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Tissue-specific effects of exercise as NAD$^+$-boosting strategy: Current knowledge and future perspectives

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Abstract

Nicotinamide adenine dinucleotide (NAD$^+$) is an evolutionarily highly conserved coenzyme with multi-faceted cell functions, including energy metabolism, molecular signaling processes, epigenetic regulation, and DNA repair. Since the discovery that lower NAD$^+$ levels are a shared characteristic of various diseases and aging per se, several NAD$^+$-boosting strategies have emerged. Other than pharmacological and nutritional approaches, exercise is thought to restore NAD$^+$ homeostasis through metabolic adaption to chronically recurring states of increased energy demand. In this review we discuss the impact of acute exercise and exercise training on tissue-specific NAD$^+$ metabolism of rodents and humans to highlight the potential value as NAD$^+$-boosting strategy. By interconnecting results from different investigations, we aim to draw attention to tissue-specific alterations in NAD$^+$ metabolism and the associated implications for whole-body NAD$^+$ homeostasis. Acute exercise led to profound alterations of intracellular NAD$^+$ metabolism in various investigations, with the magnitude and direction of changes being strongly dependent on the applied exercise modality, cell type, and investigated animal model or human population. Exercise training elevated NAD$^+$ levels and NAD$^+$ metabolism enzymes in various tissues. Based on these results, we discuss molecular mechanisms that might connect acute exercise-induced disruptions of NAD$^+$/NADH homeostasis to chronic exercise adaptions in NAD$^+$ metabolism. Taking this hypothesis-driven approach, we hope to inspire future research on the molecular mechanisms of exercise as NAD$^+$-modifying lifestyle intervention, thereby elucidating the potential therapeutic value in NAD$^+$-related pathologies.

KEYWORDS

exercise, health, metabolism, NAD$^+$, NADH, nicotinamide adenine dinucleotide

David Walzik and Wiebke Jonas contributed equally to this work.

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1 | INTRODUCTION

The first identified function of nicotinamide adenine dinucleotide (NAD^+) was its ability to reversibly bind hydride ions, which are formally defined as anions of hydrogen (H^-).[1] Today, this chemical property is known to play a crucial role in cellular metabolism, where NAD^+ serves as an electron acceptor in oxidation–reduction (redox) reactions during the breakdown of energetic substrates (e.g., glucose, fatty acids), thus generating NADH as reducing equivalent. Apart from these fundamental redox properties, numerous further processes crucial for cell vitality are dependent on NAD^+. For instance, NAD^+ serves as a substrate for NAD^+-consuming enzymes, such as sirtuins (SIRTs), poly(ADP-ribose) polymerases (PARPs), and ectoenzymes like cluster of differentiation (CD)38, CD157, or the recently discovered sterile alpha and toll interleukin receptor motif-containing protein 1 (SARM1).[2] A common feature of these enzymes is their ability to cleave NAD^+ to nicotinamide and adenosine diphosphate ribose (ADPR), the latter of which exerts target-specific downstream functions.[3] Sirtuins use ADPR to modify DNA-binding proteins like histones and nuclear transcription factors, or coactivators, thereby regulating transcription activity.[4] PARPs are involved in DNA repair processes by adding ADPR molecules to proteins in a process termed mono- or poly-(ADP-ribosyl) ation.[5] CD38, CD157, and SARM1 use ADPR or cyclic ADPR as calcium-mobilizing second messengers, outlining the importance of NAD^+ cleavage products in fundamental cell signaling processes.[6] Despite the apparent structural resemblance, $NADP^+$, the phosphorylated form of NAD^+, has rather distinct functions in cellular metabolism. $NADP^+$ is reduced to NADPH in the pentose phosphate pathway and serves as a redox partner in the reductive biosynthesis of fatty acids, cholesterol, and steroid hormones, hepatic detoxification of xenobiotics, and oxidative defenses.[7]

In this review, we aim to discuss the impact of exercise on NAD^+ metabolism, to examine its potential value as NAD^+-boosting strategy. After giving a short introduction into NAD^+ biosynthesis as well as age- and disease-associated alterations, we provide a detailed overview of studies investigating the impact of exercise on NAD^+ metabolism in both, rodents, and humans. To facilitate the interpretation of results, we have separated studies performed in an acute exercise setting from those investigating exercise training (multiple bouts of acute exercise) in a tissue-specific manner, respectively. Finally, we condense these results on a molecular level by proposing a mechanism that connects acute alterations in NAD^+/NADH homeostasis to chronic adaptions in NAD^+ metabolism.

1.1 | NAD^+ biosynthesis

NAD^+ biosynthesis can broadly be divided into the kynurenine pathway, the Preiss-Handler pathway, and a salvage pathway. While these pathways all generate NAD^+ from different nutritional precursors, the salvage pathway additionally recycles NAD^+ from nicotinamide—a cleavage product arising from the activity of NAD^+-consuming enzymes (Figure 1).

The kynurenine pathway—also termed de novo NAD^+ synthesis pathway—depicts the main catabolic route of the essential amino acid tryptophan. After cellular uptake of tryptophan through amino acid transporters (SLC7A5, SLC36A4),[8,9] it is converted to N-formylkynurenine in an initial and rate-limiting step by the enzyme indolamine 2,3-dioxygenase (IDO) 1, its homologue IDO2 or its structurally unrelated liver-specific analogue tryptophan 2,3-dioxygenase (TDO).[10] In different subsequent enzymatic reactions, N-formylkynurenine is either catabolized to kynurenic acid (KA) or—more relevant in the context of de novo NAD^+ synthesis—quinolinic acid (QA). The kynurenine pathway then converges with the Preiss-Handler pathway through formation of nicotinic acid mononucleotide (NAMN) catalyzed by the enzyme quinolinate phosphoribosyltransferase (QPRT). NAMN is also generated from its dietary precursor nicotinic acid (NA, vitamin B_3), which is taken up by SLC5A8[11] and SLC22A13.[12] Subsequently, NAMN is converted to nicotinic acid adenine dinucleotide (NAAD), a metabolite generated by compartment-specific isoforms of nicotinamide mononucleotide adenylyltransferase (NMNAT1-3).[13] NAD^+ synthetase (NADS) ultimately converts NAAD to NAD^+, which is electively phosphorylated to $NADP^+$ (Figure 1).

In contrast, NAD^+ is recycled from nicotinamide in the salvage pathway after it has been cleaved by NAD^+-consuming enzymes. This resynthesis of NAD^+ requires two enzymes: nicotinamide phosphoribosyltransferase (NAMPT) and NMNAT1-3. Notably, both nicotinamide mononucleotide (NMN) and nicotinamide are also replenished via nutrition. While nicotinamide diffuses across the plasma membrane, NMN is either shuttled into the cell via a transporter (SLC12A8) or taken up in form of its dietary precursor nicotinamide riboside (NR) (Figure 1). However, whether SLC12A8 actually encodes a cellular NMN transporter is still under debate.[14]

1.2 | Tissue-specific aspects of NAD^+ metabolism

Given the essential role of NAD^+ in cellular energy homeostasis, it is not surprising that NAD^+ metabolism is conserved across a wide range of metabolically active

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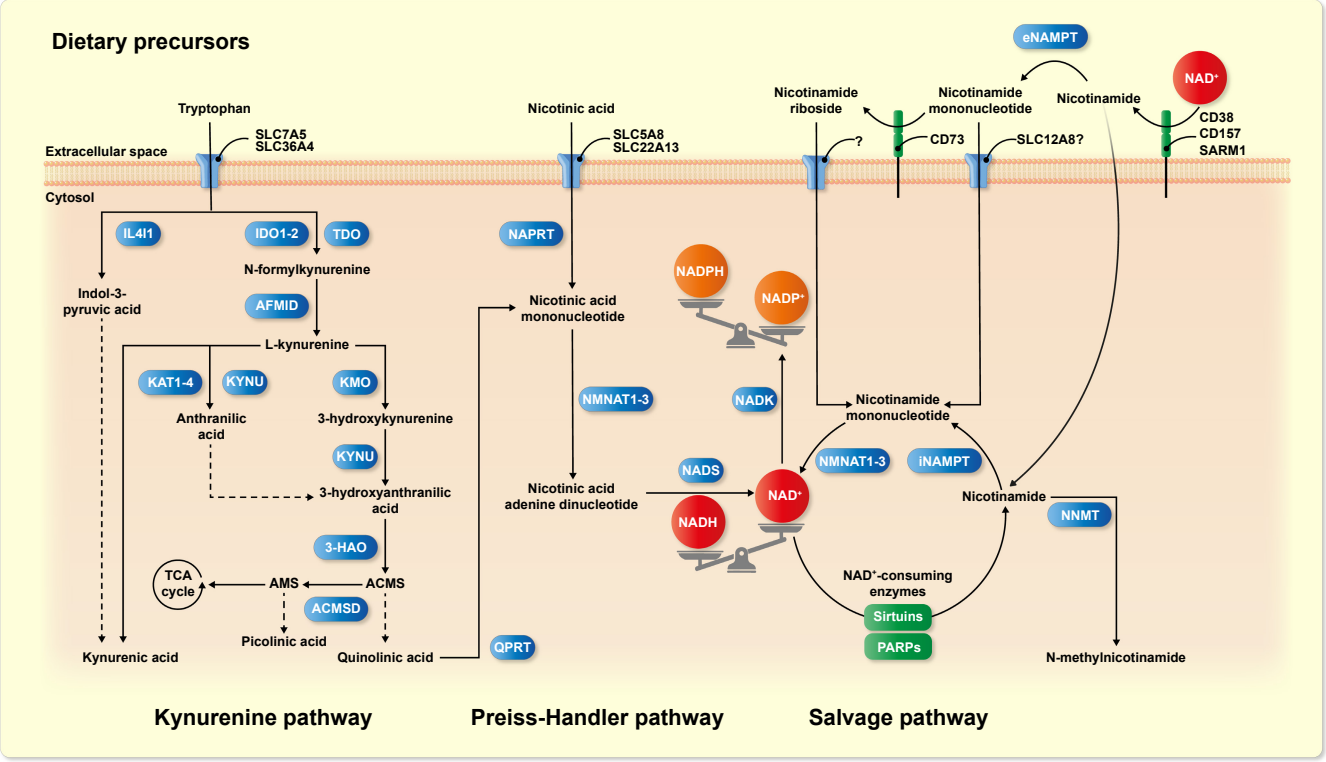

FIGURE 1 Biosynthetic pathways yielding NAD^+.
Intracellular levels of NAD^+ are held constant via three distinct pathways using the dietary precursors tryptophan, nicotinic acid, nicotinamide riboside, nicotinamide mononucleotide, and nicotinamide. The kynurenine pathway depicts the major catabolic route of the essential amino acid tryptophan, generating quinolinic acid (QA) as one of its end products. QA then converges with the Preiss-Handler pathway, ultimately yielding NAD^+. The salvage pathway either recycles NAD^+ from nicotinamide after it has been cleaved by NAD^+-consuming enzymes or incorporates dietary precursors. Note, that the illustration above serves as a schematic overview of NAD^+-synthesizing pathways. For tissue-specific and subcellular expression patterns of the corresponding enzymes please refer to Ref. 15.
3-HAO, 3-Hydroxyanthranilate 3,4-dioxygenase; ACMS, α-amino-β-carboxymuconate ε-semialdehyde; ACMSD, aminocarboxymuconate semialdehyde decarboxylase; AFMID, arylformamidase; AMS, 2-aminomuconic-6-semialdehyde; IDO, indolamine 2,3-dioxygenase; IL4I1, interleukin 4 induced 1; iNAMPT & eNAMPT, intracellular & extracellular nicotinamide phosphoribosyltransferase; KAT, kynurenine aminotransferase; KMO, kynurenin-3-monooxygenase; KYNU, kynureninase; NADK, NAD kinase; NADS, NAD synthase; NAPRT, nicotinate phosphoribosyltransferase; NMNAT, nicotinamide mononucleotide adenylyltransferase; NNMT, nicotinamide N-methyltransferase; NRK, nicotinamide riboside kinase; PARPs, poly(ADP-ribose) polymerases; QPRT, quinolinate phosphoribosyltransferase; SARM1, sterile alpha and toll interleukin receptor motif–containing protein 1; SLC, solute carrier; TCA, tricarboxylic acid; TDO, tryptophan 2,3-dioxygenase

tissues. However, the different (re)synthesis pathway enzymes are expressed in a tissue-specific manner.[15] For instance, the liver expresses almost all enzymes involved in NAD^+ metabolism, indicating its key role in whole-body NAD^+ homeostasis. Particularly high expression levels are found for kynurenine pathway enzymes, suggesting that NAD^+ biosynthesis is mainly assured via metabolization of tryptophan.[16] After de novo synthesis, NAD^+ is cleaved by hepatic NAD^+-consuming enzymes, and nicotinamide—one of the resulting cleavage products—is released into circulation to serve as NAD^+ precursor for other organs such as skeletal muscle. In this context, isotope tracing techniques and quantitative flux analyses have revealed that the liver accounts for over 95% of circulating nicotinamide in mice.[16] Thus, while hepatic NAD^+ is predominantly ensured via de novo synthesis, skeletal muscle relies more on salvage pathway activity via incorporation of dietary precursors (e.g., nicotinamide, NR, NMN) and intracellular resynthesis of NAD^+. Cell culture experiments and mouse model studies have confirmed the pivotal role of salvage pathway activity in skeletal muscle tissue.[17–19] Of note, these tissue-specific differences relate to resting conditions, under which both, metabolic flux, and enzyme expression are well investigated. The impact of acute exercise and exercise training on this fine-tuned equilibrium remains inconclusive so far. However, exercise-induced increases in kynurenine pathway metabolites

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in human blood serum[20] and altered gene expression of kynurenine pathway enzymes in human skeletal muscle,[21] make bioenergetic assumptions related to exercise as modulator of de novo NAD^+ synthesis tempting.

2 | NAD^+ METABOLISM IN AGING AND DISEASE

2.1 | Impaired NAD^+ metabolism: a common feature?

Over the last decades alterations in NAD^+ metabolism have been revealed in multiple age-associated diseases, including neoplastic,[22] metabolic,[23] and neurodegenerative diseases,[24] as well as aging per se.[25,26] Depending on the investigated population (rodents vs. humans) and tissue type, the NAD^+ levels of aged subjects were reported to range from 15% to 90% of normal tissue levels.[27] Current hypotheses for this age-dependent decline revolve around the frequently formulated "hallmarks of ageing", including loss of proteostasis and genomic stability.[28] According to this, NAD^+ biosynthesis enzymes become dysfunctional during aging, resulting in decreased NAD^+ levels. Additionally, the age-associated accumulation of DNA damage is thought to increase PARP activity, reinforcing the cellular decline of NAD^+.[29] This renders NAD^+-dependent processes such as energy metabolism and DNA repair ineffective, leading to age-related pathophysiology and tissue degeneration. Although promising as such, a recent review of studies on this topic concluded that despite overall claims of NAD^+ decline with age the scientific evidence is rather limited and often restricted to certain tissue types. Additionally, the authors highlight the potential selection bias attached to narrative literature reviews and shed light on the importance of unbiased longitudinal studies to establish an evidence-based foundation for the abovementioned claims.[30] Hence, while the exact impact of aging on tissue NAD^+ content remains unclear (especially in humans), tissue-specific alterations of NAD^+ homeostasis are frequently reported in disease settings,[31] making tissue-specific approaches indispensable when investigating NAD^+-modifying interventions.

2.2 | On the rise: therapeutic potential of NAD^+-boosting strategies

Linking the pathophysiology of different diseases to impaired NAD^+ metabolism has given rise to various new therapeutic avenues aiming to boost NAD^+ levels. Targeting specific aspects of NAD^+ metabolism, these approaches fall into three categories: supplementation of NAD^+ precursors (e.g., NR, NMN),[27] pharmacological activation of rate-limiting enzymes (e.g., NAMPT),[32] and pharmacological or genetic inhibition of NAD^+-consuming enzymes (e.g., CD38).[33] Despite decades of preclinical research on the therapeutic potential of these approaches (reviewed in Refs. [3,15,34]), there is surprisingly little evidence when it comes to well-powered clinical interventions in aged or diseased populations. In a systematic review of clinical trials investigating the therapeutic potential of supplementation with NAD^+ precursors in diseased populations, it was recently concluded that the clinical evidence is rather sparse.[35] Validating preclinical results on clinical populations is, therefore, indispensable for future research endeavors. Additionally, it must be emphasized that the effect of NAD^+-boosting strategies is strongly context-dependent. Although the predominant notion of these approaches is often connoted rather positively (e.g., increased life expectancy, health improvements, rejuvenation of target tissues, etc.),[36,37] similar underlying mechanisms might also contribute to pathological processes. For instance, the involvement of NAD^+ metabolism in cancer cell biology is well-established,[22] and it was recently reported that NAD^+ metabolism is also crucially exploited in cellular senescence of mice. In detail, senescent cells in white adipose tissue and liver induce the expression of the NAD-dependent ecto-enzyme CD38 on proinflammatory M1-like macrophages via the secretion of senescence-associated secretory phenotypes. Together with a higher NADase rate this senescence-associated upregulation of CD38 leads to an increased cleavage of the NAD^+ precursor NMN, resulting in diminished NMN availability for adjacent cells and lower tissue NAD^+ levels.[38,39] In this scenario, boosting NAD^+ levels could in fact reinforce the vicious cycle of inflammation-induced NAD^+ consumption, thus exacerbating health outcomes. Therefore, the overly uniform narrative on NAD^+-boosting strategies as having a positive impact on health outcomes must be put into perspective at least, to obtain a holistic and more realistic impression of the context-dependent impact of NAD^+-boosting strategies.

While supplemental and pharmacological interventions interfere with NAD^+ homeostasis externally (via external administration of NAD^+-modifying molecules), lifestyle interventions such as exercise hold the promise to induce internal adaptions and more sustainable, widespread long-term improvements in NAD^+ metabolism, that are additionally accompanied by a myriad of beneficial side effects.[40] Currently, diet, enhancing circadian rhythm, and exercise are considered lifestyle interventions with NAD^+-boosting properties.[3]

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Interestingly, the association between both, dietary[41] and circadian rhythm approaches[42,43] on the one hand, and NAD^+ metabolism, aging, disease, and longevity, on the other hand, has been studied extensively. In contrast, relatively little attention has been devoted to exercise as NAD^+-boosting lifestyle intervention. To fully appreciate the impact of acute exercise and exercise training regimens on NAD^+ metabolism, this review discusses both tissue-specific aspects and inter-organ crosstalk, to give a comprehensive insight into whole-body NAD^+ homeostasis in the context of exercise. Taking this approach, we also shed light on the potential application of exercise in aging and age-related pathologies that are characterized by altered NAD^+ metabolism.

3 | BOOSTING NAD^+ METABOLISM WITH EXERCISE—A FOCUS ON TISSUE-SPECIFIC DIFFERENCES

To appreciate the impact of exercise on NAD^+ metabolism, careful consideration of the exercise modality (e.g., type, intensity, duration, frequency) as well as the investigated population (rodent vs. human) and target tissue is indispensable. Therefore, we separated acute exercise effects on NAD^+ metabolism from adaptions induced by exercise training.

3.1 | Acute exercise disrupts NAD^+ homeostasis

Acute aerobic exercise profoundly alters NAD^+ metabolites and the associated enzymes in various tissues of both, rodents, and humans. While most studies focused on skeletal muscle tissue, some were also performed on other tissues such as blood, liver, leukocytes, erythrocytes, or epidermal skin cells.

3.1.1 | Liver

Considering the high relevance of hepatic metabolism in regulating whole-body NAD^+ homeostasis,[16] it is no surprise to find a distinct regulation in response to acute exercise. Results from an exercise study on rodents suggest that hepatic de novo NAD^+ synthesis from tryptophan is downregulated in response to acute exercise, as indicated by a decreased liver TDO activity in exercised rats when compared to sedentary controls.[44] Considering the redirection of blood flow to skeletal muscle during acute exercise, lower hepatic perfusion might restrict the availability of tryptophan for liver tissue. Since TDO was found to operate in dependence on local oxygen levels,[45] the lower oxygen availability induced by a decreased hepatic perfusion might additionally impair tryptophan breakdown. Although this mechanism would depend on cardiorespiratory fitness, exercise intensity, heat stress and hepatic oxygen extraction, the results suggest that the liver refrains from metabolizing tryptophan in acute exercise scenarios, potentially yielding precedence to other tissues with higher oxygen supply. Underlining this, IDO activity was found to depend on oxygen availability in a similar manner as TDO activity,[46] raising interesting questions concerning the impact of oxygen availability on de novo NAD^+ synthesis. While IDO activity was upregulated in rat macrophages in response to acute exercise (see Section 3.1.5), investigations on other tissues (e.g., skeletal muscle) are lacking so far, posing an urgent need for (re) evaluation of de novo NAD^+ synthesis in acute exercise scenarios.

3.1.2 | Skeletal muscle

Since skeletal muscle is under high metabolic demand during acute exercise, profound alterations in NAD^+ metabolism were observed in various studies. With the onset of acute exercise, the oxidation of energetic substrates inside skeletal muscle tissue rises, to cover the increased energy demand provoked by muscle contraction. This results in an increased metabolic combustion of glucose and fatty acids in glycolysis and β-oxidation, which are regulated in dependence on the applied exercise modality (e.g., intensity, duration).[47] Glycolysis and β-oxidation as well as the subsequent oxidation of acetyl coenzyme A (CoA) in the tricarboxylic acid (TCA) cycle are coupled to the reduction of NAD^+ to NADH, which is then oxidized back to NAD^+ on mitochondrial complex I. Cell metabolism and NAD^+/NADH redox homeostasis are, therefore, intimately linked.

In line with high glycolytic activity—as induced by high-intensity aerobic exercise—reduction of NAD^+ to NADH is promoted, resulting in decreased sarcoplasmic NAD^+ and increased NADH levels. These exercise-induced alterations in NAD^+/NADH redox homeostasis were confirmed in multiple studies in both rodents[48,49] and humans[50–54] exposed to acute aerobic exercise. Interestingly, NAD^+/NADH redox homeostasis was not only shifted in favor of NADH in response to severe exercise until exhaustion[52–55] but also after moderate exercise modalities. For instance, decreased NAD^+ levels were found in healthy males after cycling at 70%[51] and 75% of their maximal oxygen consumption ($\dot{V}O_{2max}$).[50] Similarly, increased NADH levels were found after cycling at 75%[53] and 85% of

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$\dot{V}O_{2max}$.[55] This suggests that moderate- and high-intensity exercise lowers intracellular NAD^+/NADH ratios (NAD^+ decreases, NADH increases) due to the high glycolytic activity provoked by the exercise regimen. However, it must be noted that the observed alterations in NAD^+/NADH redox balance were measured in cell lysates. While this obviously allows for a good overall impression of cellular NAD^+/NADH redox homeostasis, a subcellular resolution would convey a much more detailed depiction of the interconnection with energy metabolism (e.g., rising NADH levels due to high glycolytic rate in sarcoplasma). While such approaches are lacking in in-vivo experiments so far, they are a well-established method in cell culture models. In a recent analysis of sarcoplasmic NAD^+/NADH ratios of beating cardiomyocytes supplemented with glucose,[56] the high glycolytic rate resulting from glucose supplementation led to a sarcoplasmic accumulation of NADH and a decreased sarcoplasmic NAD^+/NADH ratio. In contrast, mitochondrial NAD^+/NADH ratios increased, indicating that the oxidation of NADH to NAD^+ on mitochondrial complex I outweighs the reduction to of NAD^+ in β-oxidation, and TCA cycle. Consequently, the reductive capacity of mitochondrial complex I does not seem to limit cellular metabolism and suggests a metabolic bottleneck upstream of mitochondria responsible for sarcoplasmic NADH accumulation. Since oxidative phosphorylation depends on NADH availability, a tempting explanation is saturation of mitochondrial NADH import shuttles. In this context, saturation of malate–aspartate- and glycerol-3-phosphate shuttles on the inner mitochondrial membrane could confine oxidative phosphorylation, resulting in a ceiling effect for aerobic glycolysis. At high glycolytic rates sarcoplasmic NADH generation via glycolysis exceeds mitochondrial NADH import, leading to sarcoplasmic NADH accumulation, which is partially relieved via lactate dehydrogenase-dependent oxidation to NAD^+. While this concept of mitochondrial NADH import as a metabolic bottleneck for aerobic glycolysis was previously suggested in the context of exercise,[57,58] it was recently also transferred to cancer metabolism as a molecular basis for the high glycolytic activity and lactate secretion observed in cancer cells despite sufficient oxygen availability, also known as Warburg effect.[59] In this context, a so far underappreciated alternative to NADH shuttling via malate–aspartate- and glycerol-3-phosphate shuttles is the import of lactate into mitochondria. Since mitochondria express both, monocarboxylate transporters for lactate import, and lactate dehydrogenase for subsequent oxidation to pyruvate (note, that this reaction yields NADH),[60,61] lactate mediated NADH shuttling depicts a further component of energy metabolism that might constrain oxidative phosphorylation in the context of exercise efforts marked by high glycolytic activity. While this would account for sarcoplasmic NADH buildup in a similar manner as described above, it additionally respects lactate production—a longstanding metabolic hallmark of high-intensity exercise.

In contrast to moderate- and high- intensity exercise, the energy metabolism of skeletal muscle tissue during low-intensity exercise is much more dependent on fatty acid oxidation.[62] On a molecular level, the competition for carnitine between acyl-CoA derived from mitochondrial fatty acid import and acetyl-CoA derived from oxidative decarboxylation of pyruvate, has been proposed as a mechanism that adjusts fatty acid oxidation to the rate of glycolysis.[47] As indicated by cell culture experiments on beating cardiomyocytes supplemented with either fatty acids and glucose, or glucose only, the addition of β-oxidation as a metabolic pathway reversed the sarcoplasmic accumulation of NADH and led to an increased sarcoplasmic NAD^+/NADH ratio compared to glucose-only conditions.[56] In line with these results from cell culture models, decreased NADH levels are also observed in skeletal muscle cell lysates after low-intensity exercise, as indicated by two investigations on men cycling at 40% $\dot{V}O_{2max}$.[52,53] Additionally, increased NAD^+ levels were found when measurements were not performed immediately after exercise, but in the subsequent recovery period,[63,64] underlining the characteristic switch from glucose to fatty acid oxidation after exercise cessation. However, similar to studies focusing on high-intensity exercise, it must be noted that the lack of subcellular resolution in these investigations impedes a detailed conclusion concerning the metabolic foundation of exercise-induced alterations in NAD^+/NADH redox homeostasis.

Aside from redox biochemistry, total NAD(H) concentrations might also increase in response to acute exercise via activation of NAMPT.[65,66] However, investigations on mouse[67] and rat skeletal muscle tissue[68] revealed inconsistent results. Due to similar exercise protocols and measurement time points, the investigated muscle type (quadriceps vs. plantaris) or rodent model (mouse vs. rat) might explain these discrepancies. Of interest, human studies have failed to show alterations of NAMPT protein content, and other enzymes related to NAD^+ biosynthesis in skeletal muscle in response to acute exercise.

3.1.3 | Blood

In whole blood, total NAD(H) concentrations were increased in mice exposed to acute moderate aerobic exercise when compared to sedentary controls.[69] Since aerobic exercise until exhaustion led to decreased whole blood NAD(H) concentrations in the same experiment,

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as well as other experiments,[70] an intensity-dependent regulation of systemic NAD^+ homeostasis seems plausible. Mechanistically, moderate exercise might increase NAD(H) concentrations via higher hepatic recirculation of NAD^+ precursors (e.g., nicotinamide, NR), while severe exercise could deplete precursor levels due to the high demand of energetically active peripheral tissue (e.g., skeletal muscle). This hypothesis is undermined by experiments showing increased NAD(H) consumption of (energetically active) blood cells after treadmill exercise until exhaustion in highly trained endurance athletes.[71] However, other experiments yielded elevated NAD(H) levels in whole blood of humans[69] and mice[44] exercised until exhaustion, raising questions concerning the exact dose–response relationship between exercise intensity and systemic NAD(H) levels.

3.1.4 | Erythrocytes

Since erythrocytes lack mitochondria, their energy metabolism relies solely on glycolysis, limiting NAD^+ reduction and NADH oxidation to glycolysis and lactate dehydrogenase activity, respectively. Interestingly, erythrocytes of exercise-trained individuals exposed to acute rowing exercise showed higher NAD^+ levels at rest and during submaximal exercise compared to untrained controls, potentially reflecting an adaption to exercise training.[72] However, exhaustive exercise decreased NAD^+ levels in trained individuals, indicating outperformance of glycolysis-mediated reduction of NAD^+ compared to lactate dehydrogenase-dependent oxidation of NADH. Additionally, trained individuals showed an exercise-induced decrease in $NADP^+$ levels, which might be explained by increased pentose phosphate pathway activity for generation of NADPH. Conceptually, this would give erythrocytes more reducing power to cope with reactive oxygen species (ROS) arising during acute exercise.

3.1.5 | Leukocytes

Although comparatively few studies have focused on alterations in leukocyte NAD^+ metabolism, interesting results were obtained on rats exercised until exhaustion.[44] While TDO activity was decreased in liver tissue (see Section 3.1.1), macrophage IDO activity was increased, suggesting a shift in de novo NAD^+ synthesis from the liver (at rest) to circulating macrophages (during exercise). From a bioenergetic point of view, this shift might fuel macrophage metabolism in the context of immune activation, induced by hormones and cytokines released during acute exercise.[73] Additionally, the increased de novo synthesis in macrophages could enable better distribution of NAD^+ and NAD^+-related metabolites (e.g., precursors) to other peripheral tissues, especially considering macrophage extravasation. A potential explanation for the tissue-specific regulation of de novo NAD^+ synthesis centers around the oxygen-dependent activity of the rate-limiting enzymes IDO and TDO.[45,46] Although oxygen availability is a tempting potential driving force, a causal link to exercise-induced alterations in de novo NAD^+ synthesis has not yet been established. While acute endurance and resistance exercise left IDO gene expression of human PBMCs unaltered,[20] investigations on other NAD^+-generating pathways of leukocytes (e.g., salvage pathway) are still pending. The differences between rodent and human NAD^+ metabolism,[74] additionally highlights a potential lack of translation of results obtained in rodent models to humans.

3.1.6 | Skin

Taking a different methodological approach, the impact of acute exercise until exhaustion on NADH kinetics of epidermal skin cells of highly trained athletes was investigated using the autofluorescence of NADH.[75] Interestingly, acute exercise shifted NAD^+/NADH redox homeostasis in favor of NADH, as indicated by higher baseline fluorescence compared to pre-exercise. Additionally, higher fluorescence was found during both, controlled forearm ischemia and subsequent reperfusion. This suggests that acute exercise alters the NAD^+/NADH redox homeostasis of epidermal skin cells delicately. Since similar alterations of NADH kinetics were found in skeletal muscle[76] and cardiac muscle,[77] the non-invasive assessment of skin NADH fluorescence could serve as an indirect correlate of exercise-induced alterations in skeletal muscle. However, several constraints, such as different perfusion rates during acute exercise, must be acknowledged when applying this method.[75]

In conclusion, acute aerobic exercise seemed to induce an intensity-dependent alteration in NAD^+/NADH redox homeostasis in skeletal muscle tissue. In mice, de novo NAD^+ synthesis was downregulated in hepatic tissue, but upregulated in macrophages, prompting research questions related to a tissue-specific regulation of NAD^+ metabolism in acute exercise scenarios. While endurance exercise was applied in multiple studies, other exercise types (e.g., resistance exercise) and durations were barely considered. Finally, erythrocyte NAD^+/NADH redox homeostasis was altered in a similar manner as in skeletal muscle (Figure 2).

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3.2 | Exercise training boosts NAD^+ metabolism

In contrast to acute exercise, exercise training is characterized by regularly recurring states of increased energy demand. These stimuli are sensed by metabolically stressed tissue and transduced into adaption processes to increase the biological capacity for future exposure. Besides commonly described exercise adaptions such as increased mitochondrial density and capillarization,[62] the following paragraphs are devoted to studies investigating adaptions in NAD^+ metabolism induced by exercise training. Like acute exercise studies, most investigations were performed in skeletal muscle tissue, while some also focused on blood, liver, kidney, cardiac muscle, brain, testicles, adipose tissue, or epidermal skin cells.

3.2.1 | Liver

In liver tissue, exercise training induced profound mRNA changes of kynurenine pathway enzymes.[78] These mRNA changes were also accompanied by higher NAD^+ levels, suggesting a chronic upregulation of de novo NAD^+ synthesis. Similarly, NAD^+/NADH ratios were increased in liver tissue of trained rats compared to controls.[79] Although these adaptions seem plausible against the backdrop of hepatic recirculation of NAD^+ precursors to peripheral tissues,[16] it remains inconclusive whether the results may be translated to humans due to interspecies difference in the metabolization of NAD^+ precursors.[74]

3.2.2 | Skeletal muscle

The impact of exercise training interventions on skeletal muscle tissue is largely consistent across rodents and humans. Although some rodent studies failed to induce alterations in NAD^+ metabolism,[68,80,81] several others revealed an increase in NAMPT protein[67,82] or NAD^+ concentration.[82,83] Similarly, in humans, exercise training led to an increase in NAMPT protein,[66,67,84,85] NAMPT mRNA,[66] and total NAD(H) concentrations.[64,85] This renders the NAD^+ salvage pathway a highly adaptive component of NAD^+ metabolism in skeletal muscle tissue. An interesting hypothesis for these adaptions is, whether the reduced intramuscular oxygen tension found during exercise[86–88] favors NAD^+ salvage pathway over de novo NAD^+ synthesis, especially considering the oxygen dependency of IDO[46] and KMO.[89] Along these lines, acute exercise regimens would rely more strongly on NAD^+ salvage pathway activity, ultimately resulting in chronic upregulation of NAMPT enzyme and increased NAD^+ levels.

Taking a closer look, exercise training interventions mainly increased NAD^+ levels when performed on aged rodents[82,83] and humans.[85] Since resting NAD^+ levels are

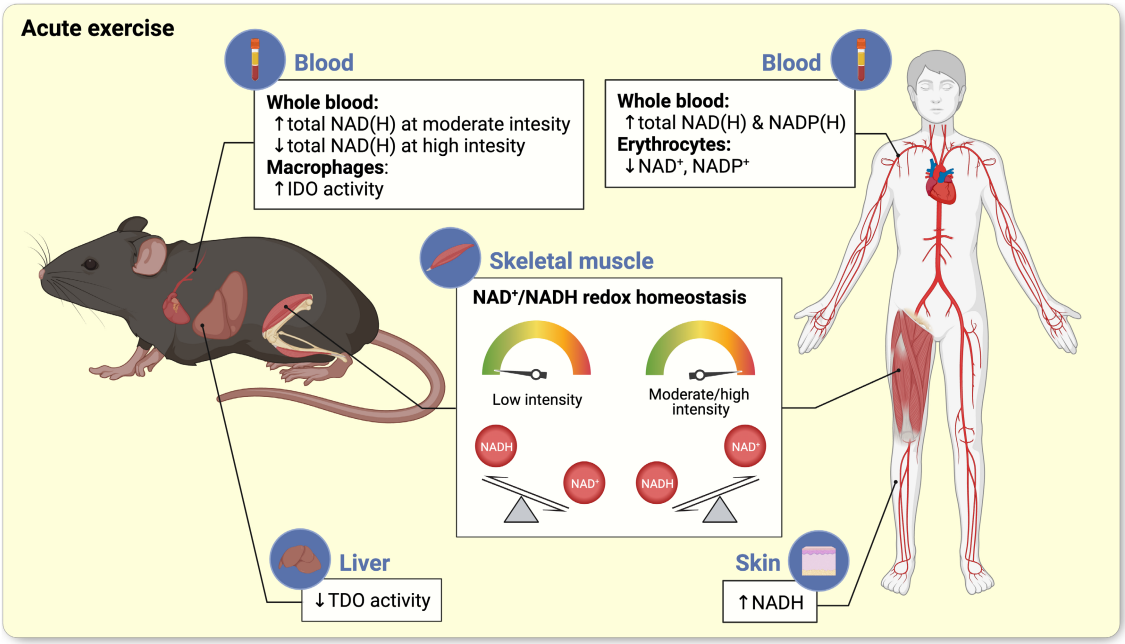

FIGURE 2 Effect of acute exercise on tissue-specific NAD^+ metabolism in rodents and humans.
IDO, indolamine 2,3-dioxygenase; KMO, kynurenine 3-monooxygenase; TDO, tryptophan 2,3-dioxygenase; Created with BioRender.com

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generally lower in these populations compared to young controls,[90,91] the observed increase might resemble an exercise-mediated adaption of senescent NAD^+ levels, resulting in a more juvenile phenotype. Limiting these results, however, none of the mentioned exercise studies compared resting NAD^+ levels of the aged subjects to young controls. In young conditions, NAD^+ levels were only altered in response to long-term regular exercise. While standard exercise interventions lasting several weeks failed to induce alterations in NAD^+ levels in rodents,[80,81] long-term exercise exposure over a year or longer increased NAD^+ levels in humans.[64] Supporting this, several cross-sectional analyses revealed a positive correlation between NAMPT protein levels and $\dot{V}O_{2max}$,[66,84] suggesting that long-term exposure to exercise increases salvage pathway activity, thereby leading to higher NAD^+ levels.

3.2.3 | Blood

With respect to systemic NAD^+ metabolism, decreased NAMPT protein levels were found in blood serum of healthy individuals after 6 months of combined strength and endurance training.[92] Since NAMPT protein levels frequently increase in response to exercise training in skeletal muscle tissue (see Section 3.2.2), these results could resemble an increased autonomy of skeletal muscle for (re)synthesis of NAD^+, rendering systemic NAD^+ supply via circulating NAMPT less necessary. However, no alterations in systemic NAMPT levels were found in response to a shorter exercise intervention of 4 weeks,[93] raising questions concerning the appropriate duration of exercise training interventions. Of note, a decreased $NAD^+/NADP^+$ ratio was found in this study, which was mainly explained by an increase in NADP(H) content. Considering the sprint interval character of the applied exercise intervention, repeated generation of ROS might have induced an increase in antioxidative defense systems like the glutathione redox system, which requires NADPH as a reducing equivalent for regeneration of glutathione.[94] Interestingly, exercise-induced alterations of systemic NAD^+ metabolism were not limited to healthy individuals but also proved beneficial in a cohort of elderly males with clinically stable heart failure. In line with other lifestyle interventions aiming to restore NAD^+ levels in old or diseased populations,[3] an increase in NAD^+ levels was found in response to 4 weeks of cycling.[95] Although these results hold promising therapeutic potential, it must be noted that the resting NAD^+ levels in the investigated population were not compared to healthy controls, thereby questioning whether the investigated population displayed decreased NAD^+ levels.

3.2.4 | Skin

Similar to the acute effects of exercise on NADH levels of epidermal skin cells of highly trained athletes, 7 weeks of sport-specific training resulted in higher baseline NADH fluorescence.[96] Since a shift in NAD^+/NADH redox homeostasis is unlikely a consequence of exercise training, these results presumably represent a chronic increase in total NAD(H) concentrations. However, since the fluorometric determination of NADH allows no direct conclusion on NAD^+ levels, these results must be seen as rather preliminary.

3.2.5 | Brain

Approaching de novo NAD^+ synthesis from a rather different angle, Liu et al. found decreased IDO levels in the brain of rats after 4 weeks of swimming exercise.[97] Though investigated in the context of neuroinflammation and depressive-like behavior, these results give an important hint at the chronic regulation of de novo NAD^+ synthesis inside the central nervous system. Since oxygen levels are considerably lower in brain compared to other tissues,[98] oxygen-dependent de novo NAD^+ synthesis seems to play a lesser role in brain NAD^+ metabolism per se. A possible explanation for the lower IDO levels in response to exercise training might be an increased reliance on salvage pathway activity, especially since this kind of adaption is also frequently observed in other tissues such as skeletal muscle (see Section 3.2.2). Reinforcing this assumption, a salvage pathway also dominates brain purine metabolism, where loss of function comes at the cost of severe neurological symptoms—circumstances most famously known from Lesch–Nyhan syndrome.[99] Since salvaging NAD^+ is bioenergetically much more efficient than de novo synthesis, these exercise adaptions seem plausible. However, very few research endeavors have focused on brain NAD^+ metabolism, posing an urgent need for scientific investigation.

In conclusion, while de novo synthesis was found to increase in hepatic tissue of rodents in response to exercise training, salvage pathway activity increased in skeletal muscle of both, rodents, and humans. Besides higher NAD(H) concentrations, increased NAMPT expression was among the most observed adaptions. On a systemic level, NAMPT concentrations decreased in blood serum of healthy individuals, while NAD^+ concentrations increased in elderly patients suffering from heart failure. In contrast to studies focusing on acute exercise, exercise training interventions frequently applied resistance exercise as training stimulus, with results being largely consistent to endurance exercise conditions (Figure 3).

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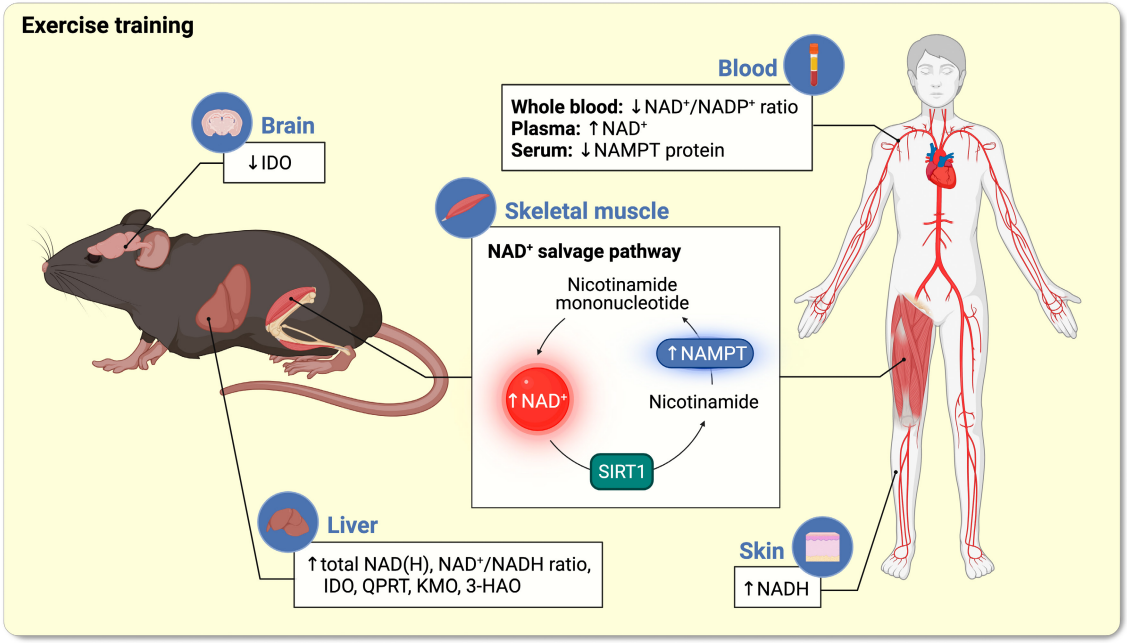

FIGURE 3 Effect of exercise training on tissue-specific NAD^+ metabolism in rodents and humans.
3-HAO, 3-Hydroxyanthranilate 3,4-dioxygenase; IDO, indolamine 2,3-dioxygenase; KMO, kynurenine 3-monooxygenase; NAMPT, nicotinamide phosphoribosyltransferase; QPRT, quinolinate phosphoribosyltransferase; SIRT1, sirtuin 1; TDO, tryptophan 2,3-dioxygenase; Created with BioRender.com

4 | SIRT1/CLOCK:BMAL1 SIGNALING AS A POTENTIAL MOLECULAR MECHANISM FOR EXERCISE-INDUCED ADAPTIONS IN NAD^+ METABOLISM

In search of molecular signaling pathways mediating adaptions of NAD^+ metabolism to exercise training, an interesting hypothesis revolves around the circadian expression of NAMPT. NAMPT expression is controlled by the two core circadian clock proteins circadian locomotor output cycles kaput (CLOCK) and brain and muscle ARNT-like 1 (BMAL1). Interestingly, SIRT1, the cytosolic and nuclear isoform of the mammalian sirtuin protein family, deacetylates both histones and BMAL1, leading to chromatin packing and reduced transcriptional activity. Conversely, low SIRT1 activity induces acetylation of the same structures, resulting in chromatin unpacking, thereby facilitating transcription.[100] Since SIRT1 activity is strongly dependent on the local NAD^+/NADH ratio, it serves as a metabolic switch, coupling NAD^+/NADH redox homeostasis to transcriptional activity. Considering that one of the gene products controlled by the CLOCK:BMAL1 dimer is NAMPT, SIRT1/CLOCK:BMAL1 signaling forms a circadian feedback loop in which NAD^+ levels are kept constant via transcriptional regulation of NAMPT.[101–103] Of note, circadian expression of NAMPT has been shown in human primary myoblasts as well as human muscle biopsies, indicating that the abovementioned mechanisms are of major relevance for human physiology.[104] While the impact of dietary approaches on these circadian rhythms has received considerable attention,[43,100] far less work has focused on other lifestyle interventions such as exercise. Due to apparent disruptions in NAD^+/NADH homeostasis in response to acute exercise (see Section 3.1), we propose SIRT1/CLOCK:BMAL1 signaling as a blueprint mechanism that connects acute exercise-induced disruptions in NAD^+ metabolism to chronic adaptions to exercise training, such as increased NAMPT expression or higher cellular NAD^+ levels (see Section 3.2). In response to low NAD^+/NADH ratios, as observed during acute high-intensity exercise,[52–55] SIRT1 activity would decrease, resulting in acetylation of histones and BMAL1. This would in turn facilitate CLOCK/BMAL1 dimerization and subsequent binding to the NAMPT promotor, which would result in increased NAMPT expression, higher salvage pathway activity and increased NAD^+ levels. On the contrary, in the recovery period following exercise, when NAD^+ levels are increased,[63,64] SIRT1 activity would also increase. While this would dampen CLOCK-BMAL1-driven NAMPT expression via deacetylation of histones and BMAL1, other target proteins such as the forkhead box O1 (FOXO1) or the peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) benefit of SIRT1-induced deacetylation.[65,105] These post-translational modifications increase the transcriptional activity of FOXO1 and PGC-1α target genes, resulting in numerous of the commonly described

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exercise adaptions such as improved lipid metabolism or mitochondrial biogenesis.[62] Besides SIRT1-dependent deacetylation, phosphorylation by 5'-AMP-activated protein kinase (AMPK) was shown to be necessary for the induction of PGC-1α.[65,105] Additionally, AMPK was shown to play a role in the post-translational regulation of NAMPT expression in skeletal muscle tissue of mice.[67] These results render both, NAD^+/NADH ratio and AMP/ATP ratio important metabolic signals in the context of acute exercise settings. Alterations in these ratios are sensed by sirtuins and AMPK, respectively, leading to the induction of exercise-related transcriptional programs. Altogether, the accumulation of acute exercise stimuli results in chronic adaption of metabolically stressed tissue, bridging the gap between acute metabolic disruptions and exercise training adaption (Figure 4). Of note, while the AMPK- and SIRT1-mediated post-translational modifications of PGC-1α and FOXO1 are well-established signaling events in the context of exercise,[65] our proposed SIRT1/CLOCK:BMAL1-mediated regulation of NAMPT and the subsequent salvage pathway activity is purely speculative so far. However, since the same transcription-translation feedback loop accounts for circadian oscillations in NAD^+ levels via NAMPT expression,[101,102] an equal regulation seems highly probable in exercise settings.

5 | LIMITATIONS AND FUTURE PERSPECTIVES

Although several studies in both rodents and humans investigated the impact of exercise on isolated aspects of NAD^+

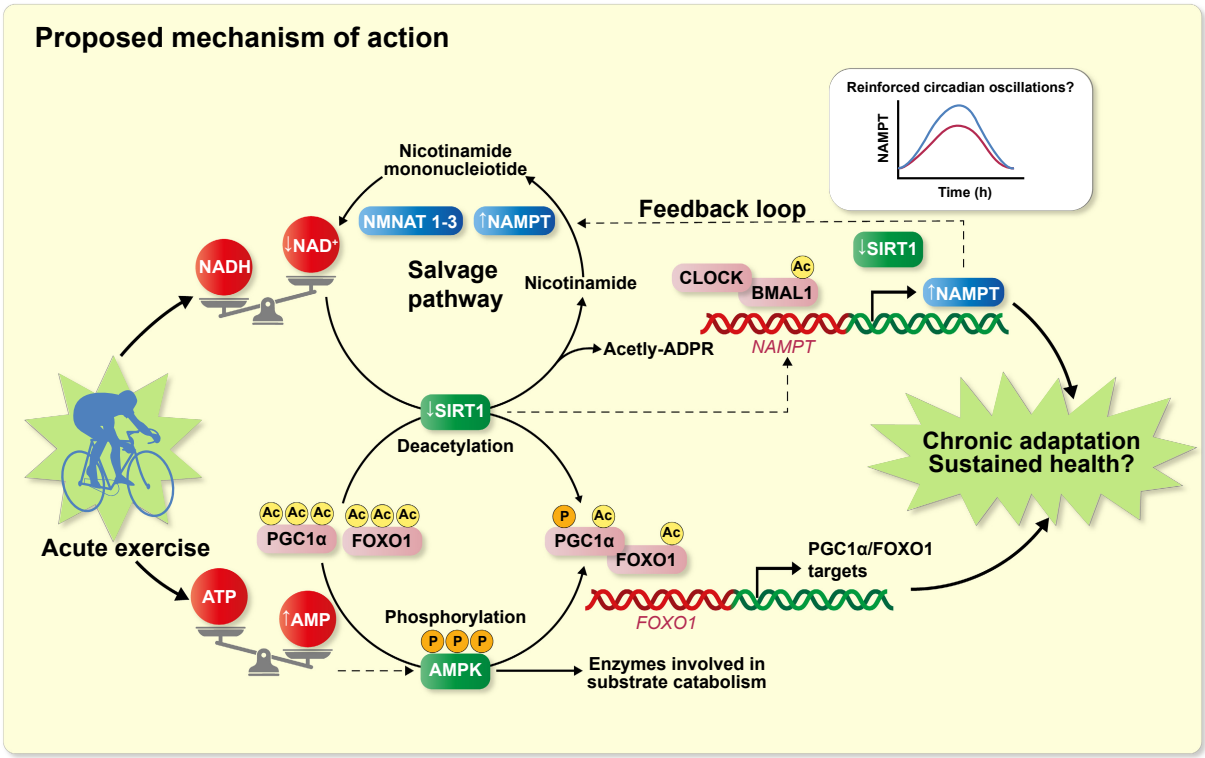

FIGURE 4 SIRT1/CLOCK:BMAL1 signaling as a potential molecular mechanism for exercise-induced adaptions in NAD^+ metabolism. Acute high-intensity exercise is characterized by profound alterations in cell metabolism, leading to a transient increase in AMP/ATP ratio and a decrease in NAD^+/NADH ratio. Higher AMP levels activate AMPK, resulting in phosphorylation of PGC-1α and increased substrate catabolism. The decreased NAD^+/NADH ratio dampens SIRT1 activity, which could result in acetylation of histones and BMAL1, thereby facilitating binding of the CLOCK:BMAL1 dimer to the NAMPT promotor. In turn, NAMPT expression would increase, which could boosts salvage pathway activity and NAD^+ levels. Therefore, SIRT1/CLOCK:BMAL1 signaling could depict a crucial component of exercise-induced adaptions in NAD^+ metabolism, which might reinforce circadian oscillations of NAMPT and/or NAD^+. In contrast, in the recovery period following acute exercise, rising NAD^+/NADH ratios would increase SIRT1 activity. While this would dampen NAMPT expression, it facilitates deacetylation of PGC-1α and FOXO1, inducing many of the commonly described adaption processes associated with exercise training.

AMP, adenosine monophosphate; AMPK, adenosine monophosphate (AMP)-activated protein kinase; ATP, adenosine triphosphate; BMAL1, brain and muscle ARNT-like 1; CLOCK, circadian locomotor output cycles kaput; FOXO1, forkhead box O1; NAMPT, nicotinamide phosphoribosyltransferase; NMNAT1-3, nicotinamide mononucleotide adenylyltransferase 1-3; PGC-1α, peroxisome proliferator-activated receptor γ coactivator 1α; SIRT1, sirtuin 1

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metabolism in specific tissues, few did so in a more holistic manner. While focusing on a single tissue of interest is a good starting point, future research should try to draw connections between different tissues to allow for a more realistic representation of whole-body NAD^+ metabolism and potential inter-organ crosstalk. Additionally, exercise was often complemented with other interventions such as nutritional programs or injections with NAD^+ precursors, rendering only the exercised, healthy control groups eligible for this review. While this obviously limited the quality of the results, it also prevented definitive conclusions concerning the impact of different exercise modalities. For mouse models, it remains questionable whether the results can be translated to humans. Mice convert tryptophan more efficiently and rely more on de novo NAD^+ synthesis from tryptophan, while humans use alternative precursors such as NA, NR, NMN or nicotinamide.[74] Since NAD(H) levels were assessed in cell lysates across all investigations, the characterization and quantification of subcellular NAD(H) pools is another promising area of research. Besides enabling insights into subcellular differences in NAD(H) concentrations, this would also help to elucidate crucial interconnections between intensity-dependent substrate catabolism and NAD^+/NADH redox homeostasis. While such subcellular measurements were recently applied in cell culture models,[56] a transfer to animal or human studies is still pending due to methodological constraints. Characterizing the subcellular NAD^+ pools would also resolve many of the uncertainties concerning cell signaling and the carryover of acute exercise bouts to exercise training adaptions. In this context, another interesting question is, whether NAD(H) levels increase as such or as a function of mitochondrial density in response to exercise training. To consolidate the observed results and evaluate the potential of exercise as a tissue-wide NAD^+-boosting lifestyle intervention, future exercise studies should investigate different populations suffering from NAD^+-related pathologies. While exercise does certainly not constitute the anecdotally praised "fountain of youth", we do believe that tissue-specific adaption processes increase cellular NAD^+ levels in a stress-response manner. Finally, it should be highlighted that this review includes studies investigating exercise-induced alterations in NAD^+ metabolism, without considering changes in NAD^+-consuming enzymes such as sirtuins, PARPs, CD38, CD157 or SARM1. While our focus was to elucidate the impact of exercise on the biosynthetic pathways yielding $NAD(P)^+$ and NAD(P)H, the impact on NAD^+-consuming enzymes is, without doubt, an interesting topic for future research.

6 | OVERALL CONCLUSION

Acute exercise led to profound disruptions of NAD^+ metabolism in various tissues of both rodents and humans, with alterations being highly dependent on the type and intensity of exercise as well as the investigated tissue. In response to exercise training, NAD^+ metabolism was most prominently marked by an increased NAMPT expression and higher NAD^+ levels, especially in muscle tissue. Although the molecular mechanisms mediating these adaptions remain to be investigated, it makes NAD^+ salvage pathway a highly responsive branch of NAD^+ metabolism and opens promising avenues for exercise as an NAD^+-boosting strategy. Future investigations should focus on replicating these results in different NAD^+-related pathologies and explore connections to clinical symptoms and markers of disease progression to assess the therapeutic potential of exercise in improving age-related symptoms and overall health.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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