-67, Cys-268, and Cys-623) of 5
 15 cysteines exposed to possible reversible thiol modification via redox balance (Autiero I 2008). Cys-67 and Cys-623 are consistent with post-translational regulation of these terminal regions, Cys-268 lies in the NAD⁺ binding region in all the members of the Sirtuin family of which the catalytic core is highly conserved. The binding of NAD⁺ results in changes in the Sirtuin conformation that allows catalysis to proceed (Zee R 2010).

20 ***Redox Biology Main Components***

There are different types of ROS and RNS (reactive nitrogen species). Together they are referred to as RONS. They include: superoxide, hydrogen peroxide, hydroxyl radicals, singlet oxygen, nitric oxide, peroxynitrite, hyperchlorite, and also lipid peroxidations "PUFA"s. There is different specificity of ROS. ROS display a type of specificity that is
 25 atomic rather than molecular. ROS most often reversibly reacts in cell signaling with Sulphur, which is one of the least abundant atoms in biological macromolecules and mostly with side chains of cysteine or methionine residues in peptides or proteins (Nathan C 2013). Endogenous enzymatic sources of ROS (multiple isoforms allow more sensitivity and specificity in regulation) include seven isoforms of NADPH oxidases (NOXs) that are
 30 differentially expressed (regulated) in diverse cells and species as well as a list of other sources (see Box 1 page 2 Nathan C 2013).

The main types of anti-oxidants enzymes (multiple isoforms allow more sensitivity and specificity in regulation) (need control and use of transition metals) are Superoxide Dismutase (SOD) 3 isoforms, Glutathione Peroxidase (GPX) 5 isoforms, and Catalase.

There are also Thioredoxin (TRX) 2 isoforms (with thioredoxin reductase) Thioredoxin may be recycled by interaction with REF-1 (REF-1 keeps Sirtuin thiols reduced), Glutaredoxin (GRX) 3 isoforms, Peroxiredoxin (PRX) 6 isoforms (responsible for reduction of 90% of Eukaryote mitochondrial and more than that of cytosol H₂O₂. This can be turned on and off with a functional loop of regulation allowing redox signaling (Sies H. 2014). Peroxiredoxin makes up a phylogenetically ancient family of proteins whose primary role is detoxification of H₂O₂. These also create a redox rhythm. It is thought that catalytic cycle of peroxiredoxin hyperoxidation and recycling by sulfiredoxin may form the basis of a transcription-independent circadian clock (Rey, G. 2013). NAD⁺ levels are correlated to the biological clock with 2 peaks in the day 12 hours apart. It is thought that because NAD⁺/NADH with a cellular ratio of >1 is higher than NADP⁺/NADPH cellular ratio of <0.01 in the cytosol that this allows the cell to segregate antioxidant and biosynthetic reducing equivalents (NADPH) from those destined for mitochondrial ATP generation (NADH). The phosphate of NADPH confers different substrate specificity but has the same electron transfer properties. Hyperoxidation of peroxiredoxins can induce chaperone function as well as signal transduction.

Antioxidant small molecules include: Glutathione (GSH), Uric acid, Bilirubin, Ascorbic Acid (Vitamin C) Vitamin E, also carotenoids, Co-Enzyme Q10, N-acetylcysteine (NAC). NAC acts as a reduced thiol donor and counters H₂O₂ that oxidizes thiols.

20 ***Methylation Theory of Aging***

Not all genes are expressed in all cells. This "selective gene expression" control of the 21,800 genes that code for proteins in human cells determines if the cell becomes a brain cell or a heart cell. This system of gene regulation is referred to as "epigenetics" (Kundaje A 2015). Epigenetics controls the rate of aging (Reynolds L 2014). One of the methods by which genes are regulated is the methylation of certain DNA residues called "cytosines." In 1967, Boris Vanyushin showed that DNA loses its methylation with aging (Vanyushin BF 2005). There are other epigenetic mechanisms involved besides DNA methylation, such as histone protein modifications, microRNA, and chromatin remodeling (heterochromatin vs euchromatin) (Kundaje A 2015). In addition, some DNA cytosines increase their methylation with aging and other sites decrease their methylation with aging. It is clear is that DNA methylation is the form of epigenetic gene regulation that correlates with aging. This has most recently been shown by Steven Horvath, who showed that a "DNA methylation clock" can be constructed from the analysis of only 353 cytosine residues and that this "DNAm clock" (Bocklandt S 2014) has a 0.96 correlation with aging.

More importantly, this "clock" keeps time much better than any other known measure of aging besides birth date. DNA methylation profiling of mesenchymal stem cells (MSCs) obtained from individuals aged from 2 to 92 years identified 18,735 hypermethylated and 45,407 hypomethylated CpG sites associated with aging. Most importantly, hypomethylated CpG sites were strongly enriched in the active chromatin mark H3K4me1 in stem and differentiated cells, suggesting this is a cell type-independent chromatin signature of DNA hypomethylation during aging. These results indicated that the dynamics of DNA methylation during aging depends on a complex mixture of factors that include the DNA sequence, cell type, and chromatin context involved and that, depending on the locus, the changes can be modulated by genetic and/or external factors (Fernandez AF 2015). It has been shown that calorie restriction prevents the age-related changes in DNA methylation in mice (Chouliaras L 2012). Two of the 7 Sirtuin enzymes have been shown to indirectly affect DNA methylation through their effects on histone deacetylation (Sirt1 and Sirt6). It was also shown that the end-product of the Sirtuin reaction, nicotinamide, needs to be methylated to 1-methylnicotinamide, otherwise the end product nicotinamide will bind inside the Sirtuin enzyme and stop its enzymatic activity (Schmeisser K 2013). Sirtuin-1 decreases the activity of NF- κ B which increases tri-methylation of lysine 36 on histone 3 (H3K36me3). This correlates to accelerated DNA methylation. Genes with a dramatic expression change during aging are marked with low or even undetectable levels of H3K36me3 in their gene bodies irrespective of their corresponding mRNA abundance (Pu M 2015). In human cells a global loss of tri-methylation of H3K9 (H3K9me3) recapitulates accelerated cellular senescence and changes in heterochromatin architecture. These findings also correlated to people ages 7 to 72 heterochromatin's disorganization with increasing age (Zhang W 2015). In January 30, 2015 DNA-methylation-age of blood was used to predict all-cause mortality in later age of humans independently of health status, lifestyle factors and known genetic factors (Marioni RE 2015). On February 19, 2015 the journal Nature published the results of 111 human epigenomes allowing future comparisons and references to be made by others (Kundaje A 2015).

Methylation Pathway

Methionine is particularly sensitive to oxidative modification. Methionine is the step after homocysteine synthesis and prior to SAM synthesis in the methylation pathway. Thus methionine is a part of the methylation pathway and is regulated by redox balance. Cysteine is synthesized from methionine and is the main precursor of hydrogen sulfide (H₂S). Elevated homocysteine levels are associated with inhibition of endogenous hydrogen sulfide

(H₂S) generation (Tang X 2011). Hydrogen sulfide (H₂S) ameliorates methionine induced oxidative stress (Tyagi N 2009). Homocysteine (Hcy) can be irreversibly degraded to hydrogen sulfide (H₂S) by a transsulfuration pathway which is activated by oxidative stress. H₂S has protective functions in hyperhomocysteinemia (Ohashi, R. 2006, Chang L 2008).

5 Adipose tissue is an important organ of methionine metabolism and is also an insulin-sensitive organ. Increases of H₂S in adipose tissue increases insulin sensitivity (Feng X 2009). High pancreatic H₂S suppresses insulin release (Wu L 2009). Blood levels of H₂S are lower in type 2 diabetes than age matched healthy subjects (Jain S 2010). Aspirin is an arachidonate inhibitor and may influence the methionine-homocysteine cycle and
10 associated one carbon metabolism and thereby both methylation and redox balance (Lupoli R 2015). H₂S therapy with H₂S donors; Na₂S or NaSH inhibits aspirin in a dose dependent manner (Zanardo RC 2006).

There is also a methylation inhibitor: S-adenosylhomocysteine (SAH). When methionine is abundant, NNMT regulates only SAH not SAM (Ulanovskaya OA 2013).

15 Radical SAM enzymes are a diverse superfamily of proteins that use radical chemistry (5'-dAdo) to effect substrate modification. Substrates of these enzymes are distinct from the nucleophilic substrates that undergo methylation by a polar mechanism. There are 4 known subclasses of these enzymes (A, B, C, D).

As a general rule age-related hypo-methylation of DNA is the dominant event
20 leading to increased expression of genes, but hyper-methylation is common in some promoter regions of DNA with age leading to promoter repression. There is a close relationship between redox balance and methylation balance (Metes-Kosik N 2012).

There is a relationship of methylation to redox balance with homocysteine going to the antioxidant glutathione when under oxidative conditions and going to SAM and
25 methylation under reduction conditions (Mosharov E 2000).

Somatic Mutation Theory of Aging

Somatic cells are cells that, when cloned, can grow to a full animal that ages normally. Somatic cells in sexual organisms live to support germline cells' attempt to have their DNA reach the next generation. Somatic cells are known to give up their own cellular
30 lives for the good of the organism. One way they do this is via programmed cell death also called apoptosis where the somatic cell dies in a way that is organized and less harmful than necrosis cell death to its cellular neighbors. Sirt1 inhibits apoptosis. Sirt2 is indicated to be involved in the regulation of necroptosis, a somewhat more organized version of necrosis (NarayanN 2012). Some viruses like vaccinia have anti-apoptosis genes so other methods

of cell death are needed. Another way is the culling of somatic cells that are not as vital as their neighboring cells. In this specific selection, cells with higher anabolic capacity and higher relative c-Myc than their neighboring cells are selected for and relatively unfit cells with lower c-Myc than their neighboring cells are eliminated (Merino M 2015). Sirt1 in humans regulates c-Myc and thus this process, as well as apoptosis. Of note is that c-Myc concentration change is opposite in direction to IL-6 level changes (Hoffman-Liebermann B 1991). Note: the "Example" herein lowers IL-6 concentrations in serum. Results from the reduction in expression of myc in mice has led to its suggestion as an anti-aging therapy (AlicN 2015).

10 ***Autophagy***

Autophagy generates amino acids, sugars, fatty acids, and nucleosides that are recycled for macromolecular synthesis and energy production which is important during starvation and stress for cell survival. NAD⁺ is intimately correlated to autophagy and NAD⁺ and its metabolism can influence autophagy. The mechanism of control of autophagy by NAD⁺ include pathways involving: a) NAD⁺/ NADH b) NADPH, c) PARylation, d) Deacetylation, e) NAADP and f) cADPR/ADPR. NAD⁺ dependent deacetylation by Sirt1 regulates multiple autophagy processes. NAD⁺ metabolites catalyzed by CD38 are also involved in multiple autophagy processes. Sirt1 regulates autophagy via p53 which has a pivotal role in sensing cellular stress, including DNA damage and oxidative stress. A link between p53 and necrosis has also been reported (Tu H 2009). Autophagy is a process of self-degradation of cellular components in which double-membrane autophagosomes sequester organelles or portions of cytosol and fuse with lysosomes or vacuoles for breakdown by resident hydrolases. Deacetylation modification of the autophagy machinery proteins is also required for autophagy and the deacetylation process is dependent on the NAD-dependent deacetylase Sirt1 (He C 2009).

Connections between various theories of aging

The various theories of aging, discussed herein have connections between them. For example, the Calorie Restriction/Sirtuin Theory of aging is connected to the Methylation Theory of Aging by the methylation of nicotinamide. Nicotinamide is produced from the use of NAD⁺ by Sirtuins, and by methylating nicotinamide, methylated-nicotinamide cannot inhibit the Sirtuins in a negative feedback loop. PARPs and CD-38 also use NAD⁺ and produce nicotinamide as an end product of their reactions, which inhibits PARP and Sirtuin activity through a negative feedback loop. Thus, methylation of nicotinamide can prevent the negative feedback loops of Sirtuin and PARP enzymes.

In addition, the Calorie Restriction/Sirtuin Theory of aging is connected to the Free Radical (now Redox) theory of aging by Sirtuins inhibition of NF- κ B, a major component of inflammation and immune defense. This process increases tri-methylation of DNA which increases DNA wrapping which decrease all-cause mortality. The thiol groups at the Sirtuin active site have to be reduced for the Sirtuin to be active directly connecting the two theories.

Furthermore, the Calorie Restriction/Sirtuin Theory of aging also connects to the Somatic Mutation Theory of Aging. If cellular damage is not repaired, it accumulates, affects the fitness of cells, and if the cellular performance falls below a critical level the individual dies. This is called the Somatic Mutation theory of aging (Kennedy S 2012 and Szilard L 1959). Sirtl as well as other Sirtuins effect the expression of Myc. Mammals are able to specifically select for cells with high anabolic capacity and elimination relatively unfit cells from their relative Myc activity (Mareno E 2014). This ability to select for more fit cells and eliminate unfit cells prolongs lifespan of flies 35% over calorie restriction (Merino M 2015).

The Methylation Theory of aging is connected to the Free Radical (now Redox) theory of aging as seen when homocysteine progresses in the synthetic pathway to S-Adenosyl-methionine (SAM), which is needed to make 1-methylnicotinamide in the example above, under the reducing effect of the antioxidant defense system but is diverted to the synthesis of glutathione, an antioxidant, when under oxidative stress.

The other theories of aging feed into the above theories as discussed in the text. The previously discussed "Rate of Living Theory of Aging" (Pearl R 1928, Rubner A 1908, Sohal R 2012) and the previously discussed "Disposable Soma Theory of Aging" (Kirkwood and Rose 1991) both feed into the "Calorie Restriction" and "Sirtuin use of NAD⁺ discussions. The Redox Stress Hypothesis of Aging (Sohal R 2012) feeds into the Free Radical Theory discussion as noted in that section. It has also been noted that Redox balance itself is related to inflammation. The results demonstrated in the "Example" herein where IL-6 and TNF- α are decreased in plasma then correlate the "Inflammaging Theory of Aging" (Franceschi C 2007, 2007, 2014) also called Para-inflammation (Medzhitov R 2008) and the "Metchnikoff's Hypothesis of Aging" which concerns the intestinal lining permeability to bacteria and their products leading to aging (Metchnikoff E 1901) and this permeability to bacteria can be reduced by fasting via a pathway involving Crtc a molecule that interacts with CREB, which is linked to Sirtl pathways, discussed here which are activated by fasting. It appears there is a link between the immune system's

attack on bacteria to its attack on mitochondria proteins such as cardiolipin which is need for Nlrp3 inflammasome activation (Iyer SS 2013).

Cellular damage is causal in aging and aging is causal in the "Diseases of Aging"

In addition to the aging itself there are diseases of aging (Goldman DP 2013). In these diseases of aging, aging is a causative factor in the disease. Diseases of aging include: inflammation, heart disease (heart attack and heart failure), stroke, neurodegenerative disease such as Alzheimer's disease, diabetes, cancer, respiratory disease, systemic autoimmune disease, and muscle wasting.

What are needed are new compositions and approaches for addressing the effects of aging. The compositions and methods disclosed herein address this need.

