VITA AETERNA: everlasting life

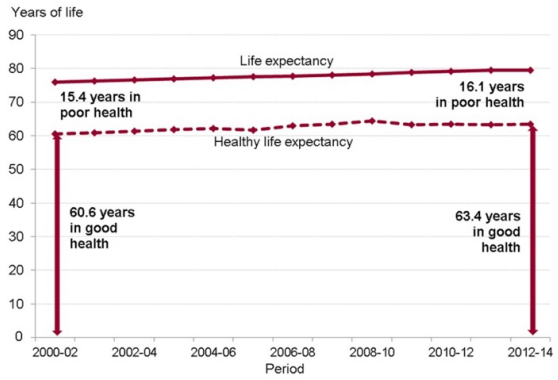
FUNDAMENTUM: basis, beginning, foundation

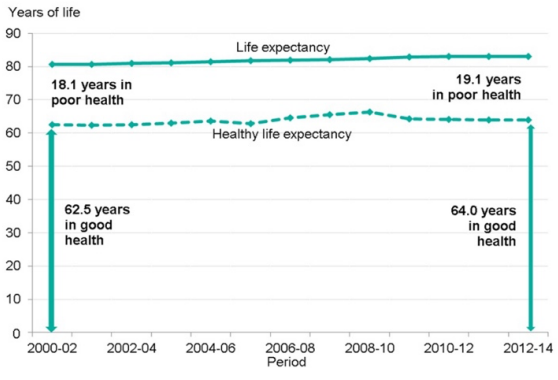
Lifespan versus healthspan

Humans are **living longer**, but despite a data trajectory of **increasing lifespan**, **healthspan has not improved**. Healthspan describes healthy life years or the '**quality-of-life**' years we live, free from disease with high functionality and independence. With advances in medicine we've been able to reduce the chance of death from infectious diseases and accidents, and increase the time we are alive, but increasing the length of life, isn't the same as increasing the time we live it. Two thirds of people aged 65 years or over suffer with **multimorbidity** (non-related illnesses). We have **improved detection of disease and can offer many treatments**, however **inflammatory mechanisms** still drive conditions for which there are few treatment options, such as lung fibrosis, Alzheimer's disease and **frailty**. Many people struggle in the last decade of their lives managing **comorbid conditions** or **disease clusters**. This is coupled with complex **polypharmacy** (multiple medicines), drug interactions and side effects, the tablets all wilfully taken without fully understanding why or without regular review. An estimation from the Royal College of Nursing for the average number of **prescribed medications** per patient **over 65 years** is **seven**. Our problem isn't necessarily getting 'old', it's becoming 'elderly' and we treat the problems in aging like we apply sticking plasters to wounds, rather than addressing the underlying cause itself.

Provisional data from Public Health England shows the average lifespan for UK 2016 was **79.5 years for males of which 63.4 years** are in good health. For **females**, lifespan was **83.1 years** with an **average of 64 years** in good health. Healthspan versus lifespan is an unethical problem.

Male and female lifespan versus healthspan UK 2000-2014. Source: Office for National Statistics.





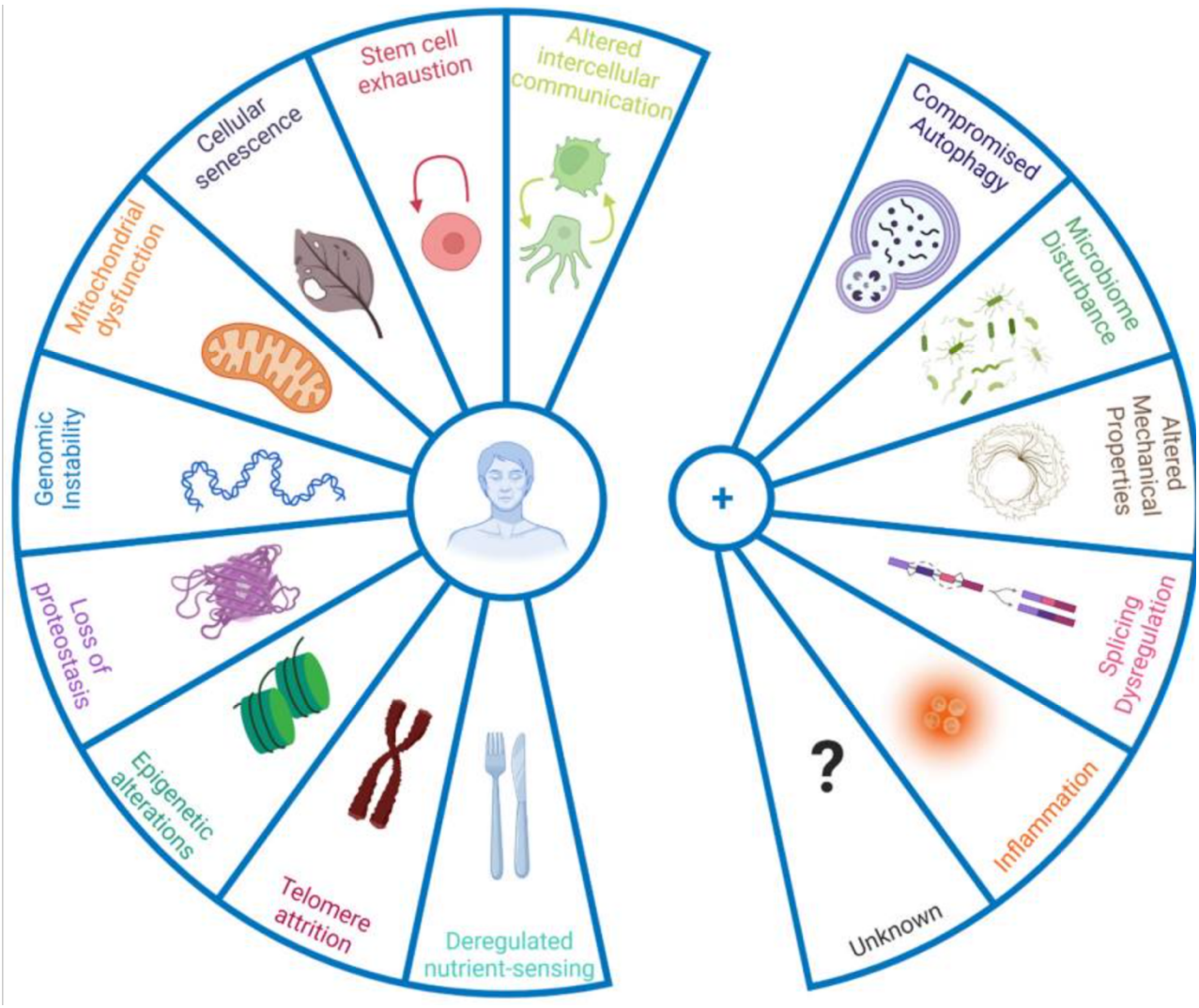
There is no denying **human aging is a complex process**, with many contributing **genetic** (5-25%), **environmental**, **socioeconomic**, luck and random chance factors. Over the past decade research into aging has rapidly increased as it is now **recognised as a disease**, with interest in therapeutic development, senolytics and drug repurposing. How we chose to live our lives plays an important part too, anywhere from 75-95% in fact. We can choose whether we smoke, the amount of **activity** and types of **exercise** we do, how much we prioritise **sleep, stress** management, our **diet**, extracurricular toxicity and percentage of our **body fat**.

