In this review, we describe the role of oxidized forms of nicotinamide adenine dinucleotide (NAD⁺) as a molecule central to health benefits as the result from observing selected healthy lifestyle recommendations. Namely, NAD⁺ level can be regulated by lifestyle and nutrition approaches such as fasting, caloric restriction, sports activity, low glucose availability, and heat shocks. NAD⁺ is reduced with age at a cellular, tissue, and organismal level due to inflammation, defect in NAMPT-mediated NAD⁺ biosynthesis, and the PARP-mediated NAD⁺ depletion. This leads to a decrease in cellular energy production and DNA repair and modifies genomic signalling leading to an increased incidence of chronic diseases and ageing. By implementing healthy lifestyle approaches, endogenous intracellular NAD⁺ levels can be increased, which explains the molecular mechanisms underlying health benefits at the organismal level. Namely, adherence to here presented healthy lifestyle approaches is correlated with an extended life expectancy free of major chronic diseases.

1. Introduction

Fasting, caloric restriction, sports activity, low glucose availability, and heat shocks are lifestyle and nutrition approaches that influence NAD⁺ levels [1–6]. Deficiency in NAMPT-mediated NAD⁺ biosynthesis, increased inflammation, and the PARP-mediated NAD⁺ depletion are causes of reduced NAD⁺ levels with age at a cellular, tissue, and organismal level [7, 8].

Coenzyme nicotinamide adenine dinucleotide (NAD⁺), which contains two covalently joined mononucleotides (nicotinamide mononucleotide or NMN, and AMP) [9], has an important role in an energy metabolism like mitochondrial electron transport, glycolysis, and citric acid cycle [10] in order to generate adenosine triphosphate (ATP) [11]. NAD⁺ is also a rate-limiting substrate for many signalling enzymes such as sirtuin (SIRT) proteins SIRT1 and SIRT3, the poly (ADP-ribose) polymerase (PARP) proteins PARP1 and PARP2, a COOH-terminal binding protein (CTBP), cyclic ADP-ribose (ADPR) synthetases CD38 and CD157, and many other NAD⁺-dependent enzymes. These enzymes are involved in important cellular processes, like DNA repair, stress response, genomic stability, chromatin remodelling, circadian rhythm regulation, cell cycle progression, insulin secretion and sensitivity, and expression of the inflammatory cytokines, thus translating changes in energy status into metabolic adaptations [12]. NAD⁺ is recycling during ATP formation in processes of glycolysis, beta-oxidation, Krebs cycle, and electron transport in cytosol and mitochondria and shifts between reduced and oxidized forms as required for the continuous flow of electrons across the metabolic pathways. Therefore, the NAD⁺ molecule is conserved during these processes. On the other hand, the NAD⁺ is consumed during cellular signalling, in adenosine diphosphate (ADP)-ribosyl transfer reactions, by poly-ADP-ribose polymerases (PARPs), sirtuin deacetylases (Sirtuins), and the cluster of differentiation 38 (CD38), i.e., the nicotinamide (NAM) unit is separated. NAD⁺ half-life is between 1–2 h in the cytoplasm and nucleus and approximately 8 h in the mitochondria [13] and can be salvaged and reused by three pathways: (1) de novo synthesis (from L-tryptophan), (2) Preiss-Handler pathway (from nicotinic acid or nicotinic
acid ribose), and (3) salvage pathway (from niacinamide/ni-cotinamide, nicotinamide riboside, and nicotinamide mononucleotide) [9, 14–18]. NAD$^+$ is mainly produced by the NAD$^+$ salvage pathway where nicotinamide phosphoribosyltransferase (NAMPT) is the rate-limiting enzyme, converting NMN into NAD$^+$ [19–21]. NAMPT regulates processes related to the pathological processes of obesity and a type 2 diabetes mellitus by influencing lipid and glucose metabolism, insulin resistance, the oxidative stress response, apoptosis, and inflammation [22, 23].

The NAD$^+/$/NADH ratio influences also the reactive oxygen species (ROS) and oxidative stress formation through regulation of intracellular ATP production, different metabolic enzymes, and redox state. An increase of NAD$^+$ and/or NAD$^+/$/NADH ratio can increase cell defence, can induce DNA repair and apoptosis through activation of PARPs and sirtuins, and thus plays an important role in the prevention of cancerogenesis and many other diseases [14, 24]. For example, cellular NAD$^+/$/NADH ratio regulates SIRT1 enzymatic activity, which further regulates a number of target proteins [25], such as FOXO family of transcription factors [26–28], p53 [29, 30], PGC-1α [31, 32], and NF-kB [33–35]. While chronic diseases and ageing are related to decreased NAD$^+$ levels [16, 36, 37], different lifestyle factors have been found that ameliorate NAD$^+$ bioavailability, which positively affects SIRT stimulation and subsequent PGC-1α and FOXO1 expression, leading to mitochondrial changes and metabolic adaptations (Figure 1) [38]. Increased available cellular energy, improved stem cell and mitochondrial function, DNA repair [39], telomere maintenance [40], and enhanced metabolic activity are prerequisites for effective health span and life span [41, 42] as demonstrated by studies where NAD$^+$ levels were intentionally increased [23, 43–48].

2. Caloric Restriction, Eating Habits, and NAD$^+$ Levels

A well-balanced diet in macro- and micronutrients represents a basis for health and well-being. Limited calorie intake continues to be the strategy supported by the greatest evidence for ensuring increased lifespan and health [49]. In different model organisms, a significant increase in life-span was reported if calories were restricted between 25–60% relative to normally fed control [50, 51]. How is caloric restriction connected with NAD$^+$ levels? CR stimulates the NAD$^+$ salvage pathway leading to increased NAD$^+$ bioavailability by activating the expression of NAMPT, which triggers the NAD$^+$ salvage pathway by transforming nicotinamide (NAM) to NAD$^+$ [52]. Caloric restriction increases NAD$^+$ levels, while lowers NADH levels and activates sirtuins [53]. For example, caloric restriction extends the yeast’s life span by lowering the level of NADH, since NADH is a competitive inhibitor of Sir2 [54]. Thus, activation of sirtuins with a sufficient amount of bioavailable NAD$^+$ is a necessary condition for the life-span extension provided by CR [55, 56]. Specifically, Sir1 regulates CR by detecting intracellular low energy levels and provoking physiological changes relevant to health and longevity [57]. On the other hand, inactivation of SIRT1 results in the prevention of CR-mediated lifespan extension [58].

Studies on caloric restriction revealed that it is more important to improve the ratio between NAD$^+$ and NADH than to raise the overall amount of cellular NAD$^+$ [59]. Namely, caloric restriction reduces NADH amount more than it influences the NAD$^+$ levels, at least in yeast [54, 60]. It seems that lowering NADH is an important factor responsible for the increased activity of the NAD$^+$-consuming enzymes, as NADH is an inhibitor of Sirtuins and PARPs [54].

Besides by caloric restriction, NAD$^+$ levels can be increased with food and commercially available supplements. Ingestion of the amino acid tryptophan or forms of vitamin B3 (niacin, nicotinic acid, niacinamide) as well as nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and nicotinic acid riboside (NaR) stimulates the formation of NAD$^+$ [61–64]. Daily requirements for NAD$^+$ synthesis can be obtained either with dietary tryptophan or with around 15 mg/d of daily niacin, a collective term for nicotinic acid (NA) and nicotinamide (NAM) [61], which can be found in meat, fish, and dairy products [65].

Small-scale human clinical studies have shown that NAD$^+$ boosters such as NMN, NR, and niacin can increase the levels of NAD$^+$ in volunteers and are relatively safe for human consumption [6, 66–72]. Most of the side effects reported during treatment with NAM, NR, and NMN are

![Figure 1: Health benefits as a result of implementing approaches to increase NAD$^+$ bioavailability.](image-url)
minor (e.g., diarrhea, nausea, rashes, hot flashes, cramps in the legs, erythema) and occur relatively rarely [73, 74]. Increased acetyl carnitine concentrations in skeletal muscle and minor changes in body composition and sleeping metabolic rate were reported in the recent study on NR supplementation in healthy obese humans [75]. The evidence for assessing the health risk is still limited, and long-term exposure to NAD⁺ booster (NR, NMN) has not yet been investigated in human clinical trials or human clinical trials are not yet completed. In addition, there is insufficient data on increasing the levels of NAD⁺ in various clinical disorders.