SUMMARY

In accordance with the purposes of the disclosed materials and methods, as embodied and broadly described herein, the disclosed subject matter, in one aspect, relates to compounds, compositions and methods of making and using compounds and compositions. In specific aspects, the disclosed subject matter relates to compositions for addressing one or more of the effects of aging. In further aspects, disclosed are compositions comprising a first compound, a second compound, and a third compound, wherein the first compound comprises nicotinamide adenine dinucleotide (NAD⁺), NAD⁺ precursor such as nicotinamide mononucleotide (NMN), a precursor or prodrug of NMN, nicotinamide riboside (NR), nicotinic acid riboside (NAR), nicotinic acid adenine mononucleotide (NaMN) (Zhou T 2002), nicotinic acid adenine dinucleotide (NaAD), analog of NAD⁺ that promotes NAD⁺ use such as 1-methylnicotinamide (NMN)(Hong S 2015), cyclic adenosine monophosphate (cAMP) (Wang Z 2015), wherein the second compound comprises S-5'-adenosyl-L-methionine (SAM), SAM precursor such as methionine, betaine, choline, folate, vitamin B12, and wherein the third compound comprises antioxidant defense activator such as Nuclear factor erythroid 2 (Nrf2) activator [including activators that increase nuclear translocation of Nrf2, increase Nrf2 mRNA transcription, increase Nrf2 protein expression, and increasing Nrf2 downstream target genes, reduce Nrf2 inhibitors (such as Bach 1, caveolae, TGF-beta)] such as H₂O₂, H₂O₂ generator, hydrogen sulfide (H₂S), H₂S Donor such as, sodium hydrosulfide (NaHS), sodium sulfide (Na₂S) and optionally, a carrier.

Also disclosed are compositions wherein the first compound, comprises NAD⁺, NMN, NR, NaMN, NaAD, NAR, NMN, cAMP, alone or in combination. Also disclosed are compositions wherein the first compound comprises NMN. Also disclosed are

compositions wherein the first compound comprises a precursor or prodrug of NMN, e.g., a compound that increases NMN production in the body or metabolizes into NMN. Also disclosed are compositions wherein the composition lowers a Surrogate Marker for Aging. Also disclosed are compositions wherein the surrogate marker is CMV IgG, C-Reactive Protein, Tumor Necrosis Factor-Alpha, or Interleukin-6 Serum. Also disclosed are compositions, where the composition comprises water. Also disclosed are compositions wherein the composition is formulated for injection. Also disclosed are compositions wherein the composition is concentrate form for dissolving in a liquid. Also disclosed are compositions wherein the composition is in tablet form or aerosol form. Also disclosed are compositions wherein the composition comprises at least 1×10^{-8} moles of the first compound, at least 1×10^{-8} moles of the second compound, and at least 1×10^{-9} moles of the third compound.

In further aspects, disclosed are methods of reducing inflammation in a subject comprising administering to the subject a compositions as disclosed herein, and optionally, a carrier. Also disclosed are methods, wherein the first compound, the second compound, and the third compound are administered at approximately the same time. Also disclosed are methods, wherein the first compound is administered within 15, 30, 60, 90, or 120 minutes of the subject's biological clock's NAD⁺ peak. Also disclosed are methods wherein the composition is administered to a subject a dosage of at least 1×10^{-6} moles/kg of the first compound to the subject, 1×10^{-6} moles/kg of the second compound to the subject, and 1×10^{-7} moles/kg of the third compound to the subject. Also disclosed are methods wherein the composition is injected over 8-12 days. Also disclosed are methods, wherein the composition is an aerosol, lyophilization, powder, or emulsion. Also disclosed are methods wherein the subject is a human. Also disclosed are methods wherein the human is treated for at least two months. Also disclosed are methods wherein the composition is a tablet that is administered orally at least once daily. Also disclosed are methods wherein the composition is administered once daily.

Additional advantages will be set forth in part in the description that follows, and in part will be obvious from the description, or may be learned by practice of the aspects described below. The advantages described below will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive.

DETAILED DESCRIPTION

The materials, compounds, compositions, and methods described herein may be understood more readily by reference to the following detailed description of specific aspects of the disclosed subject matter and the Examples included therein.

5 Before the present materials, compounds, compositions, and methods are disclosed and described, it is to be understood that the aspects described below are not limited to specific synthetic methods or specific reagents, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only and is not intended to be limiting.

10 Also, throughout this specification, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which the disclosed matter pertains. The references disclosed are also individually and specifically incorporated by reference herein for the material contained in them that is discussed in the sentence in
15 which the reference is relied upon.

 The study of caloric restriction led to the discovery of Sirtuins, which are activated by the "depleted energy" version of NADH, which is called NAD⁺. NADH is not used by sirtuins enzymes and is only inhibitory at concentrations far greater than those predicted for cells. NADH is also not used for generation of NADP⁺ by the cytosolic NADK enzyme and
20 this generated NADP⁺ is rapidly turned into NADPH (Pollak N 2007). Caloric restriction induces a "nutritional stress" that results in a depletion of the cells energy stores (ATP, NADH, etc.). The "depleted energy forms" of this stored energy are cAMP and NAD⁺.

 NAD⁺ activates a set of enzymes called Sirtuins as well as PARPs. What the data disclosed herein shows is that by providing NAD⁺ or compounds or compositions having a
25 similar activity, immune system markers are reduced, which has been shown to be associated with anti-aging. These data are consistent with an increased activation of Sirtuins, through interaction with NAD⁺, or similar acting molecules. However, also disclosed herein, the positive effect of NAD⁺ can level off, presumably because of other reactions taking place in the organism, including in the active site of the Sirtuins
30 themselves.

 Therefore, what has been additionally shown by the disclosed methods and compositions is that by adding additional molecules along with NAD⁺ or similar acting molecules, the beneficial effects can be extended by, for example, a continued, enhanced, and maintained reduction in inflammation markers, which has been linked to anti-aging.

This information has led to compositions and formulations, which contain three categories of compositions, or methods where three different categories of molecules are administered, alone, in conjunction, or in combination to a subject.

Increasing life-span and health-span by repairing cellular damage and preventing the age-related changes that can occur are disclosed. The data provided herein show that to reduce markers for inflammation three broad goals to defend against and repair deterioration from aging should be sought:

- I. NAD⁺ should be available to turn on and be used by Sirtuins,
- II. methyl donors should be available to methylate DNA and other entities needing methylation like the reaction of nicotinamide to 1-methylnicotinamide by the nicotinamide-N-methyltransferase (NNMT) enzyme, and
- III. a reducing balance should be provided so that important enzymes, such as Sirtuins, can have the thiol (sulfur) groups in their reactive sites maintained in a reduced state.

Disclosed herein are compositions, formulations, and methods that reduce markers of inflammation related to aging, and are consistent with enhancing these three goals.

Meeting these three goals is possible if oxidation, in the form pulsed low level H₂O₂, is available to turn on pre-conditioning of the anti-oxidant defense and repair system. By turning this system on, the system is protected against the down regulation of the anti-oxidant defense and repair system, which is an energy saving mechanism. In this way, when the antioxidant defense system is challenged with an oxidative assault from a larger oxidative burst, it is able to defend against this oxidation that would lead to cell damage and destruction.

In one embodiment, one provides enough oxidation from H₂O₂ to provide pre-conditioning from signaling to turn on the anti-oxidant defense and repair system but not enough to create oxidized damage like oxidizing the thiol groups in the Sirtuin active site that turns the Sirtuin enzymes activities off. The APE-1/ Ref-1 is a molecule that protects the thiol groups of amino acids in the Sirtuin active site from oxidation by H₂O₂. This can be kept active. It is theorize that the same or a similar process is needed for the nicotinamide-N-methyltransferase (NNMT) enzyme to make 1-methylnicotinamide from nicotinamide and thus to stop this feedback loop from shutting off the Sirtuin enzyme by cutting off the supply of nicotinamide that can fit into the Sirtuin enzyme and stop it's activity.

Disclosed is a usable solution for reversal of human aging by resetting the human endogenous defense and repair pathways and mechanisms. These mechanisms are normally set to preserve energy due to molecular settings set by and for evolutionary energy insufficiency, evolutionary sexual selection, and pathogen defense by diverting more usable energy and resources from defense and repair mechanisms. Through administration of the disclosed compounds, compositions, and formulations these pathways can be reset for increased repair and defense.

It is demonstrated herein that dietary NMN drunk by itself in water does turn into NAD⁺ and turns on Sirtuins in humans, but these effects are ephemeral. It is also demonstrated herein that Hormesis / feedback loops effected benefits in humans until these benefits are plateaued or reversed and even overshoot the initial beneficial effects within a three month time frame. This discovery solves this deterioration of beneficial effect problem by turning on the beneficial effects of Sirtuin enzymes, optimizing their beneficial effects, and keeping these beneficial effects turned on.

Disclosed herein are compounds, compositions, formulations, and methods, which turn on, enhance, and in some formulations keep on, the human defense and repair mechanisms involving the Sirtuin enzymes. These compounds, compositions and formulations comprise one or more items from each of three (3) categories alone or in combination, and can be administered through ingestion, injection, inhalation, application to the skin, or any other means.

When administered, the disclosed compounds, compositions, and formulations, can perform at least one of the following activities:

- A) Protect against further cellular damage from the aging process
- B) Repair cellular damage from the aging process
- C) Delay the onset of the diseases of aging where aging is a causal factor.

Diseases of aging include: inflammation, heart disease (including heart attack and heart failure), stroke, neurodegenerative disease such as Alzheimer's disease, diabetes, cancer, respiratory disease, systemic autoimmune disease (including arthritis) and muscle wasting.

- D) Promote weight loss / reduce hunger
- E) Promote more productive sleep, waking more rested

Compounds, Compositions, and Formulations

Also disclosed are compounds, compositions, and formulations falling into, or containing, one or more of the following three general categories:

Category 1 which are Repair System Activators

5 Category 2 which are Methyl Donors, and

Category 3 which are Antioxidant Defense Activators

Disclosed are compositions comprising a first compound, a second compound, and a third compound, wherein the first compound comprises nicotinamide adenine dinucleotide (NAD⁺), NAD⁺ precursor such as nicotinamide mononucleotide (NMN), a precursor or
 10 prodrug of NMN, nicotinamide riboside (NR), nicotinic acid riboside (NAR), nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), analog of NAD⁺ that promotes NAD⁺ use such as 1-methylnicotinamide (MNM), cyclic adenosine monophosphate (cAMP) wherein the second compound comprises S-5'-adenosyl-L-methionine (SAM), SAM precursor such as methionine, betaine, choline, folate, vitamin
 15 B12, and wherein the third compound comprises antioxidant defense activator such as Nuclear factor erythroid 2 (Nrf2) activator, including activators that increase nuclear translocation of Nrf2, increase Nrf2 mRNA transcription, increase Nrf2 protein expression, and increase Nrf2 downstream target genes, reduce Nrf2 inhibitors (such as Bach 1, caveolae, TGF-beta)] such as H₂O₂, H₂O₂ generator, hydrogen sulfide (H₂S), H₂S Donor
 20 such as, sodium hydrosulfide (NaHS), sodium sulfide (Na₂S) and optionally, a carrier.

Also disclosed are compositions, wherein the first compound, comprises NAD⁺ NMN, NR, NaMN, NaAD, NAR, MNM, cAMP, alone or in combination. Also disclosed are compositions wherein the first compound comprises NMN. Also disclosed are compositions wherein the first compound comprises a precursor or prodrug of NMN, e.g., a
 25 compound that increases NMN production in the body, or metabolizes to NMN. Also disclosed are compositions wherein the composition lowers a Surrogate Marker for Aging. Also disclosed are compositions wherein the surrogate marker is CMV IgG, C-Reactive Protein, Tumor Necrosis Factor-alpha, or Interleukin-6 Serum. Also disclosed are compositions, where the composition comprises water. Also, disclosed are compositions
 30 wherein the composition is formulated for injection. Also disclosed are compositions wherein the composition is concentrate form for dissolving in a liquid. Also disclosed are compositions wherein the composition is in tablet form or aerosol. Also disclosed are compositions wherein the composition comprises at least 1×10^{-8} moles of the first

compound, at least 1×10^{-8} moles of the second compound, and at least 1×10^{-9} moles of the third compound.

Category 1, Repair System Activators

The turning on and maintaining of Sirtuin activity provides the beneficial effects disclosed herein. Sirtuins require NAD⁺. Providing a repair system activator can turn on the Sirtuins. Examples of a repair system activator include NAD⁺, NAD⁺ precursor such as NMN, NR, NaMN, NaAD, NAR, analog of NAD⁺ that promotes NAD⁺ use such as MNM, and cAMP, or any combination thereof. A preferred repair system activator is the NAD⁺ precursor NMN (to make NAD⁺, to turn on, and be consumed by Sirtuins, which provides the benefit from Calorie Restriction). In humans, NAD⁺ typically naturally peaks in the morning and the evening such as at 8 AM and 8 PM, and thus the addition of NAD⁺ or precursor that would turn into NAD⁺ would be added, for example, preferentially in the 7 AM to 8 AM and the 7 PM to 8 PM time frame. In certain aspect, preferably one wants the two daily doses 12 hours apart so as not to disrupt the natural cycle of the biological clock. Typical formulations provide greater or equal to 1.19×10^{-4} moles/kg-of-subject NMN, NAD⁺, or NAD⁺ precursor when administered (NMN is 334.22 grams / mole).

One can also administer, typically through injection, NAD⁺ or use nicotinamide riboside (NR) which can be made into NMN in some cells of the body. Typically administering of NAD⁺ and NR are less preferred because NAD⁺ is not absorbed well through the digestive system and the enzymes that make NMN from NR are not found in every cell of the body. Orally delivered NR has also been shown to largely not reach muscle.

In a specific aspect, disclosed is the administration of NMN (nicotinamide mononucleotide) to humans in preferred dosages of approximately 0.08 grams / kg total body weight per day divided into two equal doses taken approximately 12 hours apart. In certain embodiments, the dosage can be adjusted for absorption. It is preferred to administer the Repair System Activator such as NMN, through water and drinking. A precursor or prodrug of NMN can also be administered, in further examples.

In certain embodiments, repair system activators are administered for reducing inflammation markers related to aging. As used herein, repair system activators are any compound, composition, formulation, molecule, biologic, or substance, which activates sirtuin enzymes. These types of enzymes prefer a redox balance near or at reducing to be optimized. Examples of such molecules that activate Sirtuin are NAD⁺, NAD⁺ precursor

such as NMN, NR, NaMN, NaAD, NAR, analog of NAD⁺ that promotes NAD⁺ use such as MNM, and cAMP.

Compounds and compositions that will activate production of NMN are disclosed. For example, Wang et al., discuss the P7C3 class of aminopropyl carbazole chemicals, compounds, and compositions which act by increasing NAD levels through its NAMPT-mediated salvage. (Wang et al. 2014)

Category 2, Methyl Donors

When adding a methyl donor for methylation, adding betaine is preferred. Betaine can bypass the need (with the use of choline) for extra NAD⁺ if used to make S-5'-adenosyl-L-methionine (SAM). SAM can provide the methyl group for nicotinamide, which has aging properties by stopping Sirtuin enzymes from working. This methylation of nicotinamide occurs via N-methyltransferase (NNMT) N-methylation to 1-methylnicotinamide. This nicotinamide with a methyl group attached provides competition to the available nicotinamide molecules that can get into the Sirtuin enzyme and decrease the Sirtuin enzyme's reactive ability; thus, preventing this process from happening in proportion to the concentration of each of the two competitors. Typically the timing for giving would be with the Repair System Activator, such as NAD⁺ or NAD⁺ precursor.