Aging is obscured by the enormity of the problem. If other conditions had caused so much of a problem for two thirds of a population, I'm fairly confident we'd have focused on doing more about it. **Aging is something that's always existed,** and we are conditioned to accept it.

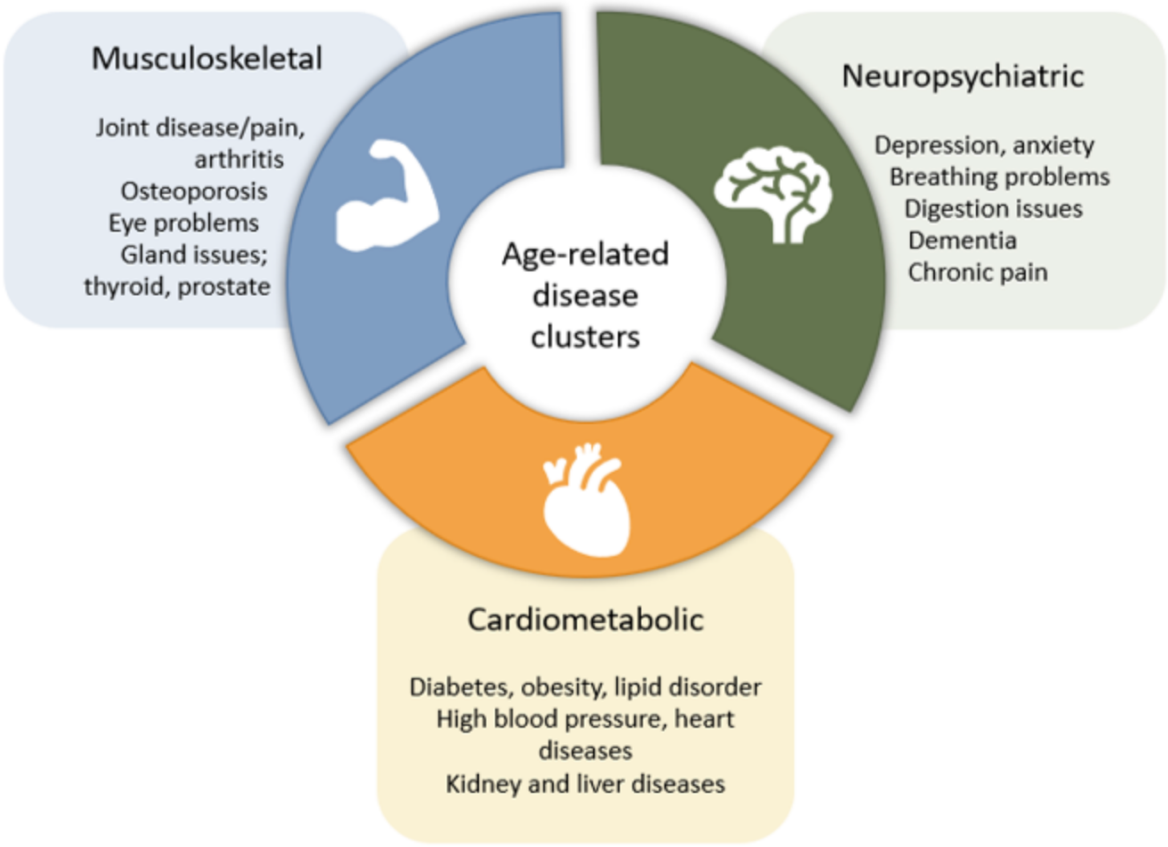
There are already a few more things we can do though. There are some well-studied nutrients and supplements with good safety-efficacy profiles to support our diets: whey protein, creatine, omega 3 and vitamins B12 and D to name a few, and there are newer and until recently less studied and generally less appreciated supplements, which are beginning to show modest-promising effects. If we can reduce the rate we age and **slow down our biological clocks,** it may give us time to benefit from the advances in anti-aging in the not-so-distant future.

Geroscience

Geroscience might not be a science you've heard of. It's a relatively new term used to study the **process of aging** that has an impact on the whole biological system rather than individual age-associated disease. It gives underlying context and relevance to seemingly unrelated illnesses. Geroscientists study the **biology of aging** and work to find ways to delay or prevent age-related diseases from occurring. They have grouped the targeted areas into **hallmarks**. The hallmarks have been defined as these are the **biological mechanisms** scientists can **alter** to **demonstrate the acceleration or slowing of aging**. There were nine hallmarks of aging proposed by Lopez-Otin and colleagues in 2013 which have recently been updated (2022) to 15, to recognise advances in the field of ageing research. Although identified as individual processes or single pathways, the hallmarks model likely fails to present the **complexity and interconnectedness of aging** or recognise how our **individual choices impact** our lives.