As data for some newly discovered NAD⁺ precursor forms are scarce, NAD⁺ supplements should be tested in a manner similar to drugs in development [72]. Niacin equivalents/precurors are found in animal and plant foods, mainly in the form of NA and NAM. Additionally, recently discovered NAD⁺ intermediates, such as NMN and NR, are also in foods, like cucumber, cabbage, and immature soybeans. Broccoli has 0.25–1.88 mg of NMN per 100 g, avocado and tomato 0.26–1.60 mg/100 g. Much less NMN can be found also in raw beef and shrimp (0.06–0.42 mg/100 g) [45] as well as human and cow milk at micromolar concentrations [76, 77]. NAD⁺ biosynthesis can be increased by direct activation of NAD⁺ biosynthetic enzymes by several AMPK and NAMPT activators, like nonflavonoid polyphenol resveratrol, metformin, 5-aminomidazole-4-carboxamide ribonucleotide, P7C3, leucine, epigallocatechin gallate, and proanthocyanidins [78–86]. CD38, its homologue CD157, and PARP-1 inhibitors could additionally increase NAD⁺ availability; however, they are registered as medical drugs for cancer treatment [24], thus beyond the scope of this review.

3. Eating Habits

NAD⁺/sirtuin pathway could be influenced also with nutritional approaches, e.g., eating habits. At what time and what and how much food we eat influence intracellular NAD⁺ bioavailability by altering electron transport in mitochondria. For example, a high-fat/sugar diet causes energy overload, culminating in reduced NAD⁺/NADH ratio [87] and decreases NAD⁺ levels [23, 63]. Also, a low AMP/ATP ratio causes a decrease in NAD⁺ or NAD⁺/NADH, in situations when enormous amounts of calorically rich food (lipids and/or carbohydrates) are eaten. This additionally leads to elevated blood sugar and insulin levels, increased NADH/NAD⁺ ratio, and increased formation of ROS, which triggers the postprandial oxidative stress and oxidative damage [88–91]. Large amounts of electrons from sugars enter the mitochondria after a large portion of food that generates more superoxide at complex I (NADH: ubiquinone oxidoreductase) and complex III (ubiquinol: cytochrome c oxidoreductase) [92]. Efficient electron flow and avoidance of electron leaks (superoxide formation) can be achieved if ATP is regularly consumed; for example, by moderate sport activity or any kind of physical work. This increases the AMP/ATP ratio and NAD⁺ availability [87, 93, 94]. The link between the metabolism and NAD⁺ is further strengthened by observations that besides overnutrition, tissue NAD⁺ levels decrease also with high-fat diets and obesity [23, 63, 95–98]. Rappou et al. [99] compared SIRT1, SIRT3, SIRT7, and NAMPT expressions and total PARP activity in lean and obese subjects. Results indicated lower sirtuins and NAMPT expressions and increased total PARP activity in obese compared to lean subjects. After a moderate weight loss, SIRT1 and NAMPT expressions increased while PARP activity significantly decreased in subjects upon the weight loss. Similar results were obtained in healthy men during lipid overfeeding [100]. Other studies observed that obesity is associated with low NAD⁺ levels or SIRT pathway expression [101]. On the other hand, supplementation with NAD⁺ precursors or intermediates activates sirtuins and oxidative metabolism resulting in the protection against high-fat diet-induced obesity [63], improved glucose tolerance and hepatic insulin sensitivity [22], and lipid metabolism [45].

A high-fat caloric diet induces obesity through the protein CD38, which is a regulator of body weight and an NAD⁺ consumer [102]. Mice deficient in CD38 are protected against the high-fat diet-induced obesity due to boosted metabolic rate in part via a NAD⁻-dependent stimulation of SIRT-PGC1α axis [102]. Adipose tissue elevates the expression of CD38 and inflammation-related genes in obese people [103, 104]. In line, low expression of CD38 protected against obesity when fed a high-fat diet in animals [102, 105].

NAD⁺ level is not only nutritionally controlled, but it depends also on the sports activities and other lifestyle factors.

4. Exercise and NAD⁺ Levels

Physical activity and exercise, as part of a healthy lifestyle, have a significant impact on health outcomes, including improved motor skills, healthy bones, enhanced aerobic fitness, efficient heart and lung function, improved cardiovascular health, lowered risk of stroke, certain types of cancer and diabetes, improved metabolic flexibility and mitochondrial function, and a positive effect on cognitive function and mental health—including on depressive symptom improvement and anxiety- or stress-related disease [38, 106–108].

How does sports activity affect NAD⁺ levels? Aerobic exercise training or any kind of exercise/sports activity increases the amount of NAD⁺ due to the induction of skeletal muscle’s NAMPT expression that was shown in rodent and human studies [109–111]. Namely, in human skeletal muscle, exercise training reverses the age-dependent decline of NAD⁺ by stimulating the NAD⁺ salvage pathway, in which nicotinamide NAMPT is a rate-limiting enzyme [112]. Exercise and aerobic sports activity increases the amount of NAD⁺ due to the induction of skeletal muscle’s NAMPT expression [109] and reverses the age-dependent decline of NAD⁺ by stimulating the NAD⁺ salvage pathway [112] through the 5′ AMP-activated protein kinase (AMPK) pathways [4].

NAD⁺ has an important role in the generation of intracellular ATP, which is required for exercise and sports activities. On the other hand, as already mentioned, ATP production in mitochondria represents the main source of
free radical generation. The reduction state of complex I in mitochondria depends strongly on the NAD⁺ and NADH levels. Ameliorating the NAD⁺/NADH ratio by elevated ATP consumption (e.g., sports activity) or decreased ATP production (e.g., intermittent fasting, consumption of small portions of food, and CR) regulates the magnitude of superoxide-generation from the transfer of electrons to molecular oxygen at mitochondrial complexes I and III and can thus ameliorate the intensity of oxidative damage [113]. Increased demand for energy during the exercise is sensed by the cell and activates AMPK, which can modulate NAD⁺ bioavailability [38]. Both exercise and caloric restriction trigger the metabolic stress that follows by adaptation by inducing NAMPT expression through the AMPK [4, 109, 114] resulting in increased NAD⁺ levels available for sirtuins and PARPs.

A recent study by de Guia et al. revealed that different exercise training methods reverse the age-dependent decline in NAD⁺ salvage capacity in the human skeletal muscle [112]. Namely, both aerobic and resistance exercise training increased NAMPT levels in young and older individuals. In aged rats, exercise training also increased NAD⁺, NAMPT levels, and SIRT1 activity [111] and accelerates the de novo biosynthesis of NAD⁺ from L-tryptophan [115].

The important function of NAD⁺ during sports activity is its role as a hydrogen/electron transfer molecule for adenosine triphosphate (ATP) production and mitochondrial biogenesis in muscle cells [116]. Additionally, sports activity increases the NAD⁺ amount also at the systemic level [117] that results in health benefits at the organismal level due to the NAD⁺ role in multiple and diverse cellular processes, in addition to redox reactions, such as deacetylation and ADP-ribosylation [116]. During the intense sports activity, ATP is consumed; thus, the need for NADH as the electron donor increases, which in the end results in the boosted formation of oxidised NAD⁺ and decreased NADH, i.e., an improved NAD⁺/NADH ratio. The total amount of NAD⁺ is not significantly changed during the redox reaction; however, the NAD⁺/NADH (and NADP to NADPH) ratio is changed in favour of NAD⁺ [61], which activates sirtuins, PARPs, CD38, and other NAD⁺-consuming reactions. Since NAD⁺-consuming enzymes intervene in many crucial cellular processes, many healthy processes at the organismal level are enhanced by the implementation of exercise and sports activity.

Surprisingly, NR, the NAD⁺ precursor, decreases exercise performance in rats [118], most likely due to the pleiotropic metabolic and redox properties of NAD⁺ and NADP+. Nicotinic acid also reduced the capacity for high-intensity exercise in humans [119], which is ascribed to lower plasma free fatty acids, leading to earlier fatigue. Studies on NAD⁺ precursor supplementation implied prevention of vascular dysfunction, oxidative stress, and muscle age-degeneration in mice [45, 46, 120]. Accordingly, it is important to preserve a high NAD⁺ to NAD⁺/NADH ratio that can be achieved also by sports activity.

5. Circadian Rhythms, Sleeping Habits, and NAD⁺ Levels

Sleep disorders predispose persons to chronic diseases like obesity, depression, diabetes, and many cardiometabolic diseases, which are significantly associated with mortality and morbidity [121, 122] [123–125]. Contrary, a steady pattern of waking and sleeping is associated with health promotion and longevity [126]. Prolonged disruptions of circadian rhythms are associated with negative health consequences [127]. NAD⁺ levels and sirtuin activity regulate a healthy circadian rhythm of sleep and wakefulness; concurrently, the NAD⁺ level is supervised by circadian rhythm and involved in the circadian clock regulation. NAD⁺ levels oscillate with a 24 h rhythm; these can be modified by feeding and sleeping time [128–130]. The central internal clock is in the hypothalamic suprachiasmatic nucleus, and the circadian rhythms are coordinated by intracellular proteins called “circadian clocks.” These proteins are regulated by a transcriptional negative feedback loop between transcriptional activators CLOCK and BMAL1 and repressors CRY and PER. CLOCK, the core circadian regulator, is a histone acetyltransferase whose activity is outweighed by the nicotinamide adenine dinucleotide- (NAD⁺-) dependent histone deacetylase SIRT1 [131, 132]. CLOCK:BMAL1 heterodimer balances the circadian expression of NAMPT, which regulates the NAD⁺ biosynthesis. The activity of NAMPT is constrained by light—or sleep—deprivation and upregulated by darkness and night [130].