SAM also provides the methyl groups to reduce the hypo-methylation seen in aging and in the right context it can to be used beneficially to combat aging, example: the need for H3K4me3 methylation (Ulanovskaya OA 2013) of DNA found especially in older people.

Methyl Donors in addition to betaine, which can be used include SAM, methionine, choline, folate, and B12. Typically these alternatives are less preferred because only about 2% of SAM get into the body when ingested (McMillan JM 2005); choline needs extra NAD⁺ to be made into betaine, which is in short supply in the body.

Dosages of betaine (trimethyl glycine) can be at least 0.03 grams/kg (3×10^{-4} mole/kg) of total body weight of the subject (calculated by 0.08 grams (from above NMN calculation) times 0.35 (for molecular weight ratio of betaine / NMN) = 0.03 grams / kg total body weight). This dose can be given over 24 hours, and can be divided into two approximately equal doses taken approximately 12 hours apart. The dose can be dissolved in water and drunk by the subject. The administration can be along with the administration of the category 1 compound or composition.

In certain embodiments, the methylation donors are administered to a subject, and these methylation donors are molecules, substances, compositions, compounds, and formulations, which increase the methylation of molecules or methylate molecules

themselves. Typically methyl donors prefer a Redox balance to be near reduction for optimal activity. S-5'Adenosyl -L- methionine (SAM) precursors include methionine, betaine, choline (a precursor of betaine), folate, Vitamin B12 alone or in combination.

Category- 3 Antioxidant Defense Activators

5 When providing a category 3 compound, composition, or formulation the antioxidant defense is turned on. Having the antioxidant defense enzymes working increases the reduction of the thiol (sulfur) groups in the reactive site of Sirtuin enzymes and others with similar regulation. This prevents the Sirtuin enzymes from turning off due to thiol oxidation.

10 **Hydrogen peroxide (H₂O₂)**

One way to create a generally reducing environment is to "shock" the organism by a pulsed burst of oxidants, such as H₂O₂. To keep the antioxidant enzymes being made and keeping them working one uses pre-conditioning with oxidants to shock on the system, and one keeps them on by additional timed shock pulses of oxidants prior to the antioxidant enzymes turning off due to their feedback loops that turn them off or down when they are not challenged by oxidants. In doing the pulse of oxidants for the preconditioning one uses a sufficient level of oxidants to turn on and keep on the antioxidant enzymes. The preferred choice for an oxidant to do the preconditioning is hydrogen peroxide (H₂O₂) due to its centrality in the redox signaling pathways and its relative stability for an oxidant and its low level of potential harmful effects compared to other oxidants that the cell deals with in its life cycle. H₂O₂ can oxidize thiol groups on proteins / enzymes thereby changing their enzymatic properties.

This pre-conditioning low level oxidation by H₂O₂ can be given in a pulsed, time controlled, and dose controlled fashion to turn on enzymes and processes without providing oxidation in excess of what is needed to turn on enzymes including anti-oxidant defense and repair systems enzymes, because excess oxidation causes cellular damage and harm. Any small molecule (non-enzyme) anti-oxidants should be taken at other time periods (other than the time period of the oxidative pulse) so as not to diminish this temporal effect of the oxidative pulse.

30 ***Hydrogen peroxide (H₂O₂) oxidation and redox signaling***

Hydrogen peroxide (H₂O₂) is a ubiquitous oxidant present in all aerobic organisms (Marino HS 2014). H₂O₂ is now appreciated as a messenger molecule and it provides sensitivity to redox signaling. H₂O₂ provides oxidative modification of amino acid side chains in proteins; in decreasing order of reactivity and biological reversibility, cysteine,

methionine, proline, histidine and tryptophan. Thiol modification is key in **H₂O₂** sensing and perception in proteins. Hydrogen peroxide has been found to mimic insulin activity, elicit arterial pulmonary relaxation, stimulate cell proliferation, and activate NF- κ B and AP-1. The functional consequences of **H₂O₂** signaling concern fundamental biological

5 processes. With recognition of the role of low level oxidants stimuli for altering the set point of gene expression for batteries of enzymes, known as Hormesis (Helmut Sies 2014). Transcriptional factors effected by **H₂O₂** include: AP-1, Nrf2, CREB, HSF1, HIF-1, TPSS, **NF-KB**, NOTCH, SPI, and CREB-1 most involved in regulation of cell damage response, cell proliferation (cell cycle regulation) differentiation and apoptosis (Albrecht SC 2011).

10 Protein acetylation is regulated by **H₂O₂** (Jung S-B 2013). Protein deacetylation is regulated by Sirtuins (Imai, S. 2000). **H₂O₂** increase acetylation and Sirtuins decrease acetylation, so **H₂O₂** and Sirtuins effects are in pushing acetylation reaction pathways in the opposite directions. Sirt1 is very sensitive to **H₂O₂** inhibition of 1 μ M of extracellular **H₂O₂** (Jung S-B 2013). Sirt1 is protected by thiol oxidation from (APE1 / Ref-1). It governs
15 the redox state and activity of Sirt1. It reduces the thiol groups in the active site of Sirt1, **H₂O₂** oxidizes the thiols in Sirt1 's active site. Sirt1 is also regulated by redox-dependent phosphorylation (Caito, S. 2010).

Need for pulsing of signaling oxidants

Low levels of **H₂O₂** increase defenses by preconditioning and thus can ultimately
20 protect against increase of oxidized thiols in Sirtuin's active site and Sirt1' s decrease in activity by an oxidative challenge. Adaptation to **H₂O₂** decrease **H₂O₂** permeability of plasma membranes. Different cell membranes have a full range of permeability to **H₂O₂**. Aquaporins also regulate **H₂O₂** transport across bio-membranes (Marinho HS 2014).

Common drugs that change H₂O₂ levels

25 Metformin, the most widely prescribed antidiabetic drug in the world, increases hydrogen peroxide (**H₂O₂**); this upregulates peroxiredoxin-2 (PRDX-2). Metformin increases lifespan in *C. elegans* and taking away the PRDX-2 gene takes away this effect. PRDX-2 appears to have the role of translating oxidative stress into a downstream pro-longevity signal. Treatment with N-acetylcysteine (NAC) and butylated hydroxyanisole
30 (BHA), which are small molecule anti-oxidants, abolished the positive effect of metformin on lifespan (De Haes W 2014). Pharmaceuticals that increase hydrogen peroxide in the body can also be used for this category either in addition to **H₂O₂** or as a substitute for adding hydrogen peroxide itself. Pharmaceuticals that increase **H₂O₂** in the body include metformin (De Haes W 2014) and acetaminophen (Hinson J 2010).

Pharmaceuticals that increase H₂O₂ in the body need also to be included in the calculation of the oxidative pulse given in category #3. An example is Acetaminophen (the ingredient in Tylenol), which is a pharmaceutical that is known to increase H₂O₂ in the body (Hinson J 2010). N-acetyl-L-cysteine (NAC) is a compound that is known to counter many effects of H₂O₂ in the body.

Timing, duration, and levels of H₂O₂

Enough oxidation to provide pre-conditioning to signal to the turn on the anti-oxidant defense and repair systems is desired; but not enough to create oxidized damage like oxidizing the thiol groups in the Sirtuin active site that turns the enzymes activities off. This level has been referred to as the "Goldilocks zone". The APE-1/ Ref-1 is a molecule that protects the thiol groups of the Sirtuin enzymes, which should remain active. The same or similar process for the nicotinamide-N-methyltransferase (NNMT) enzyme is theorized.

In certain embodiments, one can add pulsed low levels of hydrogen peroxide (H₂O₂) transiently to humans to pre-condition the anti-oxidant defense and repair systems to turn on and stay on. In certain preferred embodiments, approximately 100 μ M concentration of food grade (commercial grade has acetanilide in it as a stabilizer) H₂O₂ in the 400 to 500 mL of water per individual dose is preferred, which can be taken alone or with Category 1 and Category 2 compounds or compositions. 1 mole of H₂O₂ = 1+1+16+16 = approximately 34 grams. 50% of H₂O₂ is estimated to be absorbed by the gut so a more preferred concentration to take is approximately 200 μ M (in the 500 mL). For example, in certain embodiments, one drop of H₂O₂ is 0.05 mL. Food grade H₂O₂ comes in 35% concentrations. Taking 2 drops of 35% H₂O₂ in 500 mL distilled water (with each dose / day), gives approximately 200 μ M. H₂O₂ degrades at about 10% / year if no light and no contaminants in deionized / distilled H₂O. H₂O₂ freezes at -11 °C. So in certain embodiments, taking 4 drops / day or 0.2 mL of 35% H₂O₂ / day in 1 liter of water. 35 grams / 100 mL = 0.07 grams / 0.2 mL. In certain embodiments, a quantity of approximately 0.0008 grams of H₂O₂ /kg total body weight dosages can be used.

A preferred method of administration is to ingest H₂O₂ by dissolving H₂O₂ in deionized / distilled water and drinking. A preferred timing of dosage concentration, time taken and length of time taking is to use the same timing as #1 and #2 when in water. In certain embodiments, if H₂O₂ is partially enhanced from endurance exercise do exercise directly before or after.

Administration of metformin (De Haes W 2014), can come in liquid form, Riomet, as well as tablets. In liquid form 5 mL is equal to a 500 mg tablet. It reaches peak plasma

concentrations in 1 to 3 hours in immediate release form and a steady state in one to two days. It is typically 50 to 60% bioavailable under fasting conditions. One would need to use this data to time and dose appropriately with Metformin.

Hydrogen Sulfide (H₂S)

5 Another way to change the redox potential of oxidation-sensitive protein thiols besides using hydrogen peroxide (H₂O₂) to pre-condition the antioxidant defense system as discussed previously, is by directly augmenting the antioxidant defense system with hydrogen sulfide (H₂S). NaSH (a³⁴S donor) (0.025-0.1 millimolar /liter) treatment dose dependently countered H₂O₂ treatment. Plasma H₂S levels decrease in humans over 50 to 80
10 years of age (Chen Y 2005) and plasma levels of H₂S in patients with cardiovascular disease (CHD) show a significant inverse correlation with severity of CHD and changes in the coronary artery (Jiang H 2005). NaSH decreases ROS and enhances SOD, GPx and GST expression. Lipid and protein oxidation products decrease significantly in plasma samples of healthy volunteers with H₂S rich water (500 mL / day for 2 weeks) (Benedetti S
15 2009). A 0.1 mM NaSH / Liter can increase Sirt1 in a time dependent manner (Wu D 2015). Exogenous H₂S has a protective effect on maintaining the circadian rhythm of clock genes by changing the NAD⁺/NADH ratio and enhancing the Sirt1 protein (Shang Z 2012). H₂S is also an important endogenous inhibitor of key elements of acute inflammatory reactions (Zanardo R 2006) by down regulating NF-κB or upregulating heme oxygenase 1 expression
20 (Jin H 2008, Kim K 2008, Oh G 2006, Pan L 2011). H₂S can activate ATP-sensitive, intermediate-conductance and small-conductance potassium channels through cysteine S-sulfhydration (Mustafa A 2011, Yang G 2008) causing endothelial and smooth muscle cell hyperpolarization which in turn causes vasorelaxation of vascular endothelium and lowering of blood pressure. H₂S has a direct inhibitory effect on angiotensin-converting enzyme
25 (ACE) activity (Laggner H 2007). NaSH increases the expression of eNOS and PGC-1α (Wu D 2015), which both play a role in mitochondria biogenesis and function (Wu, CC 2013, Lagouge M 2006). H₂S upregulates the MAPK pathway (Barr LA 2014, Papapetropoulos A 2009, Yong QC 2008). It has been inferred that calorie restriction may help maintain H₂S signaling (Predmore B 2010). GYY4237 a slow releasing H₂S donor can
30 kill seven different human cancer cell lines in a concentration-dependent manner (Lee Z 2011). Sulforaphane, also a³⁴S donor, has dose-dependent antiprostata cancer (PC-3) properties (Pei Y 2011).

H₂S is a gasotransmitter. Gasotransmitters are endogenously produced at low levels and are able to freely diffuse through cell membranes to invoke cellular signaling (Calvert

JW 2010). The three gasotransmitters are nitric oxide (NO), carbon monoxide (CO), and hydrogen sulfide (H₂S).

Hydrogen sulfide is synthesized from L-cysteine. Cystathionine gamma-lyase (CSE), cystathionine beta-synthase (CBS), cysteine aminotransferase (CAT), and 3-mercaptopyruvate sulfurtransferase (MST) are endogenous enzymatic sources of hydrogen sulfide (H₂S). Liver production of H₂S to different extents has been shown by these enzymes and showed H₂S regulates lipid peroxidation and antioxidant enzyme (GPx, T-SOD, Cu/Zn-SOD, and Mn-SOD) activities in the liver, by administration of H₂S donor NaSH to the mice by injection of 0.05 mM of NaSH / kg body weight /day dissolved in 10 mL / kg body weight saline (Wu D 2015). Mitochondria are able to use H₂S under hypoxia and stress conditions to produce ATP (Fu M 2012).

Initial reports of H₂S's antioxidant ability were that H₂S can scavenge superoxide (Geng B 2004) and H₂S can upregulate glutathione (Kimura Y 2004). Later came more detailed reports of its activation of antioxidant enzymes. H₂S has been shown to activate Nuclear factor-erythroid 2-related factor 2 (Nrf2) (Peake BF 2013), which turns on antioxidant genes. Daily administration of Na₂S for 7 days increased Nrf2 expression in both cytosolic and nuclear fractions (Calvert JW 2010). Nrf2, which up regulates expression of antioxidant response element-regulated genes, is upregulated by H₂S (Islam KN 2015). H₂S activation causes Nrf2 to separate itself from its adherent inhibitor, Kelch-like ECH-associated protein 1 in the cytosol (Wakabayashi N 2004) then translocate to the nucleus and bind to a specific enhancer sequence, known as the antioxidant responsive element, in the promoter region of antioxidant genes, including HO-1 and thioredoxin 1 (Calvert JW 2009). H₂S exhibits effects on mitochondria function (Helmy N 2014, Wang CN 2014) antioxidant stress (Bos EM 2013, Du JT 2013) apoptosis (Yao LL 2010), inflammation (Lo Faro ML 2014) angiogenesis (Szabo C 2013, Coletta C 2012, Wang MJ 2010), sepsis and shock (Kida, F. 2015) and blood pressure (Polhemus DJ 2014, Ge SN 2014, Yang G 2008).

H₂S protects against NO³⁻, as does glutathione. H₂S also significantly reduces the toxic effects of HOC1. H₂S enhances the anti-oxidant effects of N-acetyl-l-cysteine (NAC).

H₂S's therapeutic effects have been most studied to date in regards to heart disease. H₂S effects on heart disease include: macrophages are able to produce H₂S endogenously (Zhu XY 2010). NaHS (a H₂S donor) inhibited pro-atherogenic oxidized low-density lipoproteins induced foam cell formation in macrophages (Wang Y 2009). H₂S is able to down regulate ROS at the mitochondria, providing protection through reduced respiration (Chen Q 2006). H₂S production (10-100 nM) enhanced mitochondrial electron transport and

cellular bioenergetics (Modis K 2013) however at high concentrations H₂S is toxic (Hill BC 1984, Nicholls P 1982). H₂S in the diet decreased adverse left ventricle (LV) remodeling during heart failure (Kondo K 2013). H₂S can upregulate endothelial nitric oxide synthase which makes NO (Kondo K 2013) and NO can upregulate the H₂S synthesis enzyme CSE (Zhao, W. 2001). Mice treated with a H₂S donor significantly increase phosphorylation effecting eNOS suggesting active cross talk between H₂S and NO (Kondo K 2013). There also appears to be cross talk between CO and H₂S (Zhange QY 2004, Majid AS 2013). H₂S induces vasodilation, leading to reduced blood pressure (Cheng Y 2004). H₂S in the form of Na₂S (10 minutes prior) prevents reperfusion injury (SodhaNR 2008). Exogenous H₂S also led to improved renal function (Xu Z 2009).