When you look at **aging** from a **statistical point of view**, it becomes fairly straight forward to understand. **Reduce the mortality rate doubling time**, and we push back the age for chronic diseases and probably death. According to the **Gompertz Law of Mortality**, the mortality rate in humans **doubles approximately every eight years**. This means the risk of diseases also accelerates after the age of about 40 and doubles at a rate equal to the doubling time. Geroscientists have proposed there is no reason for this exponential rate, thus **reducing this acceleration is entirely plausible**. For obvious reasons, most work to date has been carried out in animals, convenient as their mortality rate doubling time (lifespan) is short. They can alter the rate of senescence (aging) and see 'old' mice start to behave and appear 'young' again. They can and want to run and swim further, their ability to navigate a maze is better and their fur is thicker; **they are less frail**. Naked mole rats, some bat species and tortoises lack this predictable acceleration and exhibit negligible senescence, suggesting their mortality rate doubling time could be arbitrarily large. According to statistics, reverse engineering our underlying mechanistic biology can alter the lifelong processes which lead to diseases quite a bit.



Large cross-sectional datasets such as the UK Biobank (UKB) or National Health and Nutrition Examination Survey (NHANES), provide information on the **dynamics of human health in relation to age**. Deep dive inside the general picture of reduced physical activity as we age and there are two distinctive phases, the first **around the age of 40 years old**. **Another** is **slightly later marking healthspan**, the point in 'old age' in which the first debilitating disease appears, often followed by multiple linked morbidities known as **disease clusters**, or there's just cataracts, hearing loss, forgetfulness, 'frailty' and eventually death. A more accurate measure of aging than chronology is **biological age acceleration**, predicted by **DNA methylation** levels. This gives us predictive information which compares us to the average biological age in a sex and age-matched cohort.

There has been tremendous progress and growth of interest in the field of aging but there are many questions left to answer. Interventions in the NAD+ system and in delaying senescent cells are considered promising. **FUNDAMENTUM has been formulated with a view to influence and support some of the biological mechanisms we now understand implicated in the aging process. They have been termed the pillars or hallmarks of aging. The formulation and product guide is based on scientific studies but does not constitute medical advice. It is possible for supplements to interact with other supplements, pharmaceuticals, foods, and health conditions.**

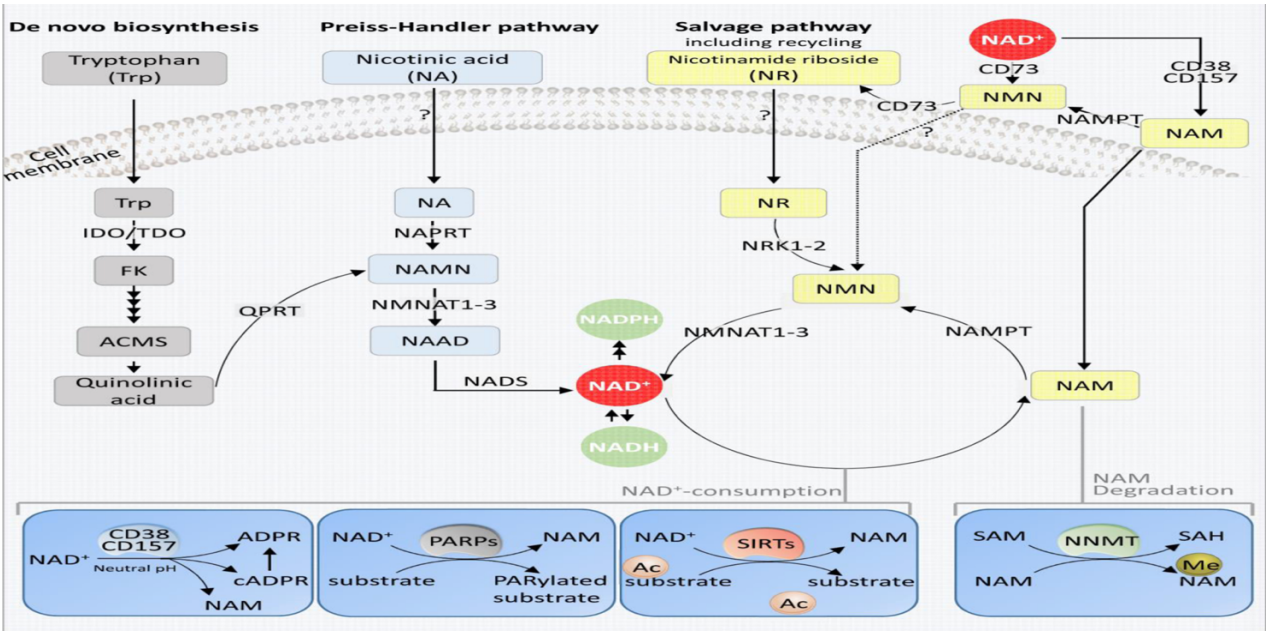
“But now I am six,
I am clever as clever.

So I think I’ll be six now
for ever and ever.”

- A.A. Milne

The fuel to boost NAD+

Nicotinamide mononucleotide (**NMN**) is obtained directly from the diet in small amounts, in various foods including **broccoli**, **tomatoes**, **avocado**, **edamame beans** and **milk**. It is also **produced** by the body from **vitamin B3** (niacin). Nicotinamide riboside (**NR**) and **NMN** are **precursors** for nicotinamide adenine dinucleotide (**NAD+**) and **NAD+** can be **synthesised** from NMN and NR in the **Salvage pathway**. It was previously thought **NMN** needed to be 'dismantled' to its components NR + phosphate prior to being **transported** into the cell, however scientists have purported the protein Slc12a8 a **direct transporter of NMN**. NMN is absorbed from the gut into blood circulation within a few minutes and transported into tissues within 30 minutes, **increasing tissue NAD+** content within an hour.



NAD+ keeps things running

NAD+ is an **organic cofactor** known as a **coenzyme** in **energy producing reactions** and is **necessary** for the **reactions to proceed**. It is also a **precursor** of cyclic ADP-ribose, an endoplasmic reticulum calcium messenger. Furthermore, **NAD+** is a **substrate** (**reactant**) **used** by **enzymes** (**proteins**). It was first reported in the early 20th century of having the ability to increase the rate of fermentation in yeast, although its importance was recognised by Dr Hans von Euler-Chelpin. In his 1930 Nobel lecture, he stated, "**cozymase NAD+ is one of the most widespread and biologically**

important activators within the plant and animal world." In 1939, it was found NAD+ cured black tongue disease in dogs, the canine equivalent of **pellagra**; a disease in humans resulting from deficiency in vitamin B3 (niacin) which causes dermatitis, diarrhoea, dementia, and death. In fact, without any NAD+, death would occur in about 30 seconds.