With ageing, NAMPT activity declines, and consequently, NAD⁺ bioavailability drops [7, 133], leading to the deterioration of the circadian rhythm (change in amplitude, period, and phase). Disrupted circadian rhythms were reported in many pathological conditions including cardiovascular diseases, diabetes, cancer, and accelerated ageing [134, 135]. On the contrary, matching the innate circadian period results in health improvements [135–140].

6. Environmental Stress: Heat/Cold Shock and NAD⁺ Levels

Exposure to the elevated heat for short time periods can result in beneficial health effects. Cardiovascular responses to long-term adaptations in response to heat stress result in reduced blood pressure and arterial stiffness and improved endothelial and microvascular function [141]. For example, regular sauna bathing may be linked to several health benefits, which include decreased risk of sudden cardiac death and cardiovascular and all-cause mortality [142], reduction in the risk of neurocognitive diseases and nonvascular conditions such as pulmonary diseases, and amelioration of conditions such as arthritis, headache, and flu [143]. What is more, heat stress cardioprotection and improved posts ischemic functional recovery in the heat-stressed hearts after cardioplogic arrest due to increased NAD⁺ and NADP⁺ concentrations were observed [144]. Heat shock triggers an increase in the NAD⁺/NADH ratio as a result of decreased NADH levels and an increase in recruitment of SIRT1 to the hsop70 promoter [25]. Enzyme nicotinamide mononucleotide adenylly-transferase (NMNAT), which catalyzes nicotinamide adenine dinucleotide (NAD⁺) synthesis, is elevated during conditions of heat shock and transcriptionally regulated by the heat shock factor (HSF) and hypoxia-inducible factor 1α (HIF1α) in vivo [145, 146].
In addition to heat stress, also cold stress-induced physiological responses and activation of brown adipose tissue (BAT) have health benefits [147]. BAT mainly burns energy in contrast to white adipose tissue (WAT), which stores fat [141]. In mouse and human BAT, cold exposure activates NAD$^+$ biosynthesis mediated by a rate-limiting enzyme, NAMPT [148]. BAT is abundant in mitochondria and plays a role in energy expenditure related to producing heat by an energy-dissipating process of nonshivering thermogenesis, leading to changes in lipid metabolism [149] and other health benefits like the absence of low-grade inflammation, increased insulin sensitivity, and decreased liver fat [150, 151]. Degradation, whitening, and impaired function of BAT promotes obesity [152–155].

The facts supporting the "NAD$^+$ > SIRTs > positive effect" pathway as the mechanism of action for the beneficial effects of NAD$^+$ repletion strategies have been presented so far. Are there indications of concerns about increasing the levels of NAD$^+$?

7. Potential Deleterious Effects of Increased NAD$^+$

As already discussed, NAD$^+$ precursors, nicotinic acid (NA), and NR decreased exercise performance in young rats [118] and reduce the capacity for high-intensity exercise in humans [119], although old individuals seem to benefit from NR supplementation. Namely, increased NAD(P)H levels, decreased oxidative stress, and improved physical performance were observed only in the old subjects [156]. Kourtzidis et al. [157] expressed concern that redox agents administered exogenously in healthy young populations (not suffering from antioxidant deficiency) might lead to adverse effects. Nicotinamide (NAM) overdose was reported to cause hepatotoxicity in rare cases [158]. In addition, it was observed that a high dose of dietary NR caused glucose intolerance and dysfunction of the white adipose tissue in mice fed a slightly obese diet [159].

Regarding longevity, overexpression of SIRT1 was found not to extend lifespan in mice fed standard diets, although they had better general health and fewer carcinomas [160]. Mitchell et al. [43] observed that supplementation with NAM in the mouse model did not change the lifespan, in spite of the improved healthspan. Additionally, Chen et al. [161] challenge the paradigm that CR induces SIRT1 activity in all tissues. Similarly, Frederick et al. [162] suggest that NMN and NR increase in NAD biosynthesis is cell- or tissue-specific.

It appears that the NAD$^+$ levels could have both pro-cancer and anticancer effects, as NAD$^+$ is a critical protective factor in early cancer development and could become a damaging factor later in the phase of cancer progression and promotion. Namely, during cancer promotion, progression, and treatment, increased NAD$^+$ levels could have adverse effects on the malignancy process due to increased cell survival, growth advantage, increased resistance to radio- and chemotherapy, and promotion of inflammation. In contrast, NAD$^+$ restoration could prevent or reverse the phenotype of malignant cells in the early stages by inducing cellular repair and adaptive stress responses and regulating cell cycle arrest and apoptotic removal of damaged cells (reviewed in [24]). In addition, the compound FK866, which inhibits the nicotinamide recycling enzyme NAMPT, is a tumor apoptosis inducer due to the NAD$^+$ depletion [163, 164] and is used as an anticancer drug.

In the area of inflammation/sepsis, there is also controversy regarding the NAD$^+$-dependent sirtuin family, as elevated NAD$^+$ levels play a different role in the different stages of sepsis. In the initial (proinflammatory) phase, which is characterized by a cytokine storm, overproduction of reactive oxygen species (ROS), and metabolic shift [165], SIRT1 activation shows positive effects, whereas the SIRT1 expression should be inhibited in the later stages of sepsis [166]. Therefore, due to the dynamic phases of sepsis, the role of SIRT1 cannot simply be defined as beneficial or detrimental. Increased NAD$^+$ might have also negative effects on inflammatory disorders, such as rheumatoid arthritis due to stimulated inflammatory cytokine secretion by leukocytes [167].

Another potential risk could be posed by the toxic degration products and metabolites of NAD$^+$ precursors, e.g., nicotinic acid adenine dinucleotide (NAAD), N-methyl nicotinamide (MeNAM), and 2-PY [71, 168]. Lastly, increased NAM levels due to the supplementation with NAD$^+$ precursors (NAM, NR, or NMN) could inhibit PARPs and CD38 activities [169], while SIRT1 feedback inhibition in vivo by NAM may not be so important [170, 171]. Increased levels of NAM might alter also the methyl pool used to methylate DNA and proteins [171].

8. Conclusions

It is not only the NAD/NADH redox role as hydride and electron transfer in redox metabolic reactions but mainly the NAD$^+$ as the signalling molecule and substrate for sirtuins and PARPs that is responsible for the health benefits and longevity. Cellular NAD$^+$ content and an adequate NAD$^+$/NADH ratio can postpone pathologic processes associated with impaired cell signalling and mitochondrial function [87, 172, 173]. Thus, for maintaining optimal cellular functioning and organismal health, it is necessary to implement the lifestyle approaches that stimulate increased NAD$^+$ levels. The synergistic effects of different measures to ensure a healthy lifestyle are important, as there is an intimate and reciprocal relationship between them. For example, sedentary lifestyle, overeating, and excessive intake of fat and sugar are associated with disturbances in circadian rhythms [174, 175] and downregulation of NAMPT gene expression [4]. Implementation of the time-restricted feeding without reducing the caloric intake (8h per day feeding/16h per day fasting) improved the robustness of circadian and metabolic rhythms and prevented metabolic diseases in mice on a high-fat diet [176]. Lifestyle approaches, such as exercise and CR, can reverse insulin resistance and type 2 diabetes mellitus (T2DM) [12]. Both manipulations increase the NAMPT-mediated NAD$^+$ generation, activate mechanistic pathways of AMPK, and enhance the SIRT1 activity and mitochondrial function [4, 114, 177, 178]. Sirtuins affect various cellular processes, including lipid metabolism, insulin
secretion, and sensitivity [179]. NAD$^+$ levels within cells are regulated by its precursors’ intake, biosynthetic pathways, and degradative enzymes [180], which can be additionally balanced by selected lifestyle factors discussed here. In order to provide sufficient NAD$^+$ bioavailability and appropriate expression of NAMPT, it is necessary to ingest sufficient amounts of NAD$^+$ precursors/intermediates in the vitamin B3 forms, preferably as a part of a normal diet, to practice regular and moderate sports activity, and to observe time intervals between darkness and light exposure as well as the appropriate time intervals between feeding and fasting.

The presented studies support the hypothesis that maintaining NAD$^+$ levels leads to healthy cell metabolism, which is beneficial in terms of amelioration of metabolic diseases and ageing. It should be stressed that NAD$^+$ is not the only factor, but rather one of the several components that influence cell health. There are many other positive effects of caloric restriction, eating habits, exercise, circadian rhythms, and environmental stress on human health that are beyond the scope of this paper. Although many animal studies have shown the link between NAD$^+$ and healthspan, the complex role of NAD$^+$ in the etiology of ageing and age-related chronic diseases in humans should be further elucidated. The current state of knowledge about NAD$^+$ positive effects on ageing and healthspan is mainly based on experiments on cell cultures and model organisms, so that the positive health effects of NAD$^+$ in humans will need to be confirmed in future in-depth studies and clinical trials.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Acknowledgments

The authors acknowledge the financial support from the Slovenian Research Agency (Research Core Funding No. P3-0388 and P3-0019).

References

[1] A. R. Hipkiss, “Energy metabolism, altered proteins, sirtuins and ageing: converging mechanisms?,” Biogerontology, vol. 9, no. 1, pp. 49–55, 2008.
[2] K. C. Morris, H. W. Lin, J. W. Thompson, and M. A. Perez-Pinzon, “Pathways for ischemic cytoprotection: role of sirtuins in caloric restriction, resveratrol, and ischemic preconditioning,” Journal of Cerebral Blood Flow & Metabolism, vol. 31, no. 4, pp. 1003–1019, 2011.
[3] E. Morselli, M. C. Maiuri, M. Markaki et al., “Caloric restriction and resveratrol promote longevity through the Sir2-dependent induction of autophagy,” Cell Death & Disease, vol. 1, no. 1, 2010.
[4] M. Fulco, Y. Cen, P. Zhao et al., “Glucose restriction inhibits skeletal myoblast differentiation by activating SIRT1 through AMPK-mediated regulation of Nampt,” Developmental Cell, vol. 14, no. 5, pp. 661–673, 2008.
[5] Y. Yang, H. Cimen, M. J. Han et al., “NAD$^+$-dependent deacetylation of the ribosomal protein MRPL10,” Journal of Biological Chemistry, vol. 285, no. 10, pp. 7417–7429, 2010.
[6] S. A. J. Trammell, M. S. Schmidt, B. J. Weidemann et al., “Nicotinamide riboside is uniquely and orally bioavailable in mice and humans,” Nature Communications, vol. 7, no. 1, article 12948, pp. 1–14, 2016.
[7] N. Braidy, G. J. Guillemín, H. Mansour, T. Chan-Ling, A. Poljak, and R. Grant, “Age related changes in NAD$^+$ metabolism oxidative stress and Sirt1 activity in Wistar rats,” PLoS One, vol. 6, no. 4, article e19194, 2011.
[8] Y. Li, J. Schoufour, D. D. Wang et al., “Healthy lifestyle and life expectancy free of cancer, cardiovascular disease, and type 2 diabetes: prospective cohort study,” BMJ, vol. 368, article 6669, 2020.
[9] S. Imai and L. Guarente, “NAD$^+$ and sirtuins in aging and disease,” Trends in Cell Biology, vol. 24, no. 8, pp. 464–471, 2014.
[10] Y. Chi and A. A. Sauve, “Nicotinamide riboside, a trace nutrient in foods, is a vitamin B3 with effects on energy metabolism and neuroprotection,” Current Opinion in Clinical Nutrition and Metabolic Care, vol. 16, no. 6, pp. 657–661, 2013.
[11] A. C. Chen and D. L. Damian, “Nicotinamide and the skin,” Australasian Journal of Dermatology, vol. 55, no. 3, pp. 169–175, 2014.
[12] Y. S. Elhassan, A. A. Philp, and G. G. Lavery, “Targeting NAD$^+$ in metabolic disease: new insights into an old molecule,” Journal of the Endocrine Society, vol. 1, no. 7, pp. 816–835, 2017.
[13] X. A. Cambronne, M. L. Stewart, D. Kim et al., “Biosensor reveals multiple sources for mitochondrial NAD$^+$,” Science, vol. 352, no. 6292, pp. 1474–1477, 2016.
[14] P. Belenky, K. L. Bogan, and C. Brenner, “NAD$^+$ metabolism in health and disease,” Trends in Biochemical Sciences, vol. 32, no. 1, pp. 12–19, 2007.
[15] L. R. Stein and S. I. Imai, “The dynamic regulation of NAD metabolism in mitochondria,” Trends in Endocrinology & Metabolism, vol. 23, no. 9, pp. 420–428, 2012.
[16] R. H. Houtkooper, C. Cantó, R. J. Wanders, and J. Auwerx, “The secret life of NAD$^+$: an old metabolite controlling new metabolic signaling pathways,” Endocrine Reviews, vol. 31, no. 2, pp. 194–223, 2010.
[17] S. I. Imai and L. Guarente, “It takes two to tango: NAD$^+$ and sirtuins in aging/longevity control,” npj Aging and Mechanisms of Disease, vol. 2, no. 1, article 16017, 2016.
[18] C. Cantó, K. J. J. Menzies, and J. Auwerx, “NAD$^+$ Metabolism and the Control of Energy Homeostasis: A Balancing Act between Mitochondria and the Nucleus,” Cell metabolism, vol. 22, no. 1, pp. 31–53, 2015.
[19] S. I. Imai, “The NAD world 2.0: the importance of the inter-tissue communication mediated by NAMPT/NAD$^+$/SIRT1 in mammalian aging and longevity control,” npj Systems Biology and Applications, vol. 2, no. 1, article 16018, pp. 1–9, 2016.
[20] J. Evans, T. C. Wang, M. P. Heyes, and S. P. Markey, “LC/MS analysis of NAD biosynthesis using stable isotope pyridine precursors,” Analytical Biochemistry, vol. 306, no. 2, pp. 197–203, 2002.
[21] J. R. Revollo, A. A. Grimm, and S. I. Imai, “The NAD biosynthesis pathway mediated by nicotinamide phosphoribosyltransferase regulates Sir2 activity in mammalian cells,”
A. Garten, S. Schuster, M. Penke, T. Gorski, T. De Giorgis, and W. Kiess, “Physiological and pathophysiological roles of NAMPT and NAD metabolism,” *Nature Reviews Endocrinology*, vol. 11, no. 9, pp. 535–546, 2015.

J. Yoshino, K. F. Mills, M. J. Yoon, and S. I. Imai, “Nicotinamide Mononucleotide, a Key NAD Intermediate, Treats the Pathophysiology of Diet- and Age-Induced Diabetes in Mice,” *Cell Metabolism*, vol. 14, no. 4, pp. 528–536, 2011.

B. Poljsak, “NAD+ in cancer prevention and treatment: pros and cons,” *Journal of Clinical & Experimental Oncology*, vol. 5, no. 4, 2016.

R. Raynes, K. M. Pombier, K. Nguyen, J. Brunquell, J. E. Mendez, and S. D. Westerheide, “The SIRT1 modulators AROS and DBC1 regulate HSFI activity and the heat shock response,” *PLoS One*, vol. 8, no. 1, article e45464, 2013.

M. Viswanathan, S. K. Kim, A. Berdichevsky, and L. Guarente, “A Role for SIR-2.1 Regulation of ER Stress Response Genes in Determining *C. elegans* Life Span,” *Developmental Cell*, vol. 9, no. 5, pp. 605–615, 2005.

A. Brunet, L. B. Sweeney, J. F. Sturgill et al., “Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase,” *Science*, vol. 303, no. 5666, pp. 2011–2015, 2004.

M. C. Motta, N. Divecha, M. Lemieux et al., “Mammalian SIRT1 represses forkhead transcription factors,” *Cell*, vol. 116, no. 4, pp. 551–563, 2004.

H. Vaziri, S. K. Dessain, E. N. Eaton et al., “hSIRT2/SIRT1 Functions as an NAD-Dependent p53 Deacetylase,” *Cell*, vol. 107, no. 2, pp. 149–159, 2001.

J. Luo, A. Y. Nikolaev, S. Imai et al., “Negative control of p53 by Sir2a promotes cell survival under stress,” *Cell*, vol. 107, no. 2, pp. 137–148, 2001.

J. T. Rodgers, C. Lerin, W. Haas, S. P. Gygi, B. M. Spiegelman, and P. Puigserver, “Nutrient control of glucose homeostasis through a complex of PGC-1α and SIRT1,” *Nature*, vol. 434, no. 7029, pp. 113–118, 2005.

J. T. Rodgers, C. Lerin, Z. Gerhart-Hines, and P. Puigserver, “Metabolic adaptations through the PGC-1α and SIRT1 pathways,” *FEBS Letters*, vol. 582, no. 1, pp. 46–53, 2008.

F. Yeung, J. E. Hoberg, C. S. Ramsey et al., “Modulation of NF-κB-dependent transcription and cell survival by the SIRT1 deacetylase,” *The EMBO Journal*, vol. 23, no. 12, pp. 2369–2380, 2004.

A. Salminen, J. Huuskonen, J. Ojala, A. Kauppinen, K. Kaarniranta, and T. Suuronen, “Activation of innate immunity system during aging: NF-kB signaling is the molecular culprit of inflamm-aging,” *Aging Research Reviews*, vol. 7, no. 2, pp. 83–105, 2008.

K. J. Jung, E. K. Lee, J. Y. Kim et al., “Effect of short term caloric restriction on pro-inflammatory NF-kB and AP-1 in aged rat kidney,” *Inflammation Research*, vol. 58, no. 3, article 7227, pp. 143–150, 2009.

J. A. Khan, F. Forouhar, X. Tao, and L. Tong, “Nicotinamide adenine dinucleotide metabolism as an attractive target for drug discovery,” *Expert Opinion on Therapeutic Targets*, vol. 11, no. 5, pp. 695–705, 2007.

W. Ying, “Therapeutic potential of NAD+ for neurological diseases,” *Future Neurology*, vol. 2, no. 2, pp. 129–132, 2007.

N. J. Connell, R. H. Houthkooper, and P. Schrauwen, “NAD+ metabolism as a target for metabolic health: have we found the silver bullet?,” *Diabetologia*, vol. 62, no. 6, pp. 888–899, 2019.

J. Li, M. S. Bonkowski, S. Moniot et al., “A conserved NAD+ binding pocket that regulates protein-protein interactions during aging,” *Science*, vol. 355, no. 6331, pp. 1312–1317, 2017.

T. Zhang and W. L. Kraus, “SIRT1-dependent regulation of chromatin and transcription: linking NAD+ metabolism and signaling to the control of cellular functions,” *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, vol. 1804, no. 8, pp. 1666–1675, 2010.

H. Zhang, D. Ryu, Y. Wu et al., “NAD+ repletion improves mitochondrial and stem cell function and enhances life span in mice,” *Science*, vol. 352, no. 6292, pp. 1436–1443, 2016.

B. H. Goodpaster and L. M. Sparks, “Metabolic flexibility in health and disease,” *Cell Metabolism*, vol. 25, no. 5, pp. 1027–1036, 2017.

S. J. Mitchell, M. Bernier, M. A. Aon et al., “Nicotinamide improves aspects of healthspan, but not lifespan, in mice,” *Cell Metabolism*, vol. 27, no. 3, pp. 667–676.e4, 2018.

T. Yamamoto, J. Byun, P. Zhai, Y. Ikeda, S. Oka, and J. Sadowshima, “Nicotinamide mononucleotide, an intermediate of NAD+ synthesis, protects the heart from ischemia and reperfusion,” *PLoS One*, vol. 9, no. 6, article e98972, 2014.

K. F. Mills, S. Yoshida, L. R. Stein et al., “Long-Term Administration of Nicotinamide Mononucleotide Mitigates Age-Associated Physiological Decline in Mice,” *Cell Metabolism*, vol. 24, no. 6, pp. 795–806, 2016.

N. E. de Picciotto, L. B. Gano, L. C. Johnson et al., “Nicotinamide mononucleotide supplementation reverses vascular dysfunction and oxidative stress with aging in mice,” *Aging Cell*, vol. 15, no. 3, pp. 522–530, 2016.

S. Tarantini, M. N. Valcarcel-Ares, P. Toth et al., “Nicotinamide mononucleotide (NMN) supplementation rescues cerebrovascular endothelial function and neurovascular coupling responses and improves cognitive function in aged mice,” *Redox Biology*, vol. 24, article 101192, 2019.

G. M. Uddin, N. A. Youngson, S. S. Chowdhury, D. A. Sinclair, and M. J. Morris, “Administration of nicotinamide mononucleotide (NMN) reduces metabolic impairment in male mouse offspring from obese mothers,” *Cells*, vol. 9, no. 4, 2020.

J. J. Carmona and S. Michán, “Biologic of healthy aging and longevity,” *Revista de investigación clinica*, vol. 68, no. 1, pp. 7–16, 2016.

R. J. Colman, R. M. Anderson, S. C. Johnson et al., “Caloric restriction delays disease onset and mortality in rhesus monkeys,” *Science*, vol. 325, no. 5937, pp. 201–204, 2009.

C. Cruzen and R. J. Colman, “Effects of caloric restriction on cardiovascular aging in non-human primates and humans,” *Clinics in Geriatri Medicine*, vol. 25, no. 4, pp. 733–743, 2009.

A. Mennsen, P. Hydbring, K. Kapelle et al., “The c-MYC oncprotein, the NAMPT enzyme, the SIRT1-inhibitor DBC1, and the SIRT1 deacetylase form a positive feedback loop,” *Proceedings of the National Academy of Sciences*, vol. 109, no. 4, pp. E187–E196, 2012.

H. Massudi, R. Grant, G. J. Guillemin, and N. Braidy, “NAD+ metabolism and oxidative stress: the golden nucleotide on a crown of thorns,” *Redox Report*, vol. 17, no. 1, pp. 28–46, 2012.
[54] S. J. Lin, E. Ford, M. Haigis, G. Liszt, and L. Guarente, “Calor- 
rie restriction extends yeast life span by lowering the level of NAD+,” Genes & Development, vol. 18, no. 1, pp. 12–16, 2004.

[55] S. J. Lin, P. A. DeFossez, and L. Guarente, “Requirement of NAD and SIRT2 for life-span extension by calorie restriction in saccharomyces cerevisiae,” Science, vol. 289, no. 5487, pp. 2126–2128, 2000.

[56] I. B. Leibiger and P. O. Berggren, “Sirt1: a metabolic master switch that modulates lifespan,” Nature Medicine, vol. 12, no. 1, pp. 34–36, 2006.

[57] L. Guarente and F. Picard, “Calorie Restriction— the SIRT2 Connection,” Cell, vol. 120, no. 4, pp. 473–482, 2005.

[58] T. O. Tollefsbol, “Dietary epigenetics in cancer and aging,” Advances in Nutrition and Cancer, vol. 159, pp. 257–267, 2014.

[59] W. Ying, “NAD'/NADH and NADP/NADPH in cellular functions and cell death: regulation and biological consequences,” Antioxidants & Redox Signaling, vol. 10, no. 2, pp. 179–206, 2008.

[60] C. Evans, K. L. Bogan, P. Song, C. F. Burant, R. T. Kennedy, and C. Brenner, “NAD+ metabolite levels as a function of vitamins and calorie restriction: evidence for different mechanisms of longevity,” BMC Chemical Biology, vol. 10, no. 1, 2010.

[61] K. L. Bogan and C. Brenner, “Nicotinic acid, nicotinamide, and nicotinamide riboside: a molecular evaluation of NAD+ precursor vitamins in human nutrition,” Annual Review of Nutrition, vol. 28, no. 1, pp. 115–130, 2008.

[62] S. I. Imai, “The NAD world: a new systemic regulatory network for metabolism and aging-Sirt1, systemic NAD biosynthesis, and their importance,” Cell Biochemistry and Biophysics, vol. 53, no. 2, pp. 65–74, 2009.

[63] C. Cantó, R. H. Houtkooper, E. Pirinen et al., “The NAD+ precursor nicotinamide riboside enhances oxidative metabolism and protects against high-fat diet-induced obesity,” Cell Metabolism, vol. 15, no. 6, pp. 838–847, 2012.

[64] A. P. Gomes, N. L. Price, A. J. Y. Ling et al., “Declining NAD+ induces a pseudohypoxic state disrupting nuclear-mitochondrial communication during aging,” Cell, vol. 155, no. 7, pp. 1624–1638, 2013.

[65] Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate, O.B.V. and C, Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothetic acid, biotin, and choline, National Academies Press, Washington (DC), 1998.

[66] J. Irie, E. Inagaki, M. Fujita et al., “Effect of oral administration of nicotinamide mononucleotide on clinical parameters and nicotinamide metabolite levels in healthy Japanese men,” Endocrine Journal, vol. 67, no. 2, pp. 153–160, 2020.

[67] C. R. Martens, B. A. Denman, M. R. Mazzo et al., “Chronic nicotinamide riboside supplementation is well-tolerated and elevates NAD+ in healthy middle-aged and older adults,” Nature Communications, vol. 9, no. 1, 2018.

[68] S. E. Airthart, L. M. Shireman, L. J. Rusler et al., “An open-label, non-randomized study of the pharmacokinetics of the nutritional supplement nicotinamide riboside (NR) and its effects on blood NAD+ levels in healthy volunteers,” PLoS One, vol. 12, no. 12, article e0186459, 2017.

[69] E. Pirinen, M. Auranen, N. A. Khan et al., “Niacin cures systemic NAD+ deficiency and improves muscle performance in adult-onset mitochondrial myopathy,” Cell Metabolism, vol. 31, no. 6, pp. 1078–1090.e5, 2020.

[70] D. Conze, C. Brenner, and C. L. Kruger, “Safety and metabo- 
lism of long-term administration of NIAGEN (nicotinamide riboside chloride) in a randomized, double-blind, placebo-controlled clinical trial of healthy overweight adults,” Scientific Reports, vol. 9, no. 1, article 9772, 2019.

[71] Y. S. Elhassan, K. Kluckova, R. S. Fletcher et al., “Nicotinamide Riboside Augments the Aged Human Skeletal Muscle NAD+ Metabolome and Induces Transcriptomic and Anti-inflammatory Signatures,” Cell Reports, vol. 28, no. 7, pp. 1717–1728.e6, 2019.

[72] B. Poljsak and I. Milisav, “Vitamin B3 forms as precursors to NAD+: are they safe?,” Trends in Food Science & Technology, vol. 79, pp. 198–203, 2018.

[73] J. Dragovic, S. H. Kim, S. L. Brown, and J. H. Kim, “Nicotinamide pharmacokinetics in patients,” Radiotherapy and Oncology, vol. 36, no. 3, pp. 225–228, 1995.

[74] N. Braidy and Y. Liu, “NAD+ therapy in age-related degenerative disorders: a benefit/risk analysis,” Experimental Gerontology, vol. 132, article 110831, 2020.

[75] C. M. E. Remie, K. H. M. Roumans, M. P. B. Moonen et al., “Nicotinamide riboside supplementation alters body composition and skeletal muscle acetyl carnitine concentrations in healthy obese humans,” The American Journal of Clinical Nutrition, vol. 112, no. 2, pp. 413–426, 2020.

[76] P. Bieganowski and C. Brenner, “Discoveries of Nicotinamide Riboside as a Nutrient and Conserved NRK Genes Establish a Preiss-Handler Independent Route to NAD+ in Fungi and Humans,” Cell, vol. 117, no. 4, pp. 495–502, 2004.

[77] S. Ummarino, M. Mozzon, F. Zamporlini et al., “Simulta- 
neous quantitation of nicotinamide riboside, nicotinamide mononucleotide and nicotinamide adenine dinucleotide in milk by a novel enzyme-coupled assay,” Food Chemistry, vol. 221, pp. 161–168, 2017.

[78] J. A. Baur, “Resveratrol, sirtuins, and the promise of a DR mimetic,” Mechanisms of Ageing and Development, vol. 131, no. 4, pp. 261–269, 2010.

[79] S. Timmers, J. Auwerx, and P. Schrauwen, “The journey of resveratrol from yeast to human,” Aging, vol. 4, no. 3, pp. 146–158, 2012.

[80] J. Kim, G. Yang, Y. Kim, J. Kim, and J. Ha, “AMPK activators: mechanisms of action and physiological activities,” Experimental & Molecular Medicine, vol. 48, no. 4, article e224, 2016.

[81] C. Cantó, Z. Gerhart-Hines, J. N. Feige et al., “AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity,” Nature, vol. 458, no. 7241, pp. 1056–1060, 2009.

[82] G. Wang, T. Han, D. Nijhawan et al., “PTC3 neuroprotective chemicals function by activating the rate-limiting enzyme in NAD salvage,” Cell, vol. 158, no. 6, pp. 1324–1334, 2014.

[83] H. Li, M. Xu, J. Lee, C. He, and Z. Xie, “Leucine supplementation increases SIRT1 expression and prevents mitochondrial dysfunction and metabolic disorders in high-fat diet-induced obese mice,” American Journal of Physiology-Endocrinology and Metabolism, vol. 303, no. 10, pp. E1234–E1244, 2012.
proanthocyanidins boost hepatic NAD⁺ metabolism and SIRT1 expression but reduced PARP activity in white adipose tissue.

Reports

NAMPT expression and NAD levels in rat liver, "Scientific Reports," vol. 6, no. 1, article 24977, 2016.

[8] A. Ribas-Latre, L. Baselga-Escudero, E. Casanova et al., "Dietary proanthocyanidins modulate BMAL1 acetylation, Nampt expression and NAD levels in rat liver," "Scientific Reports," vol. 5, no. 1, article 10954, 2015.

[9] F. Berger, C. Lau, M. Dahlmann, and M. Ziegler, "Subcellular compartmentation and differential catalytic properties of the three human nicotinamide mononucleotide adenyltransferase isoforms," "Journal of Biological Chemistry," vol. 280, no. 43, pp. 36334–36341, 2005.

[10] R. H. Houtkooper and J. Auwerx, "Hypothalamic SIRT1 prevents age-associated weight gain by improving leptin sensitivity in mice," "Diabetologia," vol. 57, no. 4, article 3140, pp. 819–831, 2014.

[11] A. I. Cederbaum, "Alcohol metabolism," "Clinics in Liver Disease," vol. 16, no. 4, pp. 667–685, 2012.

[12] K. C. McElfresh and J. F. McDonald, "The effect of alcohol stress on nicotinamide adenine dinucleotide (NAD⁺) levels in Drosophila," "Biochemical Genetics," vol. 21, no. 3–4, pp. 365–374, 1983.

[13] L. Bleier and S. Dröse, "Superoxide generation by complex III: from mechanistic rationales to functional consequences," "Biochimica et Biophysica Acta (BBA)-Bioenergetics," vol. 1827, no. 11-12, pp. 1320–1331, 2013.

[14] R. Nogueiras, K. M. Habegger, N. Chaudhary et al., "Sirtuin 1 and sirtuin 3: physiological modulators of metabolism," "Physiological Reviews," vol. 92, no. 3, pp. 1479–1514, 2012.

[15] M. J. Yoon, M. Yoshida, S. Johnson et al., "SIRT1-mediated eNAMPT secretion from adipose tissue regulates hypothalamic NAD⁺ and function in mice," "Cell Metabolism," vol. 21, no. 5, pp. 706–717, 2015.

[16] P. Bai, C. Cantó, H. Oudart et al., "PARP-1 inhibition increases mitochondrial metabolism through SIRT1 activation," "Cell Metabolism," vol. 13, no. 4, pp. 461–468, 2011.

[17] D. Kraus, Q. Yang, D. Kong et al., "Nicotinamide N-methyltransferase knockout protects against diet-induced obesity," "Nature," vol. 508, no. 7495, pp. 258–262, 2014.

[18] E. Pirinen, C. Cantó, Y. S. Jo et al., "Pharmacological inhibition of poly(ADP-ribose) polymerases improves fitness and mitochondrial function in skeletal muscle," "Cell Metabolism," vol. 19, no. 6, pp. 1034–1041, 2014.

[19] S. J. Yang, J. M. Choi, L. Kim et al., "Nicotinamide improves glucose metabolism and affects the hepatic NAD-sirtuin pathway in a rodent model of obesity and type 2 diabetes," "The Journal of Nutritional Biochemistry," vol. 25, no. 1, pp. 66–72, 2014.

[20] E. Rappou, S. Jukarainen, R. Rinnankoski-Tuikka et al., "Weight loss is associated with increased NAD⁺/SIRT1 expression but reduced PARP activity in white adipose tissue," "The Journal of Clinical Endocrinology & Metabolism," vol. 101, no. 3, pp. 1263–1273, 2016.

[21] K. Seysel, M. Alligier, E. Meugnier et al., "Regulation of energy metabolism and mitochondrial function in skeletal muscle during lipid overfeeding in healthy men," "The Journal of Clinical Endocrinology & Metabolism," vol. 99, no. 7, pp. E1254–E1262, 2014.

[22] S. Jukarainen, S. Heinonen, J. T. Rämö et al., "Obesity is associated with low NAD⁺/SIRT1 pathway expression in adipose tissue of BMI-discordant monozygotic twins," "Journal of Clinical Endocrinology & Metabolism," vol. 101, no. 1, pp. 275–283, 2016.

[23] M. T. P. Barbosa, S. M. Soares, C. M. Novak et al., “The enzyme CD38 (a NAD glycohydrolase, EC 3.2.2.5) is necessary for the development of diet-induced obesity,” "The FASEB Journal," vol. 21, no. 13, pp. 3629–3639, 2007.

[24] S. Nair, Y. H. Lee, E. Rousseau et al., “Increased expression of inflammation-related genes in cultured preadipocytes/stromal vascular cells from obese compared with non-obese Pima Indians,” "Diabetologia," vol. 48, no. 9, pp. 1784–1788, 2005.

[25] D. M. Mutch, J. Tordjman, V. Pelloux et al., "Needle and surgical biopsy techniques differentially affect adipose tissue gene expression profiles," "The American Journal of Clinical Nutrition," vol. 89, no. 1, pp. 51–57, 2009.

[26] L. F. Wang, L. J. Miao, X. N. Wang et al., "CD38 deficiency suppresses adipogenesis and lipogenesis in adipose tissues through activating Sirt1/PPARγ signaling pathway," "Journal of Cellular and Molecular Medicine," vol. 22, no. 1, pp. 101–110, 2018.

[27] WHO, "Global action plan for the prevention and control of NCDs 2013-2020," World Health Organization, Geneva, 2015.

[28] C. Malm, J. Jakobsson, and A. Isaksson, "Physical activity and sports—real health benefits: a review with insight into the public health of Sweden," "Sports," vol. 7, no. 5, 2019.

[29] C. R. Jenkin, R. M. Eime, H. Westerbeek, G. O’Sullivan, and J. G. Z. Van Uffelen, "Sport and ageing: a systematic review of the determinants and trends of participation in sport for older adults," "BMC Public Health," vol. 17, no. 1, 2017.