H₂S under in vivo conditions has an extremely short half-life which is estimated to be between seconds and minutes (Wang R 2002, Insko MA 2009). Plasma concentrations of H₂S is in the range of 0.034 to 0.065 mM (Whiteman M 2009), in the brain it is three fold higher than the plasma (Hogg P 2009, Zhao W 2001). H₂S concentration are inversely related to O₂ concentration and H₂S decrease cellular O₂ consumption (Olson K 2015). H₂S concentrations of between 0.030 and 0.300 have also been reported in the blood and plasma (Olson K 2009). H₂S donors NaHS and Na₂S increase H₂S concentration within seconds to minutes.

The physiological range of H₂S is widely variable from 0.005 to 0.300 mM (Predmore BL 2012). Endogenous levels of H₂S in the brains of humans have been detected at from 0.05 to 0.16 mM (Whiteman M 2004); in the brains of Alzheimer's patients, the H₂S concentration is lower (Seshadri S 2002, Tang X 2010). Diallyl trisulfide (DATS) is a stable H₂S donor and shows effects 30 minutes after injection and is longer lasting. NaHS can be taken in drinking water (Givvimani S 2011). NaHS (H₂S donor), in aqueous solution releases H₂S, in drinking water for 6 weeks. There was an increase in plasma H₂S concentration with exogenous supplementation (Peake BF 2013, Kondo K 2013). There was no difference in the consumption of water among the groups of mice treated with NaHS and untreated groups. Other H₂S donors include GYY 4137 (CAS# 106740-09-4) a water soluble H₂S donor that slowly releases H₂S over the course of hours (Li L 2008) and SG 1002 from Sulfagenix, Inc. AP97, AP39, AP67, and API 05 are also H₂S donors with slower release (Whiteman M 2015, Wallace J 2015, Hancock J 2014). H₂S can be ingested with foods containing organosulfides, who's polysulfides can be H₂S donors.

In addition to ingesting H₂S dissolved in water, H₂S can be inhaled and inhalation increases blood H₂S levels (40 ppm for 8 hours for 7 days was used with mice). Inhalation

can also be combined with ingestible H₂S donors such as Na₂S and NaHS (Kida K 2011 and 2015). Measurement of H₂S in blood and tissue has been done with a sensitive and reliable means (Wintner E 2010).

5 H₂S can also be stored in cells in the form of sulfane sulfur and transported and released in response to physiological stimulus (Ishigami M. 2009).

NRF2 Activators

The transcription factor NF-E2 p45-related factor 2 (Nrf2: gene name NFE212) regulates the expression of networks of genes encoding proteins with diverse cytoprotective activities. Nrf2 itself is controlled primarily at the level of protein stability. Nrf2 is a short
10 lived protein subjected to continuous ubiquitination and protease degradation. There are three known ubiquitin ligase systems that contribute to the degradation of Nrf2 a) Keap-1, a substrate adaptor protein for Cullin-3, b) glycogen synthase kinase, and c) E3 ubiquitin ligase Hrdl. Keap-1 is also a sensor for a wide array of small-molecule activators also called inducers. When Nrf2 is not degraded and is translocated to the nucleus it forms a
15 heterodimer with a small Maf protein, binds to antioxidant-response elements which are the upstream regulatory regions of its target genes and initiates transcription. Nrf2 is a master regulator of cellular redox homeostasis. (Dinkova-Kostova AT 2015). Over 50 genes are regulated by Nrf2 in humans (Pall ML 2015, Choi B-H 2014). In a direct effect of inflammation genes, without a Redox mechanism, Nrf2 also binds to the upstream region of
20 the IL6 gene and when bound can significantly disrupt the recruitment of RNA polymerase II to regulate the transcription of IL6 in human macrophage cells.

Nrf2 signaling is regulated by transcriptional, translational, posttranslational, and epigenetic mechanisms as well as by other protein partners including p62, p21 and IQ motif-containing GTPase activating protein 1 (Huand Y 2015). Nuclear factor erythroid 2
25 (Nrf2) activators include classes of activators with activities that: induce nuclear translocation of Nrf2, increase Nrf2 mRNA transcription, increase protein expression of Nrf2 and increase Nrf2 downstream target genes. There are also Nrf2 inhibitors (Bach 1, caveolae, TGF-beta) (Gegotek A 2015). The Keap1-Nrf2 pathway acts in concert with autophagy to combat proteotoxicity (Dodson M 2015).

30 Keap-1 is a zinc metalloprotein that is localized near the plasma membrane. It has three functional domains, at least 25 reactive thiols most of which are found in the intervening linker region. Keap-1 has an Nrf2 binding site on each dimer subunit forming a "latch and hinge." Keap-1 is highly sensitive to oxidation and its different thiol groups have

different redox potentials. These different cysteine residues create a sensor system (Suzuki T 2013).

Nrf2 is a 605 amino acid transcription factor composed of six domains. The N-terminal Neh2 domain is the binding site for the inhibitory protein Keap-1. The half-life of Nrf2 when separated from Keap-1 is 20 minutes (Kasper JW 2011). Keap-1 is exported out of the nucleus in 0.5 hours. Nrf2 activations enhances Sirt1 activity in mice fibroblasts cell culture (Jodar L 2010).

When Nrf2 releases Keap-1 it is available to capture IKKBeta thus inhibiting **NF- κ B** target genes. This interaction correlates the expression of antioxidant enzymes by Nrf2 and the turning on and off of the immune system by NF- κ B. Nrf2 and NF- κ B compete for CREB-binding protein (CBP) (Liu GH 2008). There are many phytochemicals that have Nrf2 activation abilities by interacting with Keap-1 in different ways. Immediate alkylators are fast activating. "Michael acceptors", which are acetylene compounds conjugated to an electron-withdrawing group, form reversible alkylating reactions with Keap-1 sensor thiols.

Phenolics that appear to act most directly on Nrf2 are ortho- or para-dihydroxyphenols which can be oxidized to quinones (Kumar H 2014). Quinones are oxidized derivatives of aromatic compounds and are often readily made from reactive aromatic compounds with electron-donating substituents such as phenols and catechols, which increase the nucleophilicity of the ring and contributes to the large redox potential needed to break aromaticity. Quinones are conjugated but not aromatic. Quinones are electrophilic Michael acceptors stabilized by conjugation. Depending on the quinone and the site of reduction, reduction can either re-aromatize the compound or break the conjugation. Conjugate addition nearly always breaks the conjugation.

H₂O₂ and H₂S are Nrf2 activators (listed separately above). Everything mentioned that is a Nrf2 activator, is also an antioxidant defense system activator although some things activated by Nrf2 may be seen as additional to antioxidant defense system activation. The activation comes from the multiple ways listed above of keeping the Nrf2 system on. One form of regulation of Nrf2 is reversible phosphorylation. Sirt1 and PARP1 as discussed before can also be reversibly phosphorylated.

Nrf2 activation and the turning on of the antioxidant defense system needs to be correlated in timing to NAD⁺ availability and methylation availability and be synced with the biological clock NAD⁺ peaks of the person. The Nrf2 system does need to turn off (example: around 2 pm when NAD⁺ concentrations normally are at their daily biological

clock low) so one's body can do the things it needs to do under a redox balance when that leans towards oxidation.

Category 3 compounds

Antioxidant defense activators such as Nuclear factor erythroid 2 (Nrf2) activators
 5 (including activities such as: nuclear translocation of Nrf2, increasing Nrf2 mRNA transcription, increasing protein expression of Nrf2 and increasing Nrf2 downstream target genes), **H2O2**, ROS, RNS, RCS, RSOH, **O2¹**, **O2**, **H2S**, **O3**, **HOC1**, HOBr, HOI, ROOH, where R is alkyl, cycloalkyl, heteralkyl, heterocycloalkyl, alkenyl, heteroalkenyl, cycloalkenyl, or heterocycloalkenyl, **H2O2** generator, such as metformin or acetaminophen,
 10 ortho hydroxyphenols which can be oxidized to quinones (Kumar H 2014), para dihydroxyphenols which can be oxidized to quinones (Kumar H 2014), quinones (are oxidized derivatives of aromatic compounds), hydrogen sulfide (**H2S**), **H2S** Donor (such as), sodium hydrosulfide (NaHS), sodium sulfide (Na₂S), diallyl trisulfide (DATS), GYY4137 (a water soluble **H2S** donor (patent # WO2014018569 A1) (Li L 2008)), SG-1002 (a **H2S**
 15 synthetic donor from SulfaGENEX) (Kondo K 2013), penicillamine-based **H2S** donors (Zhao Y 2013), polyorganosulfides (Tocmo R 2015), 2-mercaptoethanol, dithiothreitol, isothiocyanates, sulforaphane (in broccoli) (Nallasamy P 2014), glucoraphanin (broccoli) (Armah CN 2013), curcumin (in turmeric) (Pae H-0 2007, He HJ 2012, Balogun E 2003, Goel A 2007, Pyrrolidone (water soluble), Theracumin (nanoparticle), Zerumbone
 20 (Stefanson AL 2014), Cinnamate analogs that have thioketone-conjugated-Alpha-Beta-unsaturated moiety (Kumar S 2013) like, cinamic aldehyde, quercetin (in onions, apples, tea) (Magesh S 2012, Kimura S 2009), isoquercetin (2 to 6 fold better absorption), kaempferol (Kang BY 2008), ginseng (Panax ginseng and Panax quinquefolius), carnosic acid, xanthohumol, Dh404, (R)-alpha-lipoic acid (Flier J 2002, Suh JH 2004, Cao Z 2003),
 25 Isothiocyanate, benzyl isothiocyanate (Sahu RP 2009), Neoglucobrassicin (Stefanson AL 2014), Glucosinolates (Stefanson AL 2014), Hydrophilic oxidized derivatives of Lycopene (Stefanson AL 2014), (HNE) 4-hydroxynonenal (Forman HJ 2008), (15-dPGJ2) 15-deoxydelta prostaglandin J2 (Mochizuki M 2005), Falcariindiol (Stefanson AL 2014), Hydroxytyrosol (Stefanson AL 2014), Barley beta-glucan, Spermidine (Kwak MK2003),
 30 Spermine (Kwak MK 2003), luteolin (Paredes-Gonzalez X 2015), 4-methylalkylcatechol, 4-vinylcatechol, 4-ethylcatechol, pyrroloquinoline quinone (Zhang Q 2012, Liang C 2015), Mangafodipir trisodium (MnDPDP) (a contrast agent currently used in magnetic resonance imaging) (Mosbah IB 2012), N-Acetylcysteine (Wallace J 2015), ATB-346 from Antibe Therapeutics (Wallace J 2015), NBS-1120 from City College of New York (Wallace J

2015), GIC-101 from GI care Pharma (Wallace J 2015), AP39 patent number
 WO201 304595 1A1 University of Exeter, Alos AP67, AP 97 and AP105,
 WO2014018569A1, Sialor (Wallace J 2015), Sulfarlem (Wallace J 2015), and Anethole
 trithione (Wallace J 2015), DHEA (Jeon S 2015), coal tar (Van den Bogaard EH 2013),
 5 garlic (via H₂S), β -lapachone (from tree bark of a South American tree: it produces
 oxidation by cycling cellular NADH into NAD⁺), pterostilbene (McCormack D 2013,
 resveratrol (Cheng L 2015, Mokni M 2007, Kitada M 2011, apigenin (in parsley) (Paredes-
 Gonzalez X 2015 and 2014, Escande C 2013), zinc (Wang F 2015, Sternberg P 2007,
 Magesh S 2012) and optionally, a carrier.

10 *Specific Compositions*

In specific examples, the disclosed nutritional composition can comprise
 nicotinamide adenine dinucleotide (NAD⁺), Betaine, and H₂O₂. In specific examples, the
 disclosed composition can comprise nicotinamide adenine dinucleotide (NAD⁺), folate +
 Vitamin B12, and H₂O₂. In specific examples, the disclosed nutritional composition can
 15 comprise nicotinamide adenine dinucleotide (NAD⁺), Methionine, and H₂O₂. In other
 examples, the disclosed composition can comprise nicotinamide riboside (NR), Methionine,
 and H₂O₂. In specific examples, the disclosed composition can comprise nicotinamide
 adenine dinucleotide (NAD⁺), Choline, and H₂O₂.

In specific examples, the disclosed composition can comprise nicotinamide adenine
 20 dinucleotide (NAD⁺), Betaine, and NaHS. In specific examples, the disclosed composition
 can comprise nicotinamide adenine dinucleotide (NAD⁺), Folate + Vitamin B12, and
 NaHS. In specific examples, the disclosed composition can comprise nicotinamide adenine
 dinucleotide (NAD⁺), Methionine, and NaHS. In specific examples, the disclosed
 composition can comprise nicotinamide adenine dinucleotide (NAD⁺), Choline, and NaHS.

25 In specific examples, the disclosed composition can comprise nicotinamide adenine
 dinucleotide (NAD⁺), Betaine, and Na₂S. In specific examples, the disclosed composition
 can comprise nicotinamide adenine dinucleotide (NAD⁺), Folate + Vitamin B12, and Na₂S.
 In specific examples, the disclosed composition can comprise nicotinamide adenine
 dinucleotide (NAD⁺), Methionine, and Na₂S. In specific examples, the disclosed
 30 composition can comprise nicotinamide adenine dinucleotide (NAD⁺), Choline, and Na₂S.

In specific examples, the disclosed composition can comprise nicotinamide adenine
 dinucleotide (NAD⁺), Betaine, and any one or more of H₂S, O₃, metformin, acetaminophen,
 sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic
 acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -

lapachone, pterostilbene, resveratrol, apigenin, and zinc. In specific examples, the disclosed composition can comprise nicotinamide adenine dinucleotide (NAD⁺), Folate + Vitamin B12, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid,

5 Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β-lapachone, pterostilbene, resveratrol, apigenin, and zinc. In specific examples, the disclosed composition can comprise nicotinamide adenine dinucleotide (NAD⁺), Methionine, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of

10 Lycopene, N-Acetylcysteine, DHEA, garlic, β-lapachone, pterostilbene, resveratrol, apigenin, and zinc. In specific examples, the disclosed composition can comprise nicotinamide adenine dinucleotide (NAD⁺), Choline, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-

15 Acetylcysteine, DHEA, garlic, β-lapachone, pterostilbene, resveratrol, apigenin, and zinc.

In specific examples, the disclosed nutritional composition can comprise nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, Betaine, and H₂O₂. In other examples, the disclosed composition can comprise nicotinamide riboside (NR), Betaine, and H₂O₂. In other examples, the disclosed composition can comprise one

20 or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), with Betaine, and H₂O₂. In other examples, the disclosed composition can comprise 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Betaine, and H₂O₂.

In specific examples, the disclosed composition can comprise nicotinamide

25 mononucleotide (NMN) or a precursor or prodrug of NMN, folate + Vitamin B12, and H₂O₂. In other examples, the disclosed composition can comprise nicotinamide riboside (NR), folate + Vitamin B12, and H₂O₂. In other examples, the disclosed composition can comprise one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), folate + Vitamin B12, and

30 H₂O₂. In other examples, the disclosed composition can comprise 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), folate + Vitamin B12, and H₂O₂.

In specific examples, the disclosed composition can comprise nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, Betaine + Vitamin B12, and H₂O₂. In other examples, the disclosed composition can comprise nicotinamide riboside

(NR), Betaine + Vitamin B12, and H₂O₂. In other examples, the disclosed composition can comprise one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), Betaine + Vitamin B12, and H₂O₂. In other examples, the disclosed composition can comprise 1-

5 methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Betaine + Vitamin B12, and H₂O₂.

In specific examples, the disclosed nutritional composition can comprise nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, Methionine, and H₂O₂. In other examples, the disclosed composition can comprise nicotinamide riboside
10 (NR), Methionine, and H₂O₂. In other examples, the disclosed composition can comprise one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), with Methionine, and H₂O₂. In other examples, the disclosed composition can comprise 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Methionine, and H₂O₂.

15 In specific examples, the disclosed composition can comprise nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, Choline, and H₂O₂. In other examples, the disclosed composition can comprise nicotinamide riboside (NR), Choline, and H₂O₂. In other examples, the disclosed composition can comprise one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide
20 (NaAD), and nicotinic acid riboside (NAR), Choline, and H₂O₂. In other examples, the disclosed composition can comprise 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Choline, and H₂O₂.

In specific examples, the disclosed composition can comprise nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, S-Adenosyl-methionine
25 (SAM), and H₂O₂. In other examples, the disclosed composition can comprise nicotinamide riboside (NR), S-Adenosyl-methionine (SAM), and H₂O₂. In other examples, the disclosed composition can comprise one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), S-Adenosyl-methionine (SAM), and H₂O₂. In other examples, the disclosed composition can
30 comprise 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), S-Adenosyl-methionine (SAM), and H₂O₂.

In specific examples, the disclosed composition can comprise nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, Betaine, and NaHS. In other examples, the disclosed composition can comprise nicotinamide riboside (NR), Betaine, and

NaHS. In other examples, the disclosed composition can comprise one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), Betaine, and NaHS. In other examples, the disclosed composition can comprise 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Betaine, and NaHS.

In specific examples, the disclosed composition can comprise nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, Folate + Vitamin B12, and NaHS. In other examples, the disclosed composition can comprise nicotinamide riboside (NR), Folate + Vitamin B12, and NaHS. In other examples, the disclosed composition can comprise one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), Folate + Vitamin B12, and NaHS. In other examples, the disclosed composition can comprise 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Folate + Vitamin B12, and NaHS.

In specific examples, the disclosed composition can comprise nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, Betaine + Vitamin B12, and NaHS. In other examples, the disclosed composition can comprise nicotinamide riboside (NR), Betaine + Vitamin B12, and NaHS. In other examples, the disclosed composition can comprise one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), Betaine + Vitamin B12, and NaHS. In other examples, the disclosed composition can comprise 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Betaine + Vitamin B12, and NaHS.

In specific examples, the disclosed composition can comprise nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, Methionine, and NaHS. In other examples, the disclosed composition can comprise nicotinamide riboside (NR), Methionine, and NaHS. In other examples, the disclosed composition can comprise one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), Methionine, and NaHS. In other examples, the disclosed composition can comprise 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Methionine, and NaHS.

In specific examples, the disclosed composition can comprise nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, Choline, and NaHS. In other examples, the disclosed composition can comprise nicotinamide riboside (NR), Choline,

and NaHS. In other examples, the disclosed composition can comprise one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), Choline, and NaHS. In other examples, the disclosed composition can comprise 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Choline, and NaHS.

In specific examples, the disclosed composition can comprise nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, S-Adenosyl-methionine (SAM), and NaHS. In other examples, the disclosed composition can comprise nicotinamide riboside (NR), S-Adenosyl-methionine (SAM), and NaHS. In other examples, the disclosed composition can comprise one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), S-Adenosyl-methionine (SAM), and NaHS. In other examples, the disclosed composition can comprise 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), S-Adenosyl-methionine (SAM), and NaHS.

In specific examples, the disclosed composition can comprise nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, Betaine, and Na2S. In other examples, the disclosed composition can comprise nicotinamide riboside (NR), Betaine, and Na2S. In other examples, the disclosed composition can comprise one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), Betaine, and Na2S. In other examples, the disclosed composition can comprise 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Betaine, and Na2S.

In specific examples, the disclosed composition can comprise nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, Folate + Vitamin B12, and Na2S. In other examples, the disclosed composition can comprise nicotinamide riboside (NR), Folate + Vitamin B12, and Na2S. In other examples, the disclosed composition can comprise one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), Folate + Vitamin B12, and Na2S. In other examples, the disclosed composition can comprise 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Folate + Vitamin B12, and Na2S.

In specific examples, the disclosed composition can comprise nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, Betaine + Vitamin B12, and Na2S. In other examples, the disclosed composition can comprise nicotinamide riboside

(NR), Betaine + Vitamin B12, and Na2S. In other examples, the disclosed composition can comprise one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), Betaine + Vitamin B12, and Na2S. In other examples, the disclosed composition can comprise 1-

5 methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Betaine + Vitamin B12, and Na2S.

In specific examples, the disclosed composition can comprise nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, Methionine, and Na2S. In other examples, the disclosed composition can comprise nicotinamide riboside (NR), Methionine, and Na2S. In other examples, the disclosed composition can comprise one or more of
10 nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), Methionine, and Na2S. In other examples, the disclosed composition can comprise 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Methionine, and Na2S.

15 In specific examples, the disclosed composition can comprise nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, Choline, and Na2S. In other examples, the disclosed composition can comprise nicotinamide riboside (NR), Choline, and Na2S. In other examples, the disclosed composition can comprise one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide
20 (NaAD), and nicotinic acid riboside (NAR), Choline, and Na2S. In other examples, the disclosed composition can comprise 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Choline, and Na2S.

In specific examples, the disclosed composition can comprise nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, S-Adenosyl-methionine
25 (SAM), and Na2S. In other examples, the disclosed composition can comprise nicotinamide riboside (NR), S-Adenosyl-methionine (SAM), and Na2S. In other examples, the disclosed composition can comprise one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), S-Adenosyl-methionine (SAM), and Na2S. In other examples, the disclosed composition can
30 comprise 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), S-Adenosyl-methionine (SAM), and Na2S.

In specific examples, the disclosed composition can comprise nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, Betaine, and any one or more of H2S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin,

isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc. In other examples, the disclosed composition can comprise nicotinamide riboside (NR), Betaine, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc. In other examples, the disclosed composition can comprise one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), Betaine, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc. In other examples, the disclosed composition can comprise 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Betaine, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc.

In specific examples, the disclosed composition can comprise nicotinamide mononucleotide (NMN), Folate + Vitamin B12, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc. In other examples, the disclosed composition can comprise nicotinamide riboside (NR), Folate + Vitamin B12, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc. In other examples, the disclosed composition can comprise one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), Folate + Vitamin B12, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc. In other examples, the disclosed

composition can comprise 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Folate + Vitamin B12, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β-lapachone, pterostilbene, resveratrol, apigenin, and zinc.

In specific examples, the disclosed composition can comprise nicotinamide mononucleotide (NMN), Betaine + Vitamin B12, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β-lapachone, pterostilbene, resveratrol, apigenin, and zinc.

In other examples, the disclosed composition can comprise nicotinamide riboside (NR), Betaine + Vitamin B12, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β-lapachone, pterostilbene, resveratrol, apigenin, and zinc.

In other examples, the disclosed composition can comprise one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), Betaine + Vitamin B12, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β-lapachone, pterostilbene, resveratrol, apigenin, and zinc.

In other examples, the disclosed composition can comprise 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Betaine + Vitamin B12, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β-lapachone, pterostilbene, resveratrol, apigenin, and zinc.

In specific examples, the disclosed composition can comprise nicotinamide mononucleotide (NMN), Methionine, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β-lapachone, pterostilbene, resveratrol, apigenin, and zinc.

In other examples, the disclosed composition can comprise nicotinamide riboside (NR), Methionine, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized

derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc. In other examples, the disclosed composition can comprise one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), Methionine, and any one or more
 5 of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc. In other examples, the disclosed composition can comprise 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Methionine, and any one or more of H₂S,
 10 O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc.

In specific examples, the disclosed composition can comprise nicotinamide mononucleotide (NMN), Choline, and any one or more of H₂S, O₃, metformin,
 15 acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc. In other examples, the disclosed composition can comprise nicotinamide riboside (NR), Choline, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin,
 20 curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc. In other examples, the disclosed composition can comprise one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), Choline, and any one or more of
 25 H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc. In other examples, the disclosed composition can comprise 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Choline, and any one or more of H₂S, O₃,
 30 metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc.

In specific examples, the disclosed composition can comprise nicotinamide mononucleotide (NMN), S-Adenosyl-methionine (SAM), and any one or more of H₂S, O₃,

metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc. In other examples, the disclosed composition can comprise nicotinamide riboside (NR), S-Adenosyl-methionine (SAM), and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc. In other examples, the disclosed composition can comprise one or more of nicotinic acid adenine mononucleotide (NaMN),

10 nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), S-Adenosyl-methionine (SAM), and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc. In other examples, the disclosed

15 composition can comprise 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), S-Adenosyl-methionine (SAM), and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc.

20 In the disclosed compositions, the combined amount of compounds of category 1, 2, and 3 in the composition can be at least 5 wt.% of the composition. For example, the repair system activator, the methyl donor, and the antioxidant defense activator can be at least 5 wt.% of the composition. In other example, the combined amount of compounds of category 1, 2, and 3 in the composition can be at least 10, at least 15, at least 20, at least 25,

25 at least 30, at least 35, at least 40, at least 45, at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, or 100 wt.% of the composition, where any of the stated values can form an upper or lower endpoint of a range.

Delivery system for ingredients of category 1, 2, and 3

Formulations, which can be packaged in a powder or lyophilized form, which can

30 then have either hot or cold liquid added to them for reconstituting into a solution are disclosed. For example, the disclosed compositions could be mixed with compositions, such as is done in personal beverage systems, which make hot or cold coffee or tea or hot chocolate from individually packaged components and the addition of water. The disclosed compositions can be administered in vivo either alone or in a pharmaceutically acceptable

carrier. By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material can be administered to a subject, along with the composition disclosed herein, without causing any undesirable biological effects. The carrier would naturally be selected to minimize any degradation of the active ingredient and to minimize any adverse side effects in the subject, as would be well known to one of skill in the art. The materials can be in solution, suspension (for example, incorporated into microparticles, liposomes, or cells).

Microbiome interaction with delivery via digestive tract or skin

The mammalian intestinal microbiota is composed of up to 100 trillion microbes from over 500 genera of bacteria from two main phyla, namely *Bacteroidetes* and *Firmicutes*. A well-studied mammalian probiotic *Lactobacillus rhamnosus* GG is a potent inducer of ROS (Jones R 2014). Redox signaling mediates symbiosis between the gut microbiota and the intestine. In flies, increase in life span is correlated to increase formation of the oxidant H₂O₂ in the gut. H₂S protects the mucosal lining of the gastrointestinal tract against oxidative stress as well as regulates various functions including fluid transport, inflammation, acid induced HCO₃⁻ secretion (Yonezawa D 2007, Ise F 2011, Wallace J 2009+2010, Fiorucci S 2006, Kasperek M 2008, Takeuchi K 2011+2015). Gut microbiota composition in the elderly has been correlated to plasma IL-6 levels (Claesson MJ 2012).

A fasting molecule Crtc enhances immunity by making the gut barrier less permeable to bacteria. Gut bacteria that get across the gut barrier cause inflammation. This Crtc is a genetic switch in the brain that controls energy balance. This constant communication between the brain and the GI tract allows the body to keep track of energy expenditures and stores. Crtc interacts with CREB (cAMP response element-binding protein). A partner of Crtc in the human brain is neuropeptide Y, which causes mammals to search for food. CREB activity is regulated by energy sensing Sirt1 and its ability to deacetylate CREB (Paz JC 2014). This links the level of NAD⁺ and the feeling of hunger. The glucose-regulated antagonism between (yet coordinated with) CREB and Sirt1 for Hes-1 transcription participates in the metabolic regulation of neurogenesis, this is important since a decline in neurogenesis accompanies brain aging (Bondolfi L 2004) and CREB transcription factor is activated by nutrient deprivation which is correlated to Sirtuin enzyme activity.

TNF in the circulation of humans that occurs as part of the aging process impairs inflammatory monocyte development function and is detrimental to anti-pneumococcal immunity. This is reversed with pharmacological reduction of TNF.

The formulation could have organisms such as bacteria in the microbiome extrude any or all of these three categories of compounds that are desired and add them directly into the gut. These organisms could extrude the desired compounds in the quantity and with the timing desired. These organisms could be introduced to the microbiome either from a
5 selection of organisms that naturally occur in the microbiome or by the engineering of organisms that naturally occurs in the microbiome. The engineered organisms could be engineered to extrude these compounds in accordance to the introduced organism's and or the host's biological clock. The introduced organism could be engineered to extrude the desired amount of compound or compounds. Gene-drive could be used to switch all of the
10 species in the gut of this type used to the introduced organism's gene type desired. A kill switch could be engineered into this introduced species as well to allow an elimination of these engineered species if they were not desired at a later date.

Pharmaceutically Acceptable Carriers

The compositions disclosed herein can be used therapeutically in combination with a
15 pharmaceutically acceptable carrier.

Suitable carriers and their formulations are described in Remington: The Science and Practice of Pharmacy (22nd ed.) ed. L.V. Loyd Jr., CBS Publishers & Distributors Grandville MI USA 2012. Typically, an appropriate amount of a pharmaceutically - acceptable salt is used in the formulation to render the formulation isotonic. Examples of
20 the pharmaceutically-acceptable carrier include, but are not limited to, saline, Ringer's solution and dextrose solution. The pH of the solution is preferably from about 5 to about 8, and more preferably from about 7 to about 7.5. Further carriers include sustained release preparations such as semipermeable matrices of solid hydrophobic polymers, which matrices are in the form of shaped articles, e.g., films, liposomes or microparticles. It will
25 be apparent to those persons skilled in the art that certain carriers can be more preferable depending upon, for instance, the route of administration and concentration of composition being administered.

Pharmaceutical carriers are known to those skilled in the art. These most typically would be standard carriers for administration of drugs to humans, including solutions such
30 as sterile water, saline, and buffered solutions at physiological pH. The compositions can be administered intramuscularly or subcutaneously. Other compounds will be administered according to standard procedures used by those skilled in the art.

Pharmaceutical compositions can include carriers, thickeners, diluents, buffers, preservatives, surface active agents and the like in addition to the molecule of choice.

Pharmaceutical compositions can also include one or more active ingredients such as antimicrobial agents, anti-inflammatory agents, anesthetics, and the like.

The pharmaceutical composition can be administered in a number of ways depending on whether local or systemic treatment is desired, and on the area to be treated.

5 Administration can be topically (including ophthalmically, vaginally, rectally, intranasally), orally, by inhalation, or parenterally, for example by intravenous drip, subcutaneous, intraperitoneal or intramuscular injection. The disclosed compounds can be administered intravenously, intraperitoneally, intramuscularly, subcutaneously, intracavity, or transdermally.

10 Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium
15 chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives can also be present such as, for example, antimicrobials, chelating agents, and inert gases and the like.

20 Formulations for topical administration can include ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable.

Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets, or tablets. Thickeners,
25 flavorings, diluents, emulsifiers, dispersing aids or binders may be desirable.

Some of the compositions can be administered as a pharmaceutically acceptable acid- or base- addition salt, formed by reaction with inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, and phosphoric acid, and organic acids such as formic acid, acetic acid, propionic acid, glycolic
30 acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, and fumaric acid, or by reaction with an inorganic base such as sodium hydroxide, ammonium hydroxide, potassium hydroxide, and organic bases such as mono-, di-, trialkyl and aryl amines and substituted ethanolamines.

The various compounds and compositions of categories 1, 2, and 3, can be taken at the same time or in proximity, such as within 1, 5, 10, 30, 60, 90, or 120 minutes.

Dosages of each item or items from category 1, 2, and 3 that is sufficient but not in excess (described in molar terms to body weight) and the ingredients are such that the
5 interrelationship of these doses is balanced.

A delivery system in water is preferable if the preferred ingredient of category 1, 2 and 3 are used. This will help elicit the correct timing (all 3 preferred ingredients are easily absorbed and soluble in water). For some other less preferred ingredients, which are not as water soluble or are not as easily absorbed their delivery would result in a reduced benefit
10 with respect to the pulse timing of these three categories of ingredients.

Disclosed are methods of reducing inflammation in a subject comprising administering to the subject compounds, compositions, or formulations, and optionally, a carrier as described herein.

Also disclosed are methods, wherein the first compound, the second compound, and
15 the third compound are administered at approximately the same time.

Also disclosed are methods, wherein the first compound is administered within 15, 30, 60, 90, or 120 minutes of the subject's biological clock NAD⁺ peak.

Also disclosed are methods, wherein the compositions are administered to a subject a dosage of at least 1×10^{-8} moles of the first compound to the subject, 1×10^{-8} moles of the
20 second compound to the subject, and 1×10^{-9} moles of the third compound to the subject.

Also disclosed are methods, wherein the composition is injected over 8-12 days.

Also disclosed are methods, wherein the composition is an aerosol, lyophilization, powder, or emulsion.

Also disclosed are methods, wherein the subject is a human.

25 Also disclosed are methods, wherein the human is treated for at least two months.

Also disclosed are methods, wherein the composition is a tablet that is administered orally at least once daily.

Also disclosed are methods, wherein the composition is administered once daily.

The disclosed compositions can be administered at a variety of dosages. For
30 example category 1 compounds like Nicotinamide Mononucleotide (NMN), can be at dosages per day of 1×10^{-6} moles/kg to 1×10^{-2} moles/kg or 1×10^{-5} moles/kg to 1×10^{-3} moles/kg or 1×10^{-4} moles/kg to 1×10^{-3} moles/kg or 2×10^{-4} moles/kg to 7×10^{-4} moles/kg. In certain embodiments, the dosages per day of the category 1 molecule can be at least 1×10^{-6} moles/kg, 1×10^{-5} moles/kg, 1×10^{-4} moles/kg, 1×10^{-3} moles/kg or 1×10^{-2}

moles/kg. The dosages can also be at least 2.38 moles/kg per day. The same dosages are contemplated herein for other category 1 compounds NAD⁺, NR, NaMN, NaAD, NAR, MNM, and cAMP.

5 The dosage of category 2 compounds, such as betaine, can be at dosages per day of 1×10^{-6} moles/kg to 1×10^{-2} moles/kg or 1×10^{-5} moles/kg to 1×10^{-3} moles/kg or 1×10^{-4} moles/kg to 1×10^{-3} moles/kg or 2×10^{-4} moles/kg to 7×10^{-4} moles/kg. In certain embodiments, the dosages per day of the category 2 compound can be at least 1×10^{-6} moles/kg, 1×10^{-5} moles/kg, 1×10^{-4} moles/kg, 1×10^{-3} moles/kg or 1×10^{-2} moles/kg. The dosages can also be at least 5.82×10^{-4} moles / kg body weight / day.

10 The dosages of category 3 compounds, such as H₂O₂, can be at dosages per day of 1×10^{-7} moles/kg to 1×10^{-2} moles/kg or 1×10^{-6} moles/kg to 1×10^{-3} moles/kg or 1×10^{-5} moles/kg to 1×10^{-4} moles/kg or 1×10^{-5} moles/kg to 7×10^{-5} moles/kg. In certain embodiments, the dosages per day of the category 3 compound can be at least 1×10^{-7} moles/kg, 1×10^{-6} moles/kg, 1×10^{-5} moles/kg, 1×10^{-4} moles/kg or 1×10^{-3} moles/kg. The dosages can also be at least dosage 2.34×10^{-5} moles / kg body weight / day.

The dosages of category 3 compounds, such as NaSH, can be at dosages per day of 1×10^{-8} moles/kg to 1×10^{-3} moles/kg or 1×10^{-7} moles/kg to 1×10^{-4} moles/kg or 1×10^{-6} moles/kg to 1×10^{-5} moles/kg or 1×10^{-6} moles/kg to 7×10^{-6} moles/kg. In certain embodiments, the dosages per day of the category 3 compound can be at least 1×10^{-8} moles/kg, 1×10^{-7} moles/kg, 1×10^{-6} moles/kg, 1×10^{-4} moles/kg or 1×10^{-3} moles/kg. In certain embodiments, the dosages can also be at least 3.02×10^{-6} moles / Kg body weight / day.

Specific Methods

Disclosed are methods of resetting biological pathways for defending against and repairing deterioration from human aging. These methods can reduce inflammation in a subject. In specific examples, the disclosed methods can comprise administering to a subject nicotinamide adenine dinucleotide (NAD⁺), S-Adenosyl-methionine (SAM), and H₂O₂. In specific examples, the disclosed methods can comprise administering to a subject nicotinamide adenine dinucleotide (NAD⁺), S-Adenosyl-methionine (SAM), and NaSH. In specific examples, the disclosed methods can comprise administering to a subject nicotinamide adenine dinucleotide (NAD⁺), S-Adenosyl-methionine (SAM), and Na₂S. In specific examples, the disclosed methods can comprise administering to a subject nicotinamide adenine dinucleotide (NAD⁺), S-Adenosyl-methionine (SAM), and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin,

quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc.

In specific examples, the disclosed methods can comprise administering to a subject
 5 nicotinamide adenine dinucleotide (NAD⁺), Betaine, and H₂O₂. In specific examples, the disclosed methods can comprise administering to a subject nicotinamide adenine dinucleotide (NAD⁺), folate + Vitamin B12, and H₂O₂. In specific examples, the disclosed methods can comprise administering to a subject nicotinamide adenine dinucleotide (NAD⁺), Methionine, and H₂O₂. In other examples, the disclosed methods can comprise
 10 administering to a subject nicotinamide riboside (NR), Methionine, and H₂O₂. In specific examples, the disclosed methods can comprise administering to a subject nicotinamide adenine dinucleotide (NAD⁺), Choline, and H₂O₂.

In specific examples, the disclosed methods can comprise administering to a subject nicotinamide adenine dinucleotide (NAD⁺), Betaine, and NaHS. In specific examples, the
 15 disclosed methods can comprise administering to a subject nicotinamide adenine dinucleotide (NAD⁺), Folate + Vitamin B12, and NaHS. In specific examples, the disclosed methods can comprise administering to a subject nicotinamide adenine dinucleotide (NAD⁺), Methionine, and NaHS. In specific examples, the disclosed methods can comprise administering to a subject nicotinamide adenine dinucleotide (NAD⁺),
 20 Choline, and NaHS.

In specific examples, the disclosed methods can comprise administering to a subject nicotinamide adenine dinucleotide (NAD⁺), Betaine, and Na₂S. In specific examples, the disclosed methods can comprise administering to a subject nicotinamide adenine dinucleotide (NAD⁺), Folate + Vitamin B12, and Na₂S. In specific examples, the disclosed
 25 methods can comprise administering to a subject nicotinamide adenine dinucleotide (NAD⁺), Methionine, and Na₂S. In specific examples, the disclosed methods can comprise administering to a subject nicotinamide adenine dinucleotide (NAD⁺), Choline, and Na₂S.

In specific examples, the disclosed methods can comprise administering to a subject nicotinamide adenine dinucleotide (NAD⁺), Betaine, and any one or more of H₂S, O₃,
 30 metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc. In specific examples, the disclosed methods can comprise administering to a subject nicotinamide adenine dinucleotide (NAD⁺), Folate + Vitamin B12, and any one or more of

H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β-lapachone, pterostilbene, resveratrol, apigenin, and zinc.

In specific examples, the disclosed methods can comprise administering to a subject

5 nicotinamide adenine dinucleotide (NAD⁺), Methionine, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β-lapachone, pterostilbene, resveratrol, apigenin, and zinc.

In specific examples, the disclosed methods can comprise administering to a subject

10 nicotinamide adenine dinucleotide (NAD⁺), Choline, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β-lapachone, pterostilbene, resveratrol, apigenin, and zinc.

In specific examples, the disclosed methods can comprise administering to a subject

15 nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, Betaine, and H₂O₂. In other examples, the disclosed methods can comprise administering to a subject nicotinamide riboside (NR), Betaine, and H₂O₂. In other examples, the disclosed methods can comprise administering to a subject one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid
20 riboside (NAR), with Betaine, and H₂O₂. In other examples, the disclosed methods can comprise administering to a subject 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Betaine, and H₂O₂.

In specific examples, the disclosed methods can comprise administering to a subject

25 nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, folate + Vitamin B12, and H₂O₂. In other examples, the disclosed methods can comprise administering to a subject nicotinamide riboside (NR), folate + Vitamin B12, and H₂O₂. In other examples, the disclosed methods can comprise administering to a subject one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), folate + Vitamin B12, and H₂O₂. In other examples, the
30 disclosed methods can comprise administering to a subject 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), folate + Vitamin B12, and H₂O₂.

In specific examples, the disclosed methods can comprise administering to a subject

nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, Betaine + Vitamin B12, and H₂O₂. In other examples, the disclosed methods can comprise

administering to a subject nicotinamide riboside (NR), Betaine + Vitamin B12, and H₂O₂.

In other examples, the disclosed methods can comprise administering to a subject one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), Betaine + Vitamin B12, and H₂O₂.

- 5 In other examples, the disclosed methods can comprise administering to a subject 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Betaine + Vitamin B12, and H₂O₂.

- In specific examples, the disclosed methods can comprise administering to a subject nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, Methionine, and H₂O₂. In other examples, the disclosed methods can comprise administering to a subject
10 nicotinamide riboside (NR), Methionine, and H₂O₂. In other examples, the disclosed methods can comprise administering to a subject one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), with Methionine, and H₂O₂. In other examples, the disclosed methods can
15 comprise administering to a subject 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Methionine, and H₂O₂.

- In specific examples, the disclosed methods can comprise administering to a subject nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, Choline, and H₂O₂. In other examples, the disclosed methods can comprise administering to a subject
20 nicotinamide riboside (NR), Choline, and H₂O₂. In other examples, the disclosed methods can comprise administering to a subject one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), Choline, and H₂O₂. In other examples, the disclosed methods can
comprise administering to a subject 1-methylnicotinamide (MNM) and/or cyclic adenosine
25 monophosphate (cAMP), Choline, and H₂O₂.

- In specific examples, the disclosed methods can comprise administering to a subject nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, S-Adenosyl-methionine (SAM), and H₂O₂. In other examples, the disclosed methods can comprise
administering to a subject nicotinamide riboside (NR), S-Adenosyl-methionine (SAM), and
30 H₂O₂. In other examples, the disclosed methods can comprise administering to a subject one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), S-Adenosyl-methionine (SAM), and H₂O₂. In other examples, the disclosed methods can comprise administering to a

subject 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), S-Adenosyl-methionine (SAM), and H₂O₂.

In specific examples, the disclosed methods can comprise administering to a subject nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, Betaine, and NaHS. In other examples, the disclosed methods can comprise administering to a subject nicotinamide riboside (NR), Betaine, and NaHS. In other examples, the disclosed methods can comprise administering to a subject one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), Betaine, and NaHS. In other examples, the disclosed methods can comprise administering to a subject 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Betaine, and NaHS.

In specific examples, the disclosed methods can comprise administering to a subject nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, Folate + Vitamin B12, and NaHS. In other examples, the disclosed methods can comprise administering to a subject nicotinamide riboside (NR), Folate + Vitamin B12, and NaHS. In other examples, the disclosed methods can comprise administering to a subject one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), Folate + Vitamin B12, and NaHS. In other examples, the disclosed methods can comprise administering to a subject 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Folate + Vitamin B12, and NaHS.

In specific examples, the disclosed methods can comprise administering to a subject nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, Betaine + Vitamin B12, and NaHS. In other examples, the disclosed methods can comprise administering to a subject nicotinamide riboside (NR), Betaine + Vitamin B12, and NaHS. In other examples, the disclosed methods can comprise administering to a subject one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), Betaine + Vitamin B12, and NaHS. In other examples, the disclosed methods can comprise administering to a subject 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Betaine + Vitamin B12, and NaHS.

In specific examples, the disclosed methods can comprise administering to a subject nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, Methionine, and NaHS. In other examples, the disclosed methods can comprise administering to a subject nicotinamide riboside (NR), Methionine, and NaHS. In other examples, the disclosed

methods can comprise administering to a subject one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), Methionine, and NaHS. In other examples, the disclosed methods can comprise administering to a subject 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Methionine, and NaHS.

In specific examples, the disclosed methods can comprise administering to a subject nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, Choline, and NaHS. In other examples, the disclosed methods can comprise administering to a subject nicotinamide riboside (NR), Choline, and NaHS. In other examples, the disclosed methods can comprise administering to a subject one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), Choline, and NaHS. In other examples, the disclosed methods can comprise administering to a subject 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Choline, and NaHS.

In specific examples, the disclosed methods can comprise administering to a subject nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, S-Adenosyl-methionine (SAM), and NaHS. In other examples, the disclosed methods can comprise administering to a subject nicotinamide riboside (NR), S-Adenosyl-methionine (SAM), and NaHS. In other examples, the disclosed methods can comprise administering to a subject one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), S-Adenosyl-methionine (SAM), and NaHS. In other examples, the disclosed methods can comprise administering to a subject 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), S-Adenosyl-methionine (SAM), and NaHS.

In specific examples, the disclosed methods can comprise administering to a subject nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, Betaine, and Na₂S. In other examples, the disclosed methods can comprise administering to a subject nicotinamide riboside (NR), Betaine, and Na₂S. In other examples, the disclosed methods can comprise administering to a subject one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), Betaine, and Na₂S. In other examples, the disclosed methods can comprise administering to a subject 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Betaine, and Na₂S.

In specific examples, the disclosed methods can comprise administering to a subject nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, Folate + Vitamin B12, and Na2S. In other examples, the disclosed methods can comprise administering to a subject nicotinamide riboside (NR), Folate + Vitamin B12, and Na2S. In other examples, the disclosed methods can comprise administering to a subject one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), Folate + Vitamin B12, and Na2S. In other examples, the disclosed methods can comprise administering to a subject 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Folate + Vitamin B12, and Na2S.

In specific examples, the disclosed methods can comprise administering to a subject nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, Betaine + Vitamin B12, and Na2S. In other examples, the disclosed methods can comprise administering to a subject nicotinamide riboside (NR), Betaine + Vitamin B12, and Na2S. In other examples, the disclosed methods can comprise administering to a subject one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), Betaine + Vitamin B12, and Na2S. In other examples, the disclosed methods can comprise administering to a subject 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Betaine + Vitamin B12, and Na2S.

In specific examples, the disclosed methods can comprise administering to a subject nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, Methionine, and Na2S. In other examples, the disclosed methods can comprise administering to a subject nicotinamide riboside (NR), Methionine, and Na2S. In other examples, the disclosed methods can comprise administering to a subject one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), Methionine, and Na2S. In other examples, the disclosed methods can comprise administering to a subject 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Methionine, and Na2S.

In specific examples, the disclosed methods can comprise administering to a subject nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, Choline, and Na2S. In other examples, the disclosed methods can comprise administering to a subject nicotinamide riboside (NR), Choline, and Na2S. In other examples, the disclosed methods can comprise administering to a subject one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid

riboside (NAR), Choline, and Na₂S. In other examples, the disclosed methods can comprise administering to a subject 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Choline, and Na₂S.

In specific examples, the disclosed methods can comprise administering to a subject
 5 nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, S-Adenosyl-methionine (SAM), and Na₂S. In other examples, the disclosed methods can comprise administering to a subject nicotinamide riboside (NR), S-Adenosyl-methionine (SAM), and Na₂S. In other examples, the disclosed methods can comprise administering to a subject one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine
 10 dinucleotide (NaAD), and nicotinic acid riboside (NAR), S-Adenosyl-methionine (SAM), and Na₂S. In other examples, the disclosed methods can comprise administering to a subject 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), S-Adenosyl-methionine (SAM), and Na₂S.

In specific examples, the disclosed methods can comprise administering to a subject
 15 nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, Betaine, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β-lapachone, pterostilbene, resveratrol, apigenin, and zinc. In other examples, the disclosed methods can comprise administering to
 20 a subject nicotinamide riboside (NR), Betaine, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β-lapachone, pterostilbene, resveratrol, apigenin, and zinc. In other examples, the disclosed methods can comprise administering to a subject one or more of
 25 nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), Betaine, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β-lapachone, pterostilbene, resveratrol, apigenin, and zinc.
 30 In other examples, the disclosed methods can comprise administering to a subject 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Betaine, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized

derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc.

In specific examples, the disclosed methods can comprise administering to a subject nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, Folate + Vitamin B12, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc. In other examples, the disclosed methods can comprise administering to a subject nicotinamide riboside (NR), Folate + Vitamin B12, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc. In other examples, the disclosed methods can comprise administering to a subject one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), Folate + Vitamin B12, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc. In other examples, the disclosed methods can comprise administering to a subject 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Folate + Vitamin B12, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc.

In specific examples, the disclosed methods can comprise administering to a subject nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, Betaine + Vitamin B12, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc. In other examples, the disclosed methods can comprise administering to a subject nicotinamide riboside (NR), Betaine + Vitamin B12, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid,

Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc. In other examples, the disclosed methods can comprise administering to a subject one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), Betaine + Vitamin B12, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc. In other examples, the disclosed methods can comprise administering to a subject 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Betaine + Vitamin B12, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc.

15 In specific examples, the disclosed methods can comprise administering to a subject nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, Methionine, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc. In other examples, the disclosed methods can comprise administering to a subject nicotinamide riboside (NR), Methionine, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc.

20 In other examples, the disclosed methods can comprise administering to a subject one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), Methionine, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc.

25 In other examples, the disclosed methods can comprise administering to a subject 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Methionine, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc.

30 In other examples, the disclosed methods can comprise administering to a subject 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Methionine, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized

derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc.

In specific examples, the disclosed methods can comprise administering to a subject nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, Choline, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc. In other examples, the disclosed methods can comprise administering to a subject nicotinamide riboside (NR), Choline, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc. In other examples, the disclosed methods can comprise administering to a subject one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), Choline, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc. In other examples, the disclosed methods can comprise administering to a subject 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), Choline, and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc.

In specific examples, the disclosed methods can comprise administering to a subject nicotinamide mononucleotide (NMN) or a precursor or prodrug of NMN, S-Adenosyl-methionine (SAM), and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc. In other examples, the disclosed methods can comprise administering to a subject nicotinamide riboside (NR), S-Adenosyl-methionine (SAM), and any one or more of H₂S, O₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -

lapachone, pterostilbene, resveratrol, apigenin, and zinc. In other examples, the disclosed methods can comprise administering to a subject one or more of nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), and nicotinic acid riboside (NAR), S-Adenosyl-methionine (SAM), and any one or more of **H₂S**, ₀₃,
 5 metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc. In other examples, the disclosed methods can comprise administering to a subject 1-methylnicotinamide (MNM) and/or cyclic adenosine monophosphate (cAMP), S-Adenosyl-
 10 methionine (SAM), and any one or more of **H₂S**, ₀₃, metformin, acetaminophen, sulforaphane, glucoraphanin, curcumin, quercetin, isoquercetin, ginseng, (R)-alpha-lipoic acid, Hydrophilic oxidized derivatives of Lycopene, N-Acetylcysteine, DHEA, garlic, β -lapachone, pterostilbene, resveratrol, apigenin, and zinc.

Surrogate markers for aging

15 A variety of markers can be used as surrogates for monitoring aging.

DNA Methylation levels

DNA methylation levels change with age. Studies have identified biomarkers of chronological age based on DNA methylation levels called an "epigenetic clock" (Horvath S 2013 based on 353 dinucleotide CpG markers). Differences between DNA methylation
 20 age and chronological age led to the conclusion that DNA methylation-derived measures of biological aging are traits that predict mortality independently of health status, lifestyle factors, and known genetic factors (Marioni RE 2015). This epigenetic clock is tissue specific since some tissues age faster than others. The cerebellum ages more slowly than other parts of the body (Horvath S 2015). HIV-1-infected individuals show accelerated
 25 aging with this epigenetic clock (Rickabaugh TM 2015). Methylation data can be collected from circulating T cells and monocytes and was done so in a population cohort of 1264 participants (Reynolds LM 2014).

DNA breakage

Single stranded and double stranded DNA breakage has not been used as
 30 methylation has for a biological clock but it is correlated to aging (Yu Q 2015) with older age having more breakage on average. Companies such as Exogen Biotechnology are able to test for single stranded and double stranded DNA breakage. NAD⁺ is used in DNA repair by PARP and Sirtuin enzymes, thus seeing less DNA breakage is an indication that these enzyme systems are working.

Inflammation markers

Inflammation markers can be analyzed for aging including those markers found in the study by Arai in 2015. Arai found inflammation markers that were predictive of who would continue to live (life-span) and who would be physically and cognitively healthy (health-span). The markers used were CMV IgG, IL-6, TNF-alpha and CRP.

Other markers associated with aging

Global loss of H3K9me3 or the resulting heterochromatin architecture changes correlate to biological aging as was shown in the human aging caused by Werner syndrome's premature aging and this can also be analyzed (Zhang W 2015).

A variety of compounds in blood correlate to age, as well as effect age and can be measure. An example is TGF-beta, which is lower in younger individuals than older individuals.

Metabonomic measurements have been correlated to aging using a nonlinear regression technique and a 13 year follow up.

Peripheral blood leukocyte telomere length can be measured and compared to 64,637 individuals of known age (Rode L 2015), although telomere length is only modestly correlated to age ($r=0.5$) and cellular aging continues regardless of telomere length.

Definitions

In this specification and in the claims that follow, reference will be made to a number of terms, which shall be defined to have the following meanings:

Throughout the description and claims of this specification the word "comprise" and other forms of the word, such as "comprising" and "comprises," means including but not limited to, and is not intended to exclude, for example, other additives, components, integers, or steps.

As used in the description and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a composition" includes mixtures of two or more such compositions, reference to "the compound" includes mixtures of two or more such compounds, reference to "an agent" includes mixture of two or more such agents, and the like.

"Optional" or "optionally" means that the subsequently described event or circumstance can or cannot occur, and that the description includes instances where the event or circumstance occurs and instances where it does not.

As used herein, by a "subject" is meant an individual. Thus, the "subject" can include domesticated animals (e.g., cats, dogs, etc.), livestock (e.g., cattle, horses, pigs,

sheep, goats, etc.), laboratory animals (*e.g.*, mouse, rabbit, rat, guinea pig, etc.), and birds.

"Subject" can also include a mammal, such as a primate or a human.

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EXAMPLES

The following examples are set forth below to illustrate the methods, compositions, and results according to the disclosed subject matter. These examples are not intended to be inclusive of all aspects of the subject matter disclosed herein, but rather to illustrate representative methods, compositions, and results. These examples are not intended to exclude equivalents and variations of the present invention, which are apparent to one skilled in the art.

A 61 year old Caucasian male weighing 88 kg at the beginning of the treatment was treated with a regimine of category 1, category 2, and category 3 molecules as noted below.

Nicotinamide mononucleotide (NMN) (MW=334.22)

Betaine (trimethyl glycine) (MW = 117.14)

H₂O₂ (MW=34.01)

NaSH (MW=56.06)

Solutions of various compounds were produced for administering to the subject by mixing a set number of grams with 500 mL of water.

Typical final concentrations of NMN taken by subject were 3.5 grams in 500 mL H₂O, betaine were 3 grams in 500 mL H₂O, H₂O₂ were (2 drops of 35% concentration in 500 mL H₂O), and NaSH were (drops of 2 at 66uM per drop concentration in 500 mL H₂O).

The amounts of each composition were set so that by the subject drinking the full 500 mL a final dosage approximately 1.19×10^{-4} moles NMN/ kg body weight per dose, 2.91×10^{-4} moles betaine / kg body weight per dose, 1.17×10^{-5} moles of H₂O₂ / kg of body weight per dose, and 1.51×10^{-6} moles of NaSH / kg of body weight per dose was given to the subject through drinking the 500 mL solution.

By taking two similar dosages per day, the sum of the two daily equal allotments was

- Nicotinamide Mononucleotide (NMN) dosage - 2.38×10^{-4} moles / Kg body weight / day
- 5 • The betaine dosage $\sim 5.82 \times 10^{-4}$ moles / Kg body weight / day
- The Hydrogen Peroxide (**H2O2**) dosage $\sim 2.34 \times 10^{-5}$ moles / Kg body weight / day
- The Sodium Hydrogen Sulfide (NaSH) dosage -- 3.02×10^{-6} moles / Kg body weight / day

10 The subject was weighed each day.

The subject self-administered the formulations orally through drinking the solution at approximately 7AM and 7 PM each day. These times were chosen because they approximated the subjects' biological clock peaks of NAD⁺ as determined by Ramsey K 2009. This had the effect of pulsing the ingredients into the body twice a day,

15 approximately timed with the biological clock of the subject.

LabCor Inc. performed the marker testing using standard protocols on a monthly basis. Blood draw times ranged between 8:19 am and 8:54 am. Inflammatory measurements are correlated to the biological clock. LabCor tested levels of CMV IgG, C-Reactive Protein, Tumor Necrosis Factor-Alpha, and Interleukin-6 in Serum.

20 The subject also had the following data collected monthly at LabCorp, including Serum Glucose, Serum Uric Acid, BUN, Serum Creatinine, eGFR if non African American, BUN/Creatinine Ratio, Serum Sodium, Serum Potassium, Serum Chloride, Total Carbon Dioxide, Serum Calcium, Serum Phosphorus, Serum Total Protein, Serum Albumin, Serum, Total Globulin, A/G Ratio, Total Bilirubin, Serum Alkaline Phosphatase, LDH, AST

25 (SGOT), ALT (SGPT), Serum Iron, Total Cholesterol, Triglycerides, HDL Cholesterol, Calculation VLDL cholesterol Calculation LDL Cholesterol, Total Cholesterol/ HDL ratio, Estimated CHD risk, White Blood Cells, Red Blood Cells, Hemoglobin, Hematocrit, MCV, MCH, MCHC, RDW, Platelets, Neutrophils, Lymphs, Monocytes, Eos, Basos, Immature Cells, Neutrophils (Absolute). Lymphs (Absolute), Monocytes (Absolute), Eos (Absolute),

30 Baso (Absolute), Immature Granulocytes, Immature Grans (Absolute), NRBC, VAP Cholesterol Profile, LDL Cholesterol, HDL Cholesterol, VLDL Cholesterol, Cholesterol total, Triglycerides, Non HDL Cholesterol (LDL+VLDL), ApoB100=Calculation, LDL-R (Real)-C, Lp(a) Cholesterol, IDL Cholesterol, Remnant Lipo (IDL+VLDL3), Probable Metabolic Syndrome, HDL-2 (most Protective), HDL-3 (Less Protective), VLDL-3 (Small

Remnant), LDL1 Partem A, LDL2 Pattern A, LDL3 Pattern B, LDL4 Partem B, LDL Density Pattern, Glucose Tolerance (4 Sp Blood), Glucose Fasting, Glucose 1 hour, Glucose 2 hours, Glucose 3 hours, Insulin Fasting, Insulin 1 hour, Insulin 2 hours, Insulin 3 hours, Cortisol AM, Cortisol PM, IL-1b (Serum), Hemoglobin Ale, Rheumatoid Arthritis Factor, IGF-1, Cardiac, Tumor Interleukin-8 (Serum), Homocysteine (Plasma), Antinuclear Antibodies direct, Sedimentation Rate-Westergren Cortisol, (Urinary Free), Cortisol, F, ug, L, U, Cortisol, Fug, 24hr,U, Serum Immunoglobulin G, Qn, Serum Immunoglobulin A, Qn, Serum Immunoglobulin M, Qn, oxLDL, CMV IgM, Ferritin, and H. pylori IgG

University of California, San Diego measured:

- 10
 - a. Spectral 3 tesla MRI of right calf leg muscle before, during, and after exercise
 - b. Spectral 3 tesla MRI of Liver
 - c. Structural 3 tesla MRI of Liver
 - d. Spectral 3 tesla MRI of Brain (front and back)
 - e. A structural 3 tesla MRI of Brain
- 15
 - f. A structural 3 tesla MRI of the right knee (showing Arthritis)
 - g. 3-Nitrotyrosine (a marker for oxidative / nitrative stress)
 - h. Coagulation Tests (a marker for oxidative stress)
 - i. F2-isoprostanes (a marker for oxidative / nitrative stress,)
 - j. GSH: GSSH (a marker for and protection from oxidative / nitrative stress)
- 20
 - k. Urine Organic Acids
 - l. 8-hydroxydeoxyguanosine (8-OHDG) (a marker for oxidative / nitrative stress)
 - m. Malondialdehyde (a marker for oxidative / nitrative stress)
 - n. hsCRP (a marker that can be adversely affected by oxidative stress)
 - o. Proteomic profile (a marker for oxidative / nitrative stress)
- 25

A list of medical history questions (UCSD) were answered. Body fat and mineral testing was performed at private MD's office. Treadmill testing was performed at private MD's office. 4 tissue biopsy types (liver (needle biopsy), skin; adipose, muscle) were obtained (stored at -80 C at UCLA). A log of daily exercise and weight was obtained. Also weekly glucose monitoring before and after NMN and BP monitoring before and after
- 30

NMN was obtained.

Results

Table 1.

