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Review Article

Modulation of NAD metabolism by oxygen availability

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ABSTRACT

Reduced oxygen availability (hypoxia) can result in decreased energy levels, perturbed redox homeostasis and permanent cellular damage. Efficient hypoxic stress responses and the induction of protective adaptations are crucial to prevent hypoxic damage and can be targeted to improve cellular resilience and health. Nicotinamide adenine dinucleotide (NAD) metabolism is highly sensitive to changes in oxygen availability and interacts with other stress pathways, such as the oxygen-regulated transcription factors hypoxia-inducible factors (HIFs), to orchestrate cellular responses and adaptations to hypoxia. We evaluate what is known about this interaction, how it may be modulated and which benefits could be expected from related therapeutic interventions. We further discuss, which future research is needed to develop therapeutic strategies targeting the hypoxic response-NAD axis.

1. Cellular responses to hypoxia

Reduced oxygen availability (hypoxia) for cells and tissues can be a consequence of physiologically increased oxygen demand, reduced environmental oxygen availability or impaired oxygen delivery. Most cells of aerobic organisms depend on oxygen, primarily for energy production by oxidative phosphorylation, and cellular hypoxia can result in decreased energy levels, perturbed redox homeostasis, cellular damage and ultimately cell death. To counteract the detrimental consequences of hypoxia, cells dispose of a large arsenal of hypoxia responses and adaptations, including the upregulation of alternative metabolic pathways, the induction of antioxidant mechanisms and the improvement of cellular oxygen supply, for example by vascular remodeling. These processes can be highly beneficial, improving cellular resilience and health [1]. Being able to harness and modulate these responses in a controlled manner therefore has substantial therapeutic value. Changes in oxygen-availability immediately affect nicotinamide adenine dinucleotide (NAD) homeostasis, a regulatory system of cellular metabolism and redox state, that impacts health and healthy aging [2–4]. Central factors in the orchestration of cellular hypoxia responses are the oxygen-regulated transcription factors hypoxia-inducible factors (HIFs), in part by the modulation of cellular metabolism [5]. We recently reviewed how changes in oxygen availability modulate NAD levels and redox status and how supplementation with NAD can protect from hypoxic injuries, both in the context of ageing [2]. Here we aim to explore the biochemical and metabolic basis of cellular interactions of hypoxia response pathways (focusing on HIFs) and NAD metabolism to assess potentials to modulate these interactions for health promotion and to identify knowledge gaps and outstanding questions.

2. Hypoxia, HIFs and cellular energy metabolism

Oxygen availability is an essential regulator of cellular energy metabolism. While in normoxia oxygen-dependent mitochondrial oxidative phosphorylation is the preferred mode of energy production in many cells, reduced oxygen availability requires a switch to anaerobic energy metabolism [6]. The transcription factor family HIFs, especially HIF-1, is importantly involved in this metabolic reprogramming [6].

HIFs are composed of 2 subunits, the oxygen-level regulated α- and the oxygen-independent β-subunit. While HIFs are regulated by many other factors, such as other transcription factors and microRNAs [7,8], oxygen-dependent posttranslational modification and degradation are central in the role of HIFs as coordinators of cellular responses to hypoxia [9]. In the presence of α-ketoglutarate, ferrous iron and ascorbate, prolyl-4-hydroxylase domain containing proteins (PHDs 1–3) hydroxylate HIF-α subunit proline residues, which leads to recognition by the von Hippel Lindau protein and degradation by the 26s proteasome. With

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Glossary

HIFs (hypoxia-inducible factors) oxygen-regulated transcription factors that coordinate the cellular hypoxic response

Hypoxia adaptations (cellular) while this term often is used to refer to genetic adaptations for example due to transgenerational high-altitude residence, here it refers to lasting long-term changes (including biochemical and molecular) of cellular processes resulting from hypoxia exposure

Hypoxia conditioning the application of controlled hypoxia aiming to promote beneficial hypoxia adaptations (an approach that has to be distinguished from severe/uncontrolled forms of intermittent hypoxia, possibly involving detrimental adaptations)

Hypoxic response the ensemble of biochemical and molecular changes occurring as a consequence to reduced oxygen availability

NAD (nicotinamide adenine dinucleotide) NAD is used to summarize both the oxidized, electron-accepting (NAD^+) and the reduced, electron-donating (NADH) forms