NAD+ is a metabolic pathway regulator

As a **coenzyme**, **NAD+** is especially important for **hydrogen transferring** oxidation-reduction (**redox**) reactions as a **metabolic pathway regulator**. NAD+ acts as a vehicle for hydrogen, accepting and carrying, and donating hydrogen as required. In the cytosol, NAD+ receives hydrogen and is reduced to NADH by lactate dehydrogenase during anaerobic 'lactic' glycolysis (about the first 60 seconds of exercise). In **mitochondria**, NAD+ is reduced to NADH by 3 enzymes and used in the electron transport chain during the oxidative breakdown of glucose (oxidative phosphorylation) or fatty acids (β-oxidation). The resulting 'broken-down', 'acquired' energy, is **stored for use** as adenine triphosphate (**ATP**). **ATP** is **essential** for life: it 'pays' for **heart** and **muscle contractions**, **brain** and **nerve impulse transmission** and **protein synthesis**. This is why **ATP** is known as the body's '**energy currency**', and as very little **ATP** can be stored, there is **constant need** to **resynthesise**. As a **redox coenzyme**, **NAD+** is **not consumed** and the mitochondrial NAD+/ NADH ratio is separately maintained from NAD+ pools in the cytosol, although there is a decrease in NAD+/ NADH ratio in human plasma with aging, likely due to a decrease in NAD+ stores as opposed to an increase in NADH content. The phosphorylated form of NADH (**NADPH**) is also a **critical substrate** for enzymes whose role is **scavenging reactive oxygen species** (ROS) and **preventing oxidative damage** to cells.

NAD+ is a fuel for all 7 sirtuins

As a **substrate, NAD+** is **consumed** by proteins in **post-translational modifications**. Post-translational modifications are '**edits**' to a protein where one or more amino acids are **chemically altered** or **removed**, so that it can **bind with** or i**nteract** with other proteins. This regulates and silences genes, the information needed for the replacement of cell parts or cells. One group of proteins involved in post-translational modifications and highlighted in **aging** research is the **sirtuin family** of proteins: silent information regulators. **Sirtuins** are **NAD+ dependent**; **NAD+** is **needed** and **used** by the **sirtuin**s in **deacetylation** reactions, removing the acetyl group from histones. Histones are proteins found in nuclei that provide structural support for chromosomes, helping DNA fit inside the

nucleus. Histones act like spools for the DNA, organising it into a compact shape. In humans, 7 sirtuins (SIRT1-7) have been identified. **SIRT 3, 4 and 5** are within **mitochondria**, regulating **mitochondrial stress**, energy metabolism and **mitochondrial DNA replication** (identical DNA strands) **transcription** (DNA to mRNA), and **translation** (mRNA into amino acids). **SIRT1, 6 and 7** are located mainly within the nucleus and deacetylate **intracellular signalling proteins**. **SIRT2** is located in the **cytoplasm** and deacetylates **signalling proteins**, **transcription factors** in the nucleus, and **metabolic enzymes** in the mitochondria, **regulating** a plethora of **cellular processes**.

Sirtuins influence the response to **stress and inflammation**, **vascular aging and function**, control energy **efficiency** and **metabolism** and affect **circadian rhythms** in the body. They are essential for **DNA repair** and maintain **genomic stability**, **preventing errors from DNA replication**; changes in nucleic acid sequences which may lead to aging, cancer and degenerative disease. Sirtuins help regulate **autophagy** and **apoptosis**.

Autophagy, or '**self-eating**' is the catabolic process by which **damaged** proteins (enzymes), and organelles (cell parts) including mitochondria, are degraded. During this process, the damaged material is **engulfed** and sequestered (isolated) by double-membraned autophagosomes which transport the autophagic cargo to the lysosomes for **recycling**. This is necessary for the **survival** of **cells** as well as marking for **apoptosis**, **programmed cell death**. When **apoptosis** is necessary, '**death receptor**' markers signal nearby **macrophages** (white blood cells) to **engulf the dying cell**. The cell shrinks and is **recycled without inflammatory cytokines** being released from the cytoplasm. This ensures there is **no inappropriate immune reaction** that would cause unnecessary inflammation. **Without autophagy** or **apoptosis**, the cell becomes **senescent** (**necrotic**), '**zombie**'-**like**. The cytoplasm swells, the cell membrane disrupts, and the contents of the cytoplasm is released causing an **immune response** and **inflammation**.

Senescent cells and SASP

Acute cellular **senescence** is a **necessary** stress response to trigger **permanent cell cycle arrest** in **response to damage** and we must not prevent this from happening. Senescence associated secretory phenotype (**SASP**) is a bioactive secretome triggered by the senescent cell to communicate its arrest to immune cells. However, **inappropriate SASP** from lingering senescent cells may cause **damage** to neighbouring normal cells and the structures and organs to which they belong. There are thoughts SASP contributes **to inflammaging and disruption of tissue homeostasis**.

Inflammaging is chronic low-grade inflammation

Aging leads to a **misregulation** of **programmed cell death**. It becomes more active than needed in some cells, whilst less active than needed in others. Human serum generally shows a **reduction** in markers of **apoptosis** in normal aging. This disruption of apoptosis affects **immune T** and **B lymphocytes** (white blood cells), and leads to a loss of **immunological memory**, a **reduced vaccination response** and **decreased apoptosis of virus- infected** cells. **Inflammaging** is chronic low-grade **inflammation**, the build-up of inflammation from **altered autophagy**, **mitophagy**, (programmed mitochondria breakdown), apoptosis and **altered immune response**. **Programmed cell death** is required for the **normal** '**turnover**' of cells to **prevent** various **diseases** from occurring. It has become normal for us to accept the alterations in these processes with increasing age.

NAD+ dysregulation is consequential to cellular homeostasis

Altered levels of **NAD+** metabolism is reported in models of animal and human **age-related disease**. Several enzymes are involved in **managing** (limiting) **NAD+ homeostasis**, including **CD38** and its homologue **CD157**. Although NAD+ is associated with pathways of DNA repair, cell turnover and suppression of inflammation, **excessive levels** of **NAD+ may be detrimental to health**. Just as with other essential compounds within the body, more is not always better. **Appropriate dosing of supplemental NAD+ precursors is important.**

Geroscientists have found that whilst **NAD+ levels fall with age**, **CD38 levels rise**. **CD38** is increased in response to **inflammation**, and CD38 **requires** and **consumes NAD+** to do its job. CD38 is also involved in insulin secretion and calcium mobilisation from bone. **NAD+ consuming enzymes** stand in **competition** with each other and **overly high levels of CD38** may **prevent other NAD+** dependent enzymes from doing their jobs in **geroprotection**. It is purported that natural molecule **apigenin**, a nutritional flavonoid, **may inhibit CD38**. **Reducing CD38 by reducing inflammation** may help **rebalance the dysregulated NAD+ system seen in aging**.

Some stress is necessary

ROS are **important signallers** within the body and whilst an **inappropriate amount** leads to cellular **damage** and **aging**, the **right amount** activates signalling responses and **routine** '**turnover**'. This stress concept is demonstrated in **mitochondria** with '**mitohormesis**': where an **acceptable**

concentration of ROS initiates a positive cascade of adaptive cellular events which ultimately **protect the cell** from harmful effects.

Mitochondria and aging

An increasing number of studies link **impairment** of **mitochondrial activity** to **aging** and **age associated disease**, hence mitochondrial function is highlighted within the model of the hallmarks of aging. **Mitochondrial function** is influenced by damage to the inner mitochondrial membrane, alterations in the function of the electron transport chain or a reduction in the transport of critical metabolites into mitochondria. These changes can lead to a **reduction or inefficiency in the production of ATP** as well as **increased ROS** (hydrogen leakage during energy metabolism). Mitochondrial membrane potential decreases have been shown to better represent the transcriptional and functional abilities of mitochondria than chronological age. **Improvements in mitochondrial membrane potential and mitophagy** (removal of damaged mitochondria), have been shown to **reduce** a plethora of **age-associated disease** (adaptive immune response, anaemia, cognition, cardiovascular function).

What can we currently conclude?

Increasing intracellular NAD+ by either stimulating NAD+ biosynthesis or inhibiting NAD+ consuming enzymes such as CD38, and **supporting activity of sirtuins**, may provide **anti-inflammatory**, **cardioprotective**, **neuroprotective,** (endothelial, vascular) and **metabolic health benefits**. There have been an array of studies investigating these findings in animals and in the last few years trials have begun in humans. Although long-term evidence is currently unavailable, short-term safety and efficacy data is emerging. The key insights from aging science in yeast, fruit flies and mice has led to **aging being recognised as a disease**, and significant advances in understanding the enzymatic reactions and mechanisms. Scientists have been able to demonstrate the acceleration as well as the slowing and delaying of aging and recognise many of the **hallmarks of aging share similarities in their underlying causes**. The research is promising and senolytic drugs have the potential to improve healthspan and positively affect more than 20 pathologies in model organisms. **The capability of supplements to alleviate age-related conditions** is exciting and scientists are investigating the putative effects to target the many underpinning pathways of aging.

VITA AETERNA supports this view and although their products are not intended to treat, cure or prevent any disease, they have been designed to support healthy aging. Fundamentum aims to reduce the age-related NAD+ decline and promote activity of sirtuins to encourage healthy aging. It helps to support the function of mitochondria to provide healthy cognitive and metabolic function. Fundamentum offers an effective multitarget approach, which allows the bases to be covered while still allowing for additional targeted single-point supplementation.

Therapeutics for healthy aging: FUNDAMENTUM

Although clarifying, the **hallmarks of aging** model fails to show the **interconnectedness** between the categories and **how one hallmark may overlap another**. **Fundamentum recognises the interwoven pathways and aims to reduce the age-related NAD+ decline and promote activity of sirtuins to encourage healthy aging. It helps to support the function of mitochondria to provide healthy cognitive and metabolic function. Fundamentum offers an effective multitarget approach, which allows the bases to be covered while still allowing for additional targeted single-point supplementation.**

Hope, passion and purpose are key to **healthier aging**. **VITA AETERNA** looks beyond pharmaceutical medicines to influence health and aging and would like to stress the importance of lifestyle factors, behaviour and environment – **the entire exposome** – that influence health and healthy aging. We recognise the issue of lack of available data sets in human populations and are **invested** in staying up to date with **geroscience** and **aging biology**. We also look forward to the development of affordable biomarkers to measure the effectiveness of healthy aging regimes.

Rick formulated Fundamentum as a novel therapeutic to support the **homeostatic** functions of the body with **evidence-based dosing** of a **NAD+ precursor** and carefully selected **flavonoids** effective in improving healthspan. Fundamentum supports the **synthesis of NAD+ and activation of sirtuins**, providing an array of **nutraceuticals** to facilitate **mitochondrial health** and **reduce chronic inflammation** by **managing senescent burden**.

However, he takes no responsibility if you are 35, supplement with **FUNDAMENTUM** and continue to look 50…

Product Details

- **Supports NAD+ biosynthesis**
- **Supports activation of sirtuins**
- **Facilitates mitochondrial health**
- **Helps protect against cellular aging**
- **Promotes clearance of senescent cells**
- **Reduces general fatigue**
- **Facilitates energy production**
- **Supports healthy aging**

- **NMN 500 mg**
- **Verisperse Resveratrol 400 mg**
- **Fisetin 50 mg**
- **PQQ 30mg**
- **Spermidine Trihydrochloride 10 mg**

Serving size: 2 capsules daily (morning)

It is not advised to exceed the recommended dose

NMN (500 mg)

Nicotinamide mononucleotide (**NMN**) is a natural product which exists in small quantities in plant foods and milk. It is a precursor to **NAD+** synthesised in the salvage pathway and may help to reduce the age-related decline of NAD+. NAD+ is a coenzyme essential in energy metabolism for the production of ATP, the only energy capable of being used for muscle contractions in humans. NAD+ is also a substrate and is required to activate sirtuins, essential for **DNA repair** and maintaining **genomic stability**, preventing errors from DNA replication. Sirtuins help regulate **autophagy** and **apoptosis**, managing senescent burden. Lower NAD+ levels may be responsible for the deterioration in health and energy we experience as we age. Many pre-clinical studies of supplemental NMN have been reported, treating Werner syndrome and vascular dysfunction in mice and cognition in an Alzheimer's rat model, and lifespan has been extended in yeast, worms and flies. Human clinical trials with NMN are limited but findings are starting to be published.

In 2020 Japanese scientists published the first human clinical trial. They studied the safety of NMN in a group of 10 healthy men aged 40-60 years. They found single, capsulated oral administration of

100 mg, 250 mg and **500 mg of NMN to be well tolerated**. There were no deleterious effects on systemic or ophthalmic measures, and it was found safe for human consumption. Blood plasma levels of NAD+ metabolites were measured and increased but NAD+ concentrations were not reported.

Since then, various human clinical trials have investigated the effects of capsulated oral NMN. Most designs have dosed NMN around 250 mg/ day, but have looked at use in men and women, prediabetic, obese and healthy, middle-aged and older adults and reported raised NAD+ blood concentration with supplementation. It is deemed safe and well-tolerated up to 12 weeks duration and appears to **improve fatigue** and walking exercise performance but not physical strength when compared to exercise alone. There has been mixed data on the effects on insulin sensitivity. Some studies have found an improvement following 300 mg NMN, however Yi et al. (2023), found no significant improvement in the Homeostasis Model Assessment – Insulin Resistance (HOMA-IR) test, following 60 days of capsulated oral NMN in 80 middle-aged (mean age 49) healthy volunteers (59% female). This double-blind, placebo-controlled study compared doses of 300 mg, 600 mg and 900 mg daily. Blood NAD+ concentrations were significantly increased over placebo in all 3 NMN groups, with the 600 mg group having significantly higher NAD+ blood concentration at days 30 and 60 compared to the 300 mg group. There were no further statistical differences in NAD+ blood concentrations between the 600 mg or 900 mg groups. Participants in the 600 mg and 900 mg groups walked significantly further in the six-minute walking test compared to the 300 mg group, and again, no further statistical differences were found to suggest improvements between the 600 mg or 900 mg groups. All NMN treated groups improved blood biological age and quality of life scores from baseline to day 60 compared to placebo and it was well tolerated and considered safe at all doses.

In conclusion, studies to date have reported oral capsulated NMN up to 900 mg/ day to be safe and well tolerated. It appears to **raise NAD+ blood concentration** and have favourable results on blood biological age, physical endurance, fatigue and quality of life in healthy adults and doses over 600 mg/ day do not give significantly better efficacy compared to a 600 mg/ day dose. It will be interesting to follow the progress of longer trial end points and specific health improvement outcomes.

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doi: 10.1126/science.abe9985
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doi: 10.3389/fragi.2022.851698
doi: 10.1038/s41514-022-00084-z
doi: 10.3389/fnut.2022.868640
doi: 10.3390/nu14040755
doi: 10.1016/j.cmet.2018.02.011
doi: 10.1038/s41467-019-13172-8
doi: 10.1016/j.cmet.2016.09.013

VeriSperse® Resveratrol (400 mg) **VeriSperse® is a trademark of Pharmako Biotechnologies Pty Ltd**

Resveratrol is a naturally occurring polyphenol that is well known to be found in the skins of grapes, berries and wine. It was first extracted from white hellebore in the 1940s and later in the 1960s from the roots of Polygonum Cuspidatum, a plant used in Chinese and Japanese medicine. It can be extracted from plants such as Japanese Knotweed and grapeseed and can be chemically synthesised or produced via fermentation. It sparked interest in the 90's for purported benefits on the cardiovascular system and more recently in aging research, for **extending the lifespans** of lower organisms.

The mechanism by which resveratrol exerts these beneficial effects is not yet clear, and it may have interactions with a broad range of proteins as a **polyphenol** as opposed to activating specific pathways. Some claim it enhances the effects of nitric oxide by activating nitric oxide synthase (eNOS and iNOS respectively), facilitating **endothelial function** as a vasorelaxant. It may block platelet aggregation in a similar way to aspirin. Others have studied it as an activator of **AMPK**, a modulator of energy and glucose uptake typically observed during caloric restriction. While geroscience has suggested resveratrol may be an inducer of deacetylase activity (**SIRT1**); pro-apoptic and antioxidant, **reducing inflammaging** and orchestrating the recruitment of anti-inflammatory genes. As with many natural polyphenolic compounds, it is worth considering the compounding **synergistic interactions** of resveratrol with other nutraceuticals such as those found in this formulation.

One clear observation from the literature is the generally low concentrations in blood plasma following ingestion in mammals and this makes it challenging to sufficiently supply resveratrol at concentrations akin to those demonstrating favourable effects in vitro. **Fundamentum has used a novel ingredient to overcome this limitation**. **VeriSperse®** is a cold-water dispersible resveratrol powder, specifically designed to increase its **bioavailability**. Utilising exclusive patented LipiSperse® technology enhances resveratrol's normally limited absorption and efficacy as it is more readily available. In a single-dose, double blind study, healthy adults receiving 150 mg of **VeriSperse® doubled absorption and increased plasma trans-resveratrol conjugates 3x more** than another commercially available resveratrol.

doi: 10.1016/j.medici.2016.03.003
doi.org/10.1074/jbc.M501250200
doi.org/10.1016/j.cub.2005.12.038
doi.org/10.4049/jimmunol.164.12.6509
doi.org/10.1016/j.biopha.2021.112164
doi.org/10.1016/j.bbagrm.2010.06.005
doi: 10.3390/pharmaceutics12121190
doi: 10.1038/nrd2060

Fisetin (50 mg)

Fisetin is a naturally occurring flavonoid (polyphenol) found in plants most commonly known for its presence in strawberries, apples and onions. Fisetin has shown **antioxidant** and **neuroprotective** effects which may modulate **senolytic** pathways contributing to aging. Quercetin has been discussed for its senotherapeutic activity, however the primary study showing anti-aging benefits combined quercetin with dasatinib, a pharmacological compound used in the treatment of leukaemia which may cause toxicity. As such, pharmaceutical senolytic therapies have the potential to administered quarterly or annually to avoid these deleterious effects. Natural compounds have the advantage of continual administration and therefore have gained scientific interest.

After screening a selection of flavonoids, the same group of scientists investigating quercetin and dasatinib identified **fisetin as having greater therapeutic effect in cultured cells than quercetin alone**. To investigate this in vivo, they administered fisetin to a group of genetically modified mice, a group of aged wild-type mice and human fat tissue explants. They tested senescent markers, age-related histopathology, disease markers and the effects on healthspan and lifespan. They concluded **fisetin reduced senescence**, restored tissue homeostasis, reduced age-related pathology and extended median (a measure of average age) and maximum lifespan.

Another group studied the beneficial effects of fisetin on **brain aging**. They found brain oscillatory waves and cortical spectral power associated with coordination and complex behavioural performance improved following 4 weeks fisetin supplementation in age rates compared to aged controls.

Fisetin has been given to patients and improved outcomes with traditional stroke treatment. It has senotherapeutic activity and has no reported side effects. It is likely the benefits found in the limited studies to date are a combination of the **antioxidant effects** and **sirtuin activation** associated with polyphenols. There may be **synergistic interactions** with other ingredients in this formulation.

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PQQ (30 mg)

Mitochondria are found within our cells and generate most of the chemical energy called adenosine triphosphate (ATP), needed to power the cell's and hence our body's reactions. Cells which are highly active such as muscle, kidney, liver and brain, contain many mitochondria. Mitochondria contain their own special DNA (from our mother's) which make 13 proteins (enzyme 'nanobots') needed to do important work for the mitochondria. We need to replace mitochondria frequently and this is known as **mitochondrial biogenesis**. In producing cellular energy, mitochondria are exposed to constant free radical activity (ROS) which can damage the mitochondria itself as well as its DNA. Oxidants produced by the mitochondria; proton leakage across the inner membrane, appear to be the major source of oxidative lesions which decrease membrane fluidity. **Mitochondrial dysfunction** due to this oxidative damage is suggested to be a contributing factor in the **aging process**. It is thought the disruption to mitochondrial DNA (mtDNA) may be involved in the associated decline in insulin secretory capacity as we age.

Pyrroloquinoline quinine (**PQQ**) is a naturally occurring coenzyme in foods reported to support **mitochondrial biogenesis** as a result of **protection from ROS** and stimulation of the necessary pathway proteins. There have been studies in mice and rats lacking PQQ which show reduced mitochondrial content. Further research suggests PQQ an activator of **SIRT1** signalling pathways and enhancer of cellular **NAD+** formation. Scientists are interested in PQQ's potential in the protection of nerve cells by increased expression of **nerve growth factor** and nerve growth factor receptors, suppression of fibril formation and aggregation (build up from lack of sirtuin breakdown) of 'sticky' amyloid β.

PQQ may support memory and cognitive function as we age. In a double-blind study of 58 healthy participants (male and female; 40-80), 12 weeks orally administered 20 mg PQQ disodium salt demonstrated significantly improved scores in judgement, attention, reaction time, verbal memory and executive function compared to placebo. What was interesting in this study was both groups, those receiving PQQ and those not (placebo) showed cognitive improvements from base line.

PQQ may help in the aging context, supporting anti-inflammatory and SIRT1 activity in mitochondria, improving mitochondrial biogenesis, cellular energy and cognition in aging.

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Spermidine Trihydrochloride 10 mg

Spermidine is a natural **polyamine**, whose tissue **concentrations decline with age**. Polyamines play an important role in **autophagy and apoptosis**, functions necessary to maintain homeostasis in our cells. Polyamines are not considered vitamins as our body is able to synthesise them. In humans, spermidine is synthesised from ornithine or by oxidative breakdown of spermidine. The intestinal microbiota also synthesises spermidine.

Dietary spermidine may be a promising strategy supporting healthspan.

Adding spermidine to the diets of yeast, fruit flies and mice, **extended healthspan and lifespan** by a staggering 10%. In mice it was found to suppress a decline in cardiovascular function , reducing blood pressure and slowing the progression of congestive heart failure, whilst in fruit flies it reversed a decline in memory. It also appears likely from non-mammalian and mouse model organisms, that spermidine can delay neurodegeneration to some extent. It has been suggested these effects result from the stimulation of lysine deacetylases (**sirtuins**) and perhaps by a pathway the same as that of salicylic acid (**EP300**), the active metabolite of aspirin, although the details of the broad effects this has on human health has not yet been elucidated.

Recently, an epidemiological study of 829 participants (45-84) observed over 15 years, reported high nutritional spermidine intake was correlated with **reduced incidence of cancer and cardiovascular disease**. The data was collected by food frequency questionnaires every 5 years and corrected for confounding factors such as age, sex, body mass, alcohol and aspirin consumption, dietary quality, metabolic diseases, physical activity and socioeconomic status. This correlation between higher dietary **spermidine and reduced mortality** was seen in another study in which 1770 healthy participants (39-67 years) were followed for 13 years. Neither study measured plasma spermidine concentrations, autophagic flux or acetylation levels to establish an association.

In an aging society, cognitive decline resulting from age-related diseases such as Alzheimer's are commonplace. A group of scientists assessed whether 12 months of 0.9 mg supplemental spermidine would improve memory performance in a group of 100 participants (mean age 69; 49 % female). The **SmartAge study** enrolled otherwise healthy individuals who were in subjective cognitive decline. There was no statistically significant outcome, but they did demonstrate a **trend for improvement** in mnemonic discrimination and behaviour in the spermidine group from baseline. This was not in

line with their previous study which showed statistical improved in these measures. They did however find a beneficial effect of spermidine supplementation on sICAM-1 blood plasma level, a parameter of blood vessel injury and inflammation normally seen with endothelial dysfunction, inflammatory processes, aging and dementia. It is likely **the dose of spermidine was too low** as most people would normally obtain something closer to 10 mg from their diet. For comparison, the epidemiological group of 829 participants were consuming around 11 mg.

Spermidine is considered safe and tolerable in humans and has been shown to protect against **neurodegeneration and cognitive decline** in aged animal models. It has been shown to reduce a blood marker for **endothelial dysfunction and dementia in humans and to promote autophagy, mitophagy and apoptosis**.

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doi: 10.1080/15548627.2018.1454810
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FUNDAMENTUM has been formulated with a view to influence and support some of the biological mechanisms we now understand implicated in the aging process. They have been termed the pillars or hallmarks of aging. Fundamentum aims to reduce the age-related NAD+ decline and promote activity of sirtuins to encourage healthy aging. It helps to support the function of mitochondria to provide healthy cognitive and metabolic function. Fundamentum offers an effective multitarget approach, which allows the bases to be covered while still allowing for additional targeted single-point supplementation.

- **Reduce cellular senescence**
- **Reduce mitochondrial dysfunction**
- **Reduce genomic instability**

References

Age related and atherosclerosis related erythropathy in ApoE/ LDLR mice 10.1016/ j.bbadis.2020

An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA. 10.1016/j.devcel.2014.11.012

ARDD 2020: from aging mechanisms to interventions. 10.18632/aging.202454

Biological mechanisms of aging predict age-related disease co-occurrence in patients. 10.1111/acel.13524

CD38 dictates age-related NAD decline and mitochondrial dysfunction through an SIRT3-dependent mechanism. 10.1016/j.cmet.2016.05.006

Chronic inflammation in the etiology of disease across the life span. 10.1038/s41591-019-0675-0

Circadian control of the NAD^+ salvage pathway by CLOCK-SIRT1. 10.1126/science.1170803

Deacetylation assays to unravel the interplay between sirtuins (SIRT2) and specific protein-substrates. 10.3791/53563

Elevation of blood NAD level after moderate exercise in young women and mice. 10.3177/jnsv.47.177

Epigenetic changes during aging and their reprogramming potential. 10.1080/10409238.2019.1570075

Evolution of ageing. 10.1016/s0047-6374(01)00419-5

Genetically encoded biosensors for evaluating NAD+/ NADH ratio in cytosolic and mitochondrial compartments. 10.1016/jcrmeth.2021.100116

Hacking aging: A strategy to use big data from medical studies to extend human life. 10.3389/fgene.2018.00483

Healthspan versus lifespan: new medicines to close the gap. 10.1038/s43587-022-00318-5

Human age reversal: Fact or fiction? 10.1111/acel.13664

Inflammaging: a new immune-metabolic viewpoint for age-related diseases. 10.1038/s41574-018-0059-4

Long-term administration of nicotinamide mononucleotide mitigates age-associated physiological decline in mice 10.1016/j.cmet.2016.09.013

Loss of epigenetic information as a cause of mammalian aging. 10.1016/j.cell.2022.12.027

Macrophages in age-related chronic inflammatory diseases. 10.1038/npjamd.2016.18

Metabolic alterations in aging macrophages: ingredients for inflammaging? 10.1016/j.it.2018.12.007

Mitochondrial and metabolic dysfunction in ageing and age-related diseases. 10.1038/s41574-021-00626-7

Mitochondrial function and chronic disease: Treatment with natural supplements. PMID: 26770107

Mitochondrial proteostasis in the control of aging and longevity. 10.1016/j.cmet.2014.05.006

Mitohormesis: Promoting health and lifespan by increased levels of reactive oxygen species (ROS). 10.2203/dose-response.13-035.Ristow

Mitophagy in neurodegeneration and aging. 10.1016/j.neuint.2017.02.007

NAD augmentation restores mitophagy and limits accelerated aging in Werner syndrome. 10.1038/s41467-019-13172-8

NAD+ homeostasis and NAD+ consuming enzymes: Implications for vascular health. 10.3390/antiox12020376

NAD+ improves cognitive function and reduces neuroinflammation by ameliorating mitochondrial damage and decreasing ROS production in chronic cerebral hypoperfusion models through Sirt1/PGC-1α pathway. 10.1186/s12974-021-02250-8

NAD^+ in aging, metabolism, and neurodegeneration. 10.1126/science.aac4854

NAD^+ in aging: Molecular mechanisms and translational implications. **10.1016/jmolmed.2017.08.001**

NAD^+ Intermediates: The biology and therapeutic potential of NMN and NR. 10.1016/j.cmet.2017.11.002

NAD^+ in brain aging and neurodegenerative disorders. 10.1016/j.cmet.2019.09.001

NAD^+ metabolism and its roles in cellular processes during ageing. 10.1038/s41580-020-00313-x

New hallmarks of aging. 10.18632/aging.204248

Nicotinamide enhances mitochondria quality through autophagy activation in human cells. 10.1111/j.1474-9726.2009.00487.x

Nicotinamide-induced mitophagy: event mediated by high NAD^+/NADH ratio and SIRT1 protein activation. 10.1074/jbc.M112.363747

Obesity may accelerate the aging process. 10.3389/fendo.2019.00266

Preserved stem cell content and innervation profile of elderly human skeletal muscle with lifelong recreational exercise. 10.1113/JP282677

Programmed cell death in aging. 10.1016/j.arr.2015.04.002

Protecting the mitochondrial powerhouse. 10.1016/j.tcb.2014.11.002

Senescence and the SASP: many therapeutic avenues. 10.1101/gad.343129.120

Senescent cells promote tissue NAD^+ decline during ageing via the activation of CD38+ macrophages. 10.1038/s42255-020-00305-3

Senolytics and the compression of late-life mortality. 10.1016/j.exger.2021.111588

Senolytics in idiopathic pulmonary fibrosis: Results from a first-in-human, open-label, pilot study. 10.1016/j.ebiom.2018.12.052

Short-term NAD^+ supplementation prevents hearing loss in mouse models of Cockayne syndrome. 10.1038/s41514-019-0040-z

Sirtuins activators and inhibitors: Promises, achievements, and challenges. 10.1016/j.pharmthera.2018.03.004

Slc12a8 is a nicotinamide mono-nucleotide transporter. 10.1038/s42255-018-0009-4

The CD38 glycohydrolase and the NAD sink: implications for pathological conditions. 10.1152/ajpcell.00451.2021

The efficacy and safety of β-nicotinamide mononucleotide (NMN) supplementation in healthy middle-aged adults: a randomized, multicentre, double-blind, placebo-controlled, parallel-group, dose-dependent clinical trial. 10.1007/s11357-022-00705-1

The hallmarks of aging. 10.1016/j.cell.2013.05.039

The impact of aging on mitochondrial function and biogenesis pathways in skeletal muscle of sedentary high- and low-functioning elderly individuals. 10.1111/j.1474-9726.2012.00844.x

The intersection between aging and cardiovascular disease. 10.1161/CIRCRESAHA.111.246876

The role of senescent cells in ageing. 10.1038/nature13193

The science behind NMN – A stable, reliable NAD+ activator and anti-aging molecule. **PMID: 32549859**

Therapeutic potential of NAD boosting molecules: The in vivo evidence. 10.1016/j.cmet.2018.02.011

Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. 10.1038/35001622