[30] J. Brandauer, S. G. Vienberg, M. A. Andersen et al., "AMP-activated protein kinase regulates nicotinamide phosphorysyl transferase expression in skeletal muscle," "The Journal of Physiology," vol. 591, no. 20, pp. 5207–5220, 2013.

[31] S. R. Costford, S. Bajpeyi, M. Pasarica et al., "Skeletal muscle NAMPT is induced by exercise in humans," "American Journal of Physiology-Endocrinology and Metabolism," vol. 298, no. 1, pp. E117–E126, 2010.

[32] E. Koltai, Z. Szabo, M. Atalay et al., "Exercise alters SIRT1, SIRT6, NAD and NAMPT levels in skeletal muscle of aged rats," "Mechanisms of Ageing and Development," vol. 131, no. 1, pp. 21–28, 2010.

[33] R. M. de Guía, M. Agerholm, T. S. Nielsen et al., "Aerobic and resistance exercise training reverses age-dependent decline in NAD⁺ salvage capacity in human skeletal muscle," "Physiological Reports," vol. 7, no. 12, article e14139, 2019.

[34] B. Poljsak and I. Milisavl, "NAD⁺ as the link between oxidative stress, inflammation, caloric restriction, exercise, DNA repair, longevity, and health span," "Rejuvenation Research," vol. 19, no. 5, pp. 406–413, 2016.

[35] C. Cantó, L. Q. Jiang, A. S. Deshmukh et al., "Interdependence of AMPK and SIRT1 for metabolic adaptation to
fasting and exercise in skeletal muscle,” *Cell Metabolism*, vol. 11, no. 3, pp. 213–219, 2010.

[115] Y. Ito, R. Yonekura, Y. Nakagami et al., “Tryptophan metabolism was accelerated by exercise in rat,” in *Developments in Tryptophan and Serotonin Metabolism*, vol. 527 of Advances in Experimental Medicine and Biology, pp. 531–535, Springer, Boston, MA, 2003.

[116] M. F. Goody and C. A. Henry, “A need for NAD⁺ in muscle development, homeostasis, and aging,” *Skeletal Muscle*, vol. 8, no. 1, 2018.

[117] O. Bugaj, J. Zieliński, K. Kusy, A. Kantanista, D. Wielinski, and P. Guzik, “The effect of exercise on the skin content of the reduced form of NAD and its response to transient ischemia and reperfusion in highly trained athletes,” *Frontiers in Physiology*, vol. 10, 2019.

[118] I. A. Kourtzidis, A. T. Stoupas, I. S. Gioris et al., “The NAD⁺ precursor nicotinamide riboside decreases exercise performance in rats,” *Journal of the International Society of Sports Nutrition*, vol. 13, no. 1, 2016.

[119] R. Murray, W. P. Bartoli, D. E. Eddy, and M. K. Horn, “Physiological and performance responses to nicotinic-acid ingestion during exercise,” *Medicine & Science in Sports & Exercise*, vol. 27, no. 7, pp. 1057–1062, 1995.

[120] D. W. W. Frederick, E. Loro, L. Liu et al., “Loss of NAD homeostasis leads to progressive and reversible degeneration of skeletal muscle,” *Cell Metabolism*, vol. 24, no. 2, pp. 269–282, 2016.

[121] M. A. Dew, C. C. Hoch, D. J. Buysse et al., “Healthy older adults’ sleep predicts all-cause mortality at 4 to 19 years of follow-up,” *Psychosomatic Medicine*, vol. 65, no. 1, pp. 63–73, 2003.

[122] D. R. Mazzotti, C. Guindalini, A. L. Sosa, C. P. Ferri, and S. Tufik, “Prevalence and correlates for sleep complaints in older adults in low and middle income countries: a 10/66 Dementia Research Group study,” *Sleep Medicine*, vol. 13, no. 6, pp. 697–702, 2012.

[123] V. Beest, “Statin users risk heart attacks by dropping treatment or taking low doses doctors must emphasise importance of complying with treatment say researchers,” *Heart*, vol. 91, pp. 250–256, 2006.

[124] P. L. Enright, A. B. Newman, P. W. Wahl, T. A. Manolio, F. E. Haponik, and P. J. R. Boyle, “Prevalence and correlates of snoring and observed apneas in 5,201 older adults,” *Sleep*, vol. 19, no. 7, pp. 531–538, 1996.

[125] F. P. Cappuccio, L. D’Elia, P. Strazzullo, and M. A. Miller, “Quantity and quality of sleep and incidence of type 2 diabetes: a systematic review and meta-analysis,” *Diabetes Care*, vol. 33, no. 2, pp. 414–420, 2010.

[126] L. Klein, T. Gao, N. Barzilai, and S. Milman, “Association between sleep patterns and health in families with exceptional longevity,” *Frontiers in Medicine*, vol. 4, 2017.

[127] S. Hood and S. Amir, “The aging clock: circadian rhythms and later life,” *Journal of Clinical Investigation*, vol. 127, no. 2, pp. 437–446, 2017.

[128] Y. Nakahata, S. Sahar, G. Astarita, M. Kaluzova, and P. Sassone-Corsi, “Circadian control of the NAD⁺ salvage pathway by CLOCK-SIRT1,” *Science*, vol. 324, no. 5927, pp. 654–657, 2009.

[129] G. Asher, H. Reinke, M. Altmeyer, M. Gutierrez-Arcelus, M. O. Hottiger, and U. Schibler, “Poly(ADP-ribose) polymerase 1 participates in the phase entrainment of circadian clocks to feeding,” *Cell*, vol. 142, no. 6, pp. 943–953, 2010.

[130] K. M. Ramsey, J. Yoshino, C. S. Brace et al., “Circadian clock feedback cycle through NAMPT-mediated NAD⁺ biosynthesis,” *Science*, vol. 324, no. 5927, pp. 651–654, 2009.

[131] B. Poljsak, “NAMPT-mediated NAD biosynthesis as the internal timing mechanism: in NAD⁺ world, time is running in its own way,” *Rejuvenation Research*, vol. 21, no. 3, pp. 210–224, 2018.

[132] B. Poljsak, S. Ribarič, and I. Milisav, “Yin and Yang: why did evolution implement and preserve the circadian rhythmicity?,” *Medical Hypotheses*, vol. 131, article 109306, 2019.

[133] H. Pelicano, R. H. Xu, M. du et al., “Mitochondrial respiration defects in cancer cells cause activation of Akt survival pathway through a redox-mediated mechanism,” *Journal of Cell Biology*, vol. 175, no. 6, pp. 913–923, 2006.

[134] J. C. Milne, P. D. Lambert, S. Schenk et al., “Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes,” *Nature*, vol. 450, pp. 712–716, 2007.

[135] T. Roenneberg and M. Merrow, “The circadian clock and human health,” *Current Biology*, vol. 26, no. 10, pp. R432–R443, 2016.

[136] S. Libert, M. S. Bonkowski, K. Pointer, S. D. Pletcher, and L. Guarante, “Deviation of innate circadian period from 24 h reduces longevity in mice,” *Aging Cell*, vol. 11, no. 5, pp. 794–800, 2012.

[137] M. Pittelli, R. Felici, V. Pitozz et al., “Pharmacological effects of exogenous NAD on mitochondrial bioenergetics, DNA repair, and apoptosis,” *Molecular Pharmacology*, vol. 80, no. 6, pp. 1136–1146, 2011.

[138] R. V. Kondratchova, A. A. Kondratchova, V. Y. Gorbacheva, O. V. Vykhovanes, and M. P. Antoch, “Early aging and age-related pathologies in mice deficient in Bmal1, the core component of the circadian clock,” *Genes & Development*, vol. 20, no. 14, pp. 1868–1873, 2006.

[139] S. Libert, K. Pointer, E. L. Bell et al., “SIRT1 activates MAO-A in the brain to mediate anxiety and exploratory drive,” *Cell*, vol. 147, no. 7, pp. 1459–1472, 2011.

[140] T. Kishi, R. Yoshimura, T. Kitajima et al., “SIRT1 gene is associated with major depressive disorder in the Japanese population,” *Journal of Affective Disorders*, vol. 126, no. 1-2, pp. 167–173, 2010.

[141] I. Heinonen and J. A. Laukkanen, “Effects of heat and cold on health, with special reference to Finnish sauna bathing,” *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, vol. 314, no. 5, pp. R629–R638, 2018.

[142] T. Laukkanen, H. Khan, F. Zaccardi, and J. A. Laukkanen, “Association between sauna bathing and fatal cardiovascular and all-cause mortality events,” *JAMA Internal Medicine*, vol. 175, no. 4, pp. 542–548, 2015.

[143] J. A. Laukkanen, T. Laukkanen, and S. K. Kunutsor, “Cardiovascular and other health benefits of sauna bathing: a review of the evidence,” *Mayo Clinic Proceedings*, vol. 93, no. 8, pp. 1111–1121, 2018.

[144] C. C. Gray, M. Amrani, R. T. Smolenski, G. L. Taylor, and M. H. Yacob, “Age dependence of heat stress mediated cardioprotection,” *The Annals of Thoracic Surgery*, vol. 70, pp. 621–626, 2000.

[145] J. M. Brazill, C. Li, Y. Zhu, and R. G. Zhai, “NMNAT: it’s an NAD⁺ synthase… it’s a chaperone… it’s a neuroprotector,”
Adipose tissue NAD+-homeostasis, sirtuins and poly(ADP-ribose) polymerases - important players in mitochondrial metabolism and metabolic health, *Biochemical Journal*, vol. 360, pp. 1518–1525, 1999.

[150] P. Björntorp, C. Bengtsson, G. Blohmé et al., “Adipose tissue fat cell size and number in relation to metabolism in randomly selected middle-aged men and women,” *Metabolism*, vol. 20, pp. 927–935, 1971.

[151] S. Heinonen, L. Saarinen, J. Naukkarinen et al., “Adipocyte morphology and implications for metabolic derangements in acquired obesity,” *International Journal of Obesity*, vol. 38, pp. 1423–1431, 2014.

[152] A. E. Goodbody and P. Trayhurn, “GDP binding to brown-adipose-tissue mitochondria of diabetic-obese (db/db) mice. Decreased binding in both the obese and pre-obese states,” *Biochemical Journal*, vol. 194, pp. 1019–1022, 1981.

[153] J. Himms-Hagen and M. Desautels, “A mitochondrial defect in brown adipose tissue of the obese (ob/ob) mouse: reduced binding of purine nucleotides and a failure to respond to cold by an increase in binding,” *Biochemical and Biophysical Research Communications*, vol. 83, pp. 628–634, 1978.

[154] I. Shimizu, T. Aprahramian, R. Kikuchi et al., “Vascular rarefaction mediates whitening of brown fat in obesity,” *Journal of Clinical Investigation*, vol. 124, pp. 2099–2112, 2014.

[155] G. H. E. J. Vijgen, N. D. Bouvy, G. J. J. Teule, B. Brans, P. Schrauwen, and W. D. van Marken Lichtenbelt, “Brown adipose tissue in morbibly obese subjects,” *PLoS One*, vol. 6, 2011.

[156] C. F. Dolopikou, I. A. Kourtzidis, N. V. Margaritellis et al., “Acute nicotinamide riboside supplementation improves redox homeostasis and exercise performance in old individuals: a double-blind cross-over study,” *European Journal of Nutrition*, vol. 59, pp. 505–515, 2020.

[157] I. A. Kourtzidis, C. F. Dolopikou, A. N. Tsifis et al., “Nicotinamide riboside supplementation dysregulates redox and energy metabolism in rats: implications for exercise performance,” *Experimental Physiology*, vol. 103, pp. 1357–1366, 2018.

[158] M. Knip, I. F. Douek, W. P. T. Moore et al., “Safety of high-dose nicotinamide: a review,” *Diabetologia*, vol. 43, pp. 1337–1345, 2000.

[159] W. Shi, M. A. Hegeman, A. Doncheva, M. Bekkenkamp-Grovenstein, V. C. J. de Boer, and J. Keijer, “High dose of dietary nicotinamide riboside induces glucose intolerance and white adipose tissue dysfunction in mice fed a mildly obesogenic diet,” *Nutrients*, vol. 11, no. 10, 2019.

[160] D. Herranz, M. Muñoz-Martin, M. Cañamero et al., “Sirt1 improves healthy ageing and protects from metabolic syndrome-associated cancer,” *Nature Communications*, vol. 1, p. 3, 2010.

[161] D. Chen, J. Bruno, E. Easlon et al., “Tissue-specific regulation of SIRT1 by calorie restriction,” *Genes & Development*, vol. 22, pp. 1753–1757, 2008.

[162] D. W. Frederick, J. G. Davis, A. Dávila et al., “Increasing NAD synthesis in muscle via nicotinamide phosphoribosyltransferase is not sufficient to promote oxidative metabolism,” *Journal of Biological Chemistry*, vol. 290, pp. 1546–1558, 2015.

[163] M. Muruganandham, A. A. Aliferay, C. Matei et al., “Metabolic signatures associated with a NAD synthesis inhibitor-induced tumor apoptosis identified by 1H-decoupled-31P magnetic resonance spectroscopy,” *Clinical Cancer Research*, vol. 11, pp. 3503–3513, 2005.

[164] A. Pogrebniak, I. Schemainda, K. Azzam, R. Pelka-Fleischer, V. Nüssler, and M. Hasmann, “Chemopotentiation effects of a novel NAD biosynthesis inhibitor, FK866, in combination with antineoplastic agents,” *European Journal of Medical Research*, vol. 11, pp. 313–321, 2006.

[165] T. F. Liu, C. M. Brown, M. El Gazzar et al., “Fueling the flame: bioenergy couples metabolism and inflammation,” *Journal of Leukocyte Biology*, vol. 92, pp. 499–507, 2012.

[166] L. Li, Z. Chen, W. Fu, S. Cai, and Z. Zeng, “Emerging evidence concerning the role of sirtuins in sepsis,” *Critical Care Research and Practice*, vol. 2018, Article ID 5489571, 8 pages, 2018.

[167] N. Busso, M. Karababa, M. Nobile et al., “Pharmacological inhibition of nicotinamide phosphoribosyltransferase/visfatin enzymatic activity identifies a new inflammatory pathway linked to NAD,” *PLoS One*, vol. 3, no. 5, article e2267, 2008.

[168] S. A. J. Trammell, B. J. Weidemann, A. Chadda et al., “Nicotinamide riboside opposes type 2 diabetes and neuropathy in mice,” *Scientific Reports*, vol. 6, no. 1, 2016.

[169] M. Bockwoldt, D. Houry, M. Niere et al., “Identification of evolutionary and kinetic drivers of NAD-dependent signaling,” *Proceedings of the National Academy of Sciences*, vol. 116, no. 32, pp. 15957–15966, 2019.

[170] E. S. Hwang and S. B. Song, “Nicotinamide is an inhibitor of SIRT1 in vitro, but can be a stimulator in cells,” *Cellular and Molecular Life Sciences*, vol. 74, no. 18, pp. 3347–3362, 2017.

[171] E. S. Hwang and S. B. Song, “Possible adverse effects of high-dose nicotinamide: mechanisms and safety assessment,” *Biomedicines*, vol. 10, no. 5, p. 687, 2020.

[172] C. Sebastián, F. K. Satterstrom, M. C. Haigis, and R. Mostoslavsky, “From sirtuin biology to human diseases: an update,” *Journal of Biological Chemistry*, vol. 287, no. 51, pp. 42444–42452, 2012.

[173] S.-i. Imai, “A possibility of nutriceuticals as an anti-aging intervention: activation of sirtuins by promoting mammalian NAD biosynthesis,” *Pharmacological Research*, vol. 62, no. 1, pp. 42–47, 2010.

[174] I. S. Puig, M. Valera-Alberni, C. Cantó, and N. J. Pillon, “Circadian rhythms and mitochondria: connecting the dots,” *Frontiers in Genetics*, vol. 9, p. 452, 2018.

[175] A. Kojisaka, A. D. Laposky, K. M. Ramsey et al., “High-fat diet disrupts behavioral and molecular circadian rhythms in mice,” *Cell Metabolism*, vol. 6, no. 5, pp. 414–421, 2007.

[176] M. Hatori, C. Vollmers, A. Arrinpar et al., “Time-restricted feeding without reducing caloric intake prevents metabolic...
diseases in mice fed a high-fat diet,” *Cell Metabolism*, vol. 15, no. 6, pp. 848–860, 2012.

[177] R. C. R. Meex, V. B. Schrauwen-Hinderling, E. Moonen-Kornips et al., “Restoration of muscle mitochondrial function and metabolic flexibility in type 2 diabetes by exercise training is paralleled by increased myocellular fat storage and improved insulin sensitivity,” *Diabetes*, vol. 59, no. 3, pp. 572–579, 2010.

[178] E. Phielix, R. Meex, E. Moonen-Kornips, M. K. C. Hesselink, and P. Schrauwen, "Exercise training increases mitochondrial content and ex vivo mitochondrial function similarly in patients with type 2 diabetes and in control individuals," *Diabetologia*, vol. 53, no. 8, pp. 1714–1721, 2010.

[179] N. Dali-Youcef, M. Lagouge, S. Froelich, C. Koehl, K. Schoonjans, and J. Auwerx, “Sirtuins: the “magnificent seven”, function, metabolism and longevity,” *Annals of Medicine*, vol. 39, pp. 335–345, 2009.

[180] K. M. Ralto, E. P. Rhee, and S. M. Parikh, “NAD’ homeostasis in renal health and disease,” *Nature Reviews Nephrology*, vol. 16, no. 2, pp. 99–111, 2020.