NAD metabolism highly compartmentalized metabolic system related to the biosynthesis and catabolism of NAD

Oxidative phosphorylation refers to the oxidation of reduced substrates via the transfer of electrons to oxygen (oxidative) and linked phosphorylation of adenosine diphosphate (ADP) to adenosine triphosphate (ATP)

Substrate-level phosphorylation an alternative energy production to oxidative phosphorylation in which ATP or guanosine triphosphate (GTP) are produced from ADP and a phosphorylated intermediate

Vascular remodeling structural changes in blood vessels as an adaptive response to a physiological or pathophysiological stimulation

decreasing oxygen availability, PHD and factor inhibiting HIF (FIH) [10] reduce their negative regulation, allowing HIF-α to stabilize, bind HIF-β and orchestrate a large transcriptional response via hypoxia response elements (HRE) [6].

HIF-1 coordinates a metabolic switch from oxidative energy metabolism to glycolysis, upregulating components of glycolysis, lactate dehydrogenase A and glucose transporters [11]. HIF-1 is thought to mediate acute cellular responses to hypoxia (during the first hours of hypoxia exposure), after which a switch from HIF-1 to HIF-2 occurs (the "HIF-switch") [12]. HIF-2 orchestrates adaptive responses like erythropoiesis and vascular/extracellular matrix remodeling [13].

3. NAD biosynthesis and metabolism

NAD is a crucial regulator of cellular redox homeostasis and metabolism [14] and thereby of health and senescence but also of central aspects of the hypoxia response [2]. It exists oxidized, electron-accepting (NAD^+) and reduced, electron-donating (NADH). NAD^+ can be oxidized from NADH by dehydrogenases and phosphorylated to $NADP^+$ by NAD^+ kinases [15]. A redox site in NAD's nicotinamide base allows the transfer of hydride from one molecule to another. This function underlies the role of NAD^+/NADH in many biochemical reactions depending on electron transfer. NAD^+ is an essential cofactor for numerous enzymes, such as alcohol and aldehyde dehydrogenases, glyceraldehyde phosphate dehydrogenase (glycolysis), pyruvate dehydrogenase, α-ketoglutarate, isocitrate and malate dehydrogenases of the tricarboxylic acid cycle (TCA) and 3-hydroxyacyl-CoA dehydrogenase (fatty acid oxidation) (for review see Ref. [16]). In normoxia, one of the metabolic functions of the TCA is the reduction of NAD^+ to NADH, which is used by the electron transport system (oxidation of NADH by NADH dehydrogenase/mitochondrial complex I) to contribute to the electrochemical gradient that is required for ATP production by oxidative phosphorylation. NADH-levels thereby are an important factor in the control of cellular respiration in both normoxia and hypoxia [17]. Low oxygen availability reduces oxidative phosphorylation mainly via HIF-1 [6] and less of the NADH produced in the TCA will be oxidized. NADH is not only essential for oxidative phosphorylation, it is also required for the reduction of pyruvate to lactate by lactate dehydrogenase A, in both process NAD^+ is regenerated. In addition, NADH/NAD^+ ratios modulate reactive oxygen species (ROS) production by the mitochondrial electron transport system [18], regulating cellular redox homeostasis.

About 10 % of the cellular NAD pool is converted to NADP ($NADP^+$ and NADPH) [19]. NADP exerts different functions than NAD and plays a major role, for example in the biosynthesis of fatty acids, cholesterol and steroids, the antioxidant (glutathione reductase) and immune (NADPH oxidase) defenses, all processes playing roles in cellular adaptations to hypoxia [6].

The cellular homeostasis of NAD is determined by NAD biosynthesis, NAD^+ regeneration and consumption. In mammalian cells, 3 pathways of NAD biosynthesis are known [14] (Fig. 1): (i) De-novo biosynthesis from tryptophan (via the kynurenine pathway) or aspartic acid. (ii) Synthesis through the deamidated salvage pathway (Preiss-Handler pathway) from nutritive compounds - such as nicotinic acid (niacin) - or from kynurenin pathway-derived nicotinic acid mononucleotide (NAMN). (iii) Synthesis via the amidated salvage pathway from different forms of vitamin B3: e.g., conversion of nicotinamide (NAM) to nicotinamide mononucleotide (NMN) via nicotinamide phosphoribosyltransferase (NAMPT) or of nicotinamide riboside (NR) to NMN by NR kinase.