						61 year old Male Caucasian				
With the additions of										
NMN					X	X	X	X	X	X
Betaine								X	X	X
H ₂ O ₂									X	
NaSH										X
		Normal Range Low	Normal Range High	Baseline						
CMV IgG		0	0	0	0	0	0	0	0	0
C-Reactive Protein	mg/L	0	3	2.77	3.25	0.43	0.53	0.85	0.21	0.40
Tumor Necrosis Factor-Alpha	pg/mL	0	8.1	1.1	0.9	1.1	1.1	1	0.5	0.3
Interleukin-6 Serum	pg/mL	0	15.5	1.3	4.4	<0.7	0.9	3.1	<0.7	<0.7
Inflammation Score		0	26.6	5.17	8.55	2.23	2.53	4.95	1.41	1.40

The results of the monthly administration schedule and testing for the subject are presented in Table 1. Table 1 shows that the subject was provided a formulation on a monthly basis, where the formulation included NMN alone for 3 months, NMN+ betaine for one month, NMN+ betaine + H₂O₂ for one month and NMN+ betaine +NaSH for one month.

Other observations of interest during study are that the subject was healthy during the full duration of the study. Photos depicted that aged skin cells on hand became youthful in appearance. The subject's complexion of facial skin improved during study. The subject had significant weight loss and apatite was lowered during study. The subject had an elimination of pain from arthritis in right knee during study. The subject had more restful sleep during study. The subject had increased energy during study. The subject had better vision at eye exam.

Discussion

The age of 61 correlates to the age of unrelated and offspring families in the Arai Y 2015 study detailed herein. The results of this study, in light of the Arai Y 2015 study, show that the triple therapy with the three categories of compounds change the predicted outcome, as identified by Arai 2015, of this 61 year old 88 kg Caucasian male from unsuccessful aging to a prediction of successful aging. In the baseline condition for the subject, both C-reactive Protein (2.77 mg/L) and Interleukin-6 (1.3 pg/mL) measurements

were above the "unrelated family" level (0.7 mg/l and 1.13 pg/mL) (Arai Y 2015, Table 1) as well as the "offspring" level (0.7 mg/l and 1.03 pg/mL) (Arai Y. 2015, Table 1) respectively. The 61 male subject of this study has a similar age to the "offspring" group and the "unrelated family" group of Arai. These two inflammation test scores effect the prediction algorithm to predict a worse aging outcome for the 61 year old subject than the "offspring" or "unrelated family" groups of Arai at baseline.

After two months of treatment with NMN, however, the markers of the 61 year old subject were brought to levels better than the "offspring" group of Arai (CRP, 0.43 mg/l and IL-6, less than 0.7 pg/mL). While both of these markers do rise slightly in month one, the overall effect of the NMN treatment is to reduce the levels of these markers. The lower or approximately similar levels to the "offspring" group of Arai continued to be produced by administration of NMN through months 3, but the effect seemingly plateaus in the 61 year old male.

All three inflammatory markers drop to their lowest level with the addition of all three categories of ingredients. IL-6 drops to undetectable levels, TNF-alpha drops by over 50%, and CRP drops to about a tenth of the original value. When H2O2 is used for the category 3 ingredient in this example CRP drops more than when NaSH is used and when NaSH is used as the category 3 ingredient TNF-alpha dropped more than when H2O2 is used. In both cases of triple therapy the results are far below the necessary levels to predict very successful aging. CMV titers were not discussed here since this 61 year old male had no or undetectable levels of CMV IgG and this is as good as the measured value of this variable can get.

When the interventional therapy for this 61 year old male in this experiment is compared to the results gained by one or two years of calorie restriction one can see that the results are far greater with this triple category therapy and they are far easier to obtain (Di Francesco A 2015, Ravussin E 2015).

Correlations by other authors to human health improvements from the lowering TNF-alpha and IL-6 which were lowered in this example;

Other studies (similar to Arai Y 2015)

Immune markers (a simple index of serum interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-alpha) two of the Arai 4 markers) were found to be the best predictor of mortality in 1,155 older adults in a 10 year all-cause mortality study after adjusting for variables already known to cause death (Varadhan R 2014). A single immune marker (Serum IL-6) predicted all-cause mortality, cancer, cardiovascular disease and liver disease

in a 1843 person prospective cohort study (Lee JK 2012). These studies confirmed results in smaller prior studies (Derhovanessian E 2010, Reuben DB 2002, Taaffe DR 2000).

Possible mechanism of action:

In December 2013, A. Gomes et al, published a study demonstrating that raising the
5 levels of NAD⁺ with precursor NMN in old mice restores mitochondrial function to that of a young mouse. C. Correia-Melo showed with age mitochondria drive a cellular pro-inflammatory phenotype including IL-6 secretion.

Immune Dysfunction:

In July 2014, I. V. Astrakhanseva et al, issued a report showing the benefits of
10 reducing the levels of TNF and IL-6 as effective ways to control inflammation symptoms such as joint destruction and autoimmune diseases. A. Puchta et al, hypothesized a molecular mechanism using these two inflammation variables (TNF and IL-6) for predictive effects on life span and health span. The study showed how TNF increasingly drives immune dysfunction with age and that lowering the levels of TNF decrease this impairment.

Brain Disease:

In September 2014 Brianne Bettcher et al, published a study indicating that at older
ages, there is a positive correlation between increased levels of IL-6 and lowered white matter function in the brain. In February 2015, Brianne Bettcher et al, published a study showing that reducing systemic inflammation had a positive effects on cognition and brain
20 structure which may reverse neurodegenerative disease processes.

Heart disease:

In 2000, Paul Ridker et al, published 2 studies concluding that in apparently healthy
men, elevated levels of IL-6 is associated in increased risk of future Myocardial Infarction and TNF increases the risk of recurrent coronary events after Myocardial Infarction. In
25 August 2005, NJ Goodson et al, published a study linking increased levels of C-Reactive Protein with a prediction of death from cardiovascular disease.

Kidney Disease:

In 2015, Belinda Lee et al, published a study demonstrating the association between
elevated levels of CRP, TNF and IL-6 with chronic kidney disease.

Alzheimer's Disease:

Lowering TNF-alpha and IL-6 lowers the chance of getting Alzheimer's disease and
lowers the negative effects of Alzheimer's disease (Butchart J 2015, Holmes C 2011).
Adding NMN in a mouse Alzheimer's disease model was beneficial (Long AN 2015).

Research into the potential benefits of lowering TNF-alpha and IL-6 for a more effective immune response to viruses and bacteria:

McElroy AK, after analyzing the kinetics of inflammatory signaling in life threatening human Ebola Virus disease, proposed the possible therapeutic benefit of lowering the proinflammatory signaling of IL-6 for clinical intervention of these patients. A. Puchta proposed the possible therapeutic benefit of lowering IL-6 and TNF alpha to increase the ability to fight Streptococcus pneumoniae.

Research correlation of the potential benefits of lowering TNF-alpha and IL-6 to better physical performance.

Cesari M in 2004 concluded higher levels of IL-6 was correlated to lower physical performance in older adults and a target for intervention. Puzianowska-Kuznicka M showed IL-6 and CRP were good predictors of physical and cognitive performance and the risk of mortality in 3496 individuals.

Sleep:

Irwin MR correlated sleep disturbances to increased CRP and IL-6 but not TNF in a meta-analysis of 72 previous sleep studies.

CLAIMS

What is claimed is:

1. A nutritional composition for administering to a subject, composition, comprising:
a repair system activator chosen from, nicotinamide adenine dinucleotide (NAD⁺),
nicotinamide mononucleotide (NMN), nicotinamide riboside (NR), nicotinic acid
adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD),
nicotinic acid riboside (NAR), 1-methylnicotinamide (MNM), cyclic adenosine
monophosphate (cAMP), and any combination thereof;
a methyl donor chosen from, S-5'-adenosyl-L-methionine (SAM), methionine,
betaine, choline, folate, vitamin B12, and any combination thereof; and
an antioxidant defense activator chosen from **H2O2**, **H2S**, NaSH, **Na2S**, ROS, RNS,
RCS, RSOH, $O_2^{\cdot-}$, OH^{\cdot} , 1O_2 , O_3 , HOC1, HOBr, HOI, ROOH, where R is alkyl,
cycloalkyl, heteralkyl, heterocycloalkyl, alkenyl, heteroalkenyl, cycloalkenyl, or
heterocycloalkenyl, metformin, acetaminophen, diallyl trisulfide, isothiocyanate,
curcumin, sulforaphane, quercetin, isoquercetin, apigenin, luteolin, ginseng,
carnosic acid, 4-methylalkylcatechol, 4-vinylcatechol, 4-ethylcatechol,
xanthohumol, β -lapachone, pterostilbene, resveratrol, zinc, and any combination
thereof.
2. The composition of claim 1, wherein the repair system activator, the methyl donor,
and the antioxidant defense activator are at least 5 wt.% of the composition.
3. The composition of claim 1, wherein the repair system activator is nicotinamide
mononucleotide (NMN), nicotinamide riboside (NR), or both.
4. The composition of claim 1, wherein the methyl donor is methionine, betaine, or
both.
5. The composition of claim 1, wherein the antioxidant defense activator is **H2O2**, **H2S**,
or NaSH.
6. The composition of claim 1, wherein the repair system activator, methyl donor, and
antioxidant defense activator are in an amount sufficient to beneficially change a
surrogate marker for aging level in a human when compared to the surrogate marker
level prior to administration.

7. The composition of claim 6 wherein the change in the level of the surrogate marker is a lowered.
8. The composition of claim 7, wherein the surrogate marker is CMV IgG, C-Reactive Protein, Tumor Necrosis Factor- Alpha, or Interleukin-6.
9. The composition of claim 6, wherein the change in the level of the surrogate marker is increased.
10. The composition of claim 9, wherein the surrogate marker is DNA methylation.
11. The composition of claim 1, where the composition further comprises water.
12. The composition of claim 1, wherein the composition comprises at least 1×10^{-8} moles of the repair system activator, at least 1×10^{-8} moles of the methyl donor, and at least 1×10^{-9} moles of the antioxidant defense activator.
13. The composition of claim 1, wherein the composition comprises nicotinamide mononucleotide (NMN), Betaine, and H₂O₂.
14. An injectable formulation, comprising the composition of claim 1.
15. A tablet comprising the composition of claim 1.
16. A method of reducing inflammation in a subject, comprising: administering to the subject the composition of claim 1.
17. The method of claim 16, wherein the composition is administered to a subject at a dosage of at least 1×10^{-6} moles /kg of the repair system activator to the subject, 1×10^{-6} moles /kg of the methyl donor to the subject, and 1×10^{-7} moles /kg of the antioxidant defense activator to the subject.
18. The method of claim 16, wherein the composition is injected over 8-12 days.
19. The method of claim 16, wherein the composition is in an aerosol, lyophilized, powder, or emulsion form.
20. The method of claim 16, wherein the subject is a human.

21. The method of claim 20, wherein the composition is administered to the human for at least two months.
22. The method of claim 16, wherein the composition is in a tablet that is administered orally at least once daily.
23. The method of claim 16, wherein the composition further comprises water.
24. The method of claim 16, wherein the composition is administered to the subject once daily.
25. The method of claim 16, wherein the composition comprises nicotinamide mononucleotide (NMN), Betaine, and H₂O₂.
26. A method of reducing inflammation in a subject, comprising: administering to the subject
 - a repair system activator chosen from nicotinamide adenine dinucleotide (NAD⁺), nicotinamide mononucleotide (NMN), nicotinamide riboside (NR), nicotinic acid adenine mononucleotide (NaMN), nicotinic acid adenine dinucleotide (NaAD), nicotinic acid riboside (NAR), 1-methylnicotinamide (MNM), cyclic adenosine monophosphate (cAMP), and any combination thereof;
 - a methyl donor chosen from, S-5'-adenosyl-L-methionine (SAM), methionine, betaine, choline, folate, vitamin B12, and any combination thereof; and
 - an antioxidant defense activator chosen from H₂O₂, H₂S, NaSH, Na₂S, ROS, RNS, RCS, RSOH, O₂^{·-}, OH[·], ¹O₂, O₃, HOC1, HOBr, HOI, ROOH, where R is alkyl, cycloalkyl, heteralkyl, heterocycloalkyl, alkenyl, heteroalkenyl, cycloalkenyl, or heterocycloalkenyl, metformin, acetaminophen, diallyl trisulfide, isothiocyanate, curcumin, sulforaphane, quercetin, isoquercetin, apigenin, luteolin, ginseng, carnosic acid, 4-methylalkylcatechol, 4-vinylcatechol, 4-ethylcatechol, xanthohumol, β-lapachone, pterostilbene, resveratrol, zinc, and any combination thereof.
27. The method of claim 26, wherein the repair system activator, the methyl donor, and the antioxidant defense activator are administered at approximately the same time.
28. The method of claim 26, wherein the repair system activator is administered within 15, 30, 60, 90, or 120 minutes of the subject's biological clock NAD⁺ peak.

29. The method of claim 26, wherein the repair system activator, the methyl donor, and the antioxidant defense activator are administered at different times.
30. The method of claim 26, wherein the subject is a human.
31. The method of claim 30, wherein the repair system activator, the methyl donor, and the antioxidant defense activator are administered to the human for at least two months.
32. The method of claim 26, wherein the repair system activator, the methyl donor, and the antioxidant defense activator are administered to the human once daily.
33. A composition comprising a precursor or prodrug of nicotinamide mononucleotide (NMN)
 - a methyl donor chosen from, S-5'-adenosyl-L-methionine (SAM), methionine, betaine, choline, folate, vitamin B12, and any combination thereof; and
 - an antioxidant defense activator chosen from H₂O₂, H₂S, NaSH, Na₂S, ROS, RNS, RCS, RSOH, O₂^{·-}, OH[·], ¹O₂, O₃, HOC1, HOBr, HOI, ROOH, where R is alkyl, cycloalkyl, heteralkyl, heterocycloalkyl, alkenyl, heteroalkenyl, cycloalkenyl, or heterocycloalkenyl, metformin, acetaminophen, diallyl trisulfide, isothiocyanate, curcumin, sulforaphane, quercetin, isoquercetin, apigenin, luteolin, ginseng, carnosic acid, 4-methylalkylcatechol, 4 vinylcatechol, 4-ethylcatechol, xanthohumol, β-lapachone, pterostilbene, resveratrol, zinc, and any combination thereof.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/55173

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61P 39/00; A23L 33/13; A23L 33/175; A61K 36/48 (2016.01)

CPC - A23L33/13; A23L33/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8): A61P 39/00; A23L 33/13; A23L 33/175; A61K 36/48 (2016.01)

CPC: A23L33/13; A23L33/17

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 424/757; 424/725 (Keyword limited, terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Patents, Google Scholar (NPL); Keywords: nutritional supplement, anti-ageing/anti-aging, nicotinamide mononucleotide/nicotinamide riboside, methionine/betaine, antioxidant defense activator, hydrogen peroxide/hydrogen sulfide/sodium hydrogen sulfide

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2015/0072950 A1 (Sauve et al.) 12 March 2015 (12.03.2015) abstract, para [0001], [0003], [0024], [0031], [0069], [0121], [0233], [0258]-[0260]	1-33
Y	US 2009/0214628 A1 (de Rijk) 27 August 2009 (27.08.2009) para [0002], [0102], [0242], [0301], [0394]	1-33
Y	US 2004/0204746 A1 (Ovokaitys et al.) 14 October 2004 (14.10.2004) para [0032], [0098], [0105], [0111], [0217], [0264], [0283]	6-10
A	US 2007/0253919 A1 (Boyd) 01 November 2007 (01.11.2007) para [0040], [0041], [0043]	1-33

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

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22 November 2016

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