Vitamin B3 forms (like niacin, NAM and NR) from which NAD^+ can be produced are termed NAD^+ precursors. All 3 NAD biosynthesis pathways are regulated by hypoxia. The kynurenine pathway enzymes indolamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO) require oxygen, but IDO also works with superoxide, which may explain observations of increased IDO activity in hypoxia [15]. TDO levels have additionally been demonstrated to be controlled by HIF-1 in glioblastoma cell lines [20]. Accordingly, the exposure to severe hypoxia (simulating an altitude of 7620 m) was recently shown to profoundly perturb the kynurenine pathway [21]. NAMPT expression is under the control of both HIF-1 and HIF-2 [2,15,22]. Reversely, NAMPT via the regulation of mRNA levels of various metalloproteinases was shown to increase HIF-2α-mediated expression of catabolic enzymes in articular chondrocytes [22]. However, chronic intermittent hypoxia (8 cycles of 1 % oxygen) may attenuate NAMPT enzyme activity, NAD^+ biosynthesis and the NAD^+/NADH ratio, as shown in human umbilical vein endothelial cells [23]. In contrast, serum NAMPT levels of patients with obstructive sleep apnea (OSA), a disease characterized by chronic intermittent hypoxia, increased with the severity of the disease and were associated with the risk of cardiovascular morbidity [24]. This indicates that different types of hypoxia exposure (continuous or intermittent, acute or chronic) as well as the hypoxic dose (severity and duration of hypoxia), potentially exert differential effects on NAD metabolism, and furthermore vary according to the tissue/cell-type. Divergent effects of hypoxia on circulating and tissue levels of components of NAD metabolism have previously been reported (e.g., Ref. [21]). Circulating NAD metabolites or related enzymes may fulfill hitherto underappreciated signaling or support function, possibly conferring hypoxic stress information or protective factors to tissues not directly affected. This is suggested by findings of mouse astrocytes that released vesicles containing NAMPT as a response to hypoxic stress, resulting in neuroprotection (Deng et al., 2022). Also, NMN adenylyltransferase (NMNAT) levels, catalyzing the conversion of NMN and NAMN to NAD^+ in the

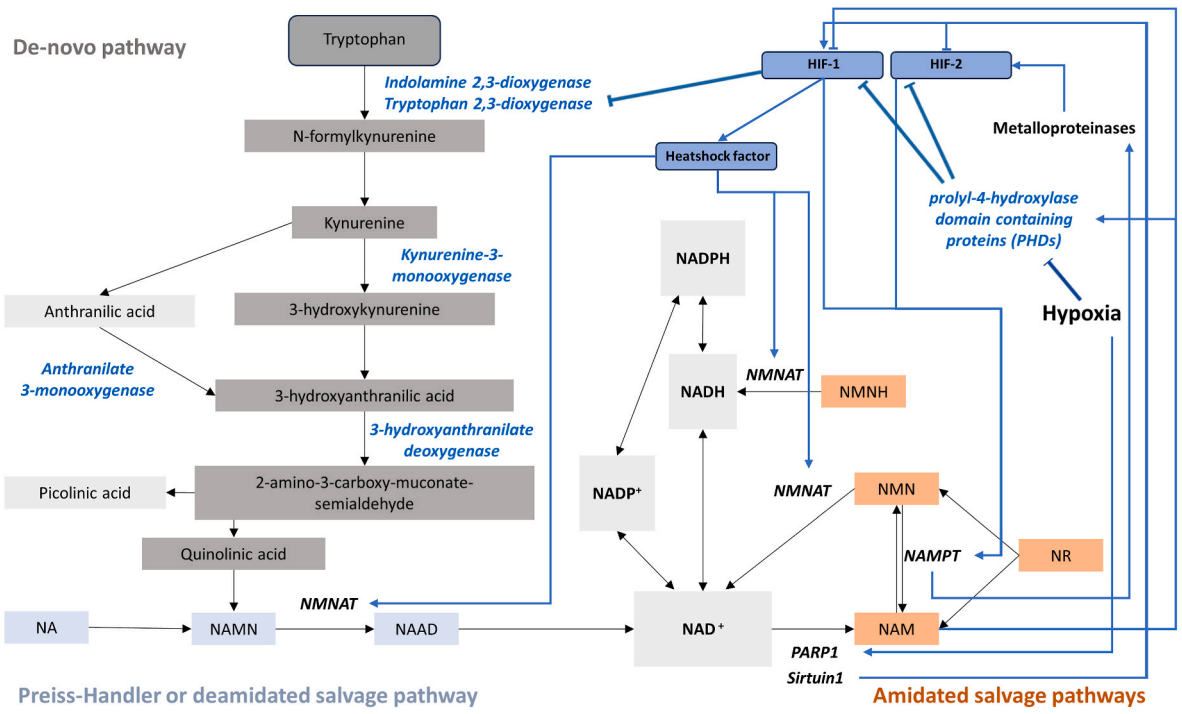

Fig. 1. Regulation of nicotinamide adenine dinucleotide (NAD) biosynthesis pathways by oxygen levels.

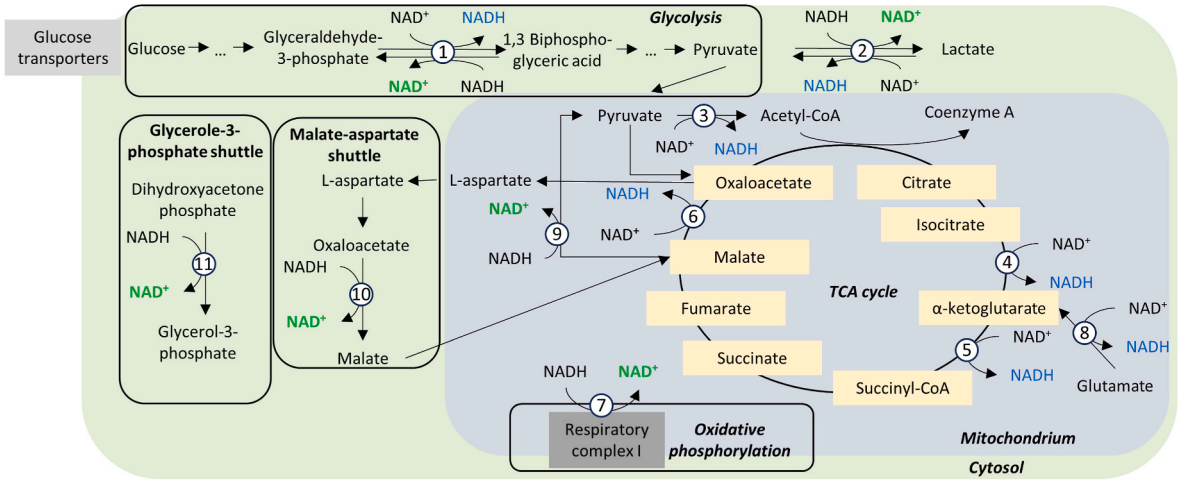

Fig. 2. Simplified overview of some relevant cellular reactions consuming (blue NADH) or regenerating (green NAD^+) oxidized nicotinamide adenine dinucleotide (NAD^+). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

amidated and deamidated salvage pathway, respectively, increased in hypoxia, dependent on HIF-1 and heat shock factor (HSF) [25]. Moreover, NAD^+ precursors interact with HIF pathways. Treatment with NAM, for example, promoted PHD production and reduced HIF-1α levels in human monocyte-derived macrophages [26].

NAD levels can be regulated by dietary intake of tryptophan and NAD^+ precursors, but also various environmental and behavioral factors. Among them, caloric restriction, exercise and circadian rhythms are well established [16]. Energy limitation by fasting or exercise is associated with increased NAD^+ levels, while high energy intake decreases NAD^+ availability in muscle [27] and other tissues. In addition, NAD biosynthesis and metabolism fluctuate in a circadian fashion [2]. As expected from the role of oxygen in regulating NAD metabolism, oxygen availability is a central parameter controlling NAD metabolism. The oxygen-dependent regulation of NAD might constitute a poorly explored factor underlying some of the described health benefits of moderate hypoxia [1]. Relevant cellular metabolism processes depending on NAD and oxygen are depicted in Fig. 2.

The total intracellular NAD pool is spatially separated and this metabolic compartmentalization relies on dedicated transporters and localized signaling processes that orchestrate organelle activities and are still under intense investigation. SLC25A51 or MCART1 has only recently been discovered as major mammalian NAD^+ transporter, maintaining the mitochondrial NAD^+ pool [4,28,29], a pool that has been recently suggested to serve as a buffer for cellular disturbances of NAD homeostasis [30]. NAD^+ is present at high levels into the cytosol, nucleus, and mitochondria [31], but has also been detected in other organelles, the endoplasmic reticulum, Golgi apparatus, and peroxisome [32]. The involved transport routes for these organelles, however, remain poorly understood. Therefore, cellular compartmentalization allows cells to safeguard crucial NAD^+ pools. While a general decline in NAD^+ can be tolerated, the mitochondrial NAD^+ pool is critically

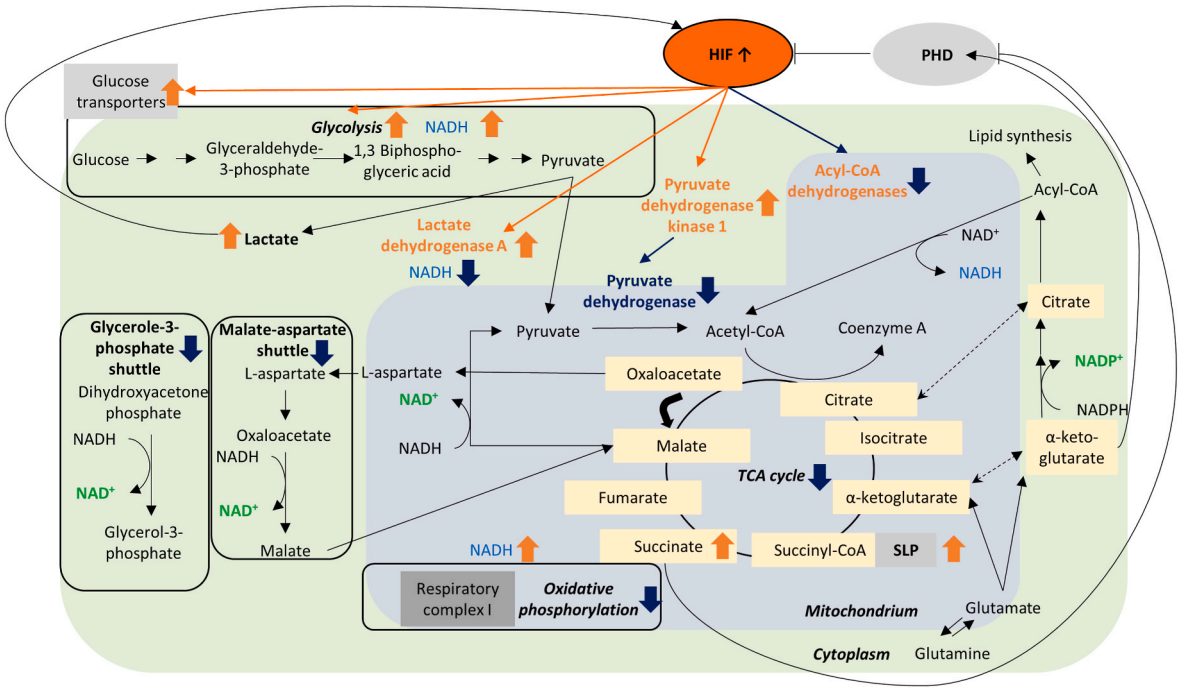

Fig. 3. *Interactions of hypoxia inducible factors (HIFs) and nicotinamide adenine dinucleotide (NAD).* PHD: prolyl-4-hydroxylase domain containing proteins, TCA: tricarboxylic acid cycle.

important. Mitochondria act as a central hub for NAD^+ homeostasis, are capable of buffering NAD^+ levels and interconnected with other subcellular pools, thus mitigating the impact of NAD^+ depletion in other cell compartments. However, direct depletion of mitochondrial NAD^+ has detrimental effects on cellular function [30]. It has been also shown that cellular perturbations such as genotoxic stress [33], or chemical NAD^+ depletion [34] affect mitochondrial NAD^+ levels less than those in the nucleus or cytoplasm. This suggests a protective mechanism, where mitochondrial NAD^+ is only depleted once other cellular compartments are exhausted. Conversely, it has been demonstrated that whole-cell NADH/NAD^+ ratios increased during respiratory chain dysfunction by a maximum of 2-fold following inhibition of mitochondrial complex I or III. In contrast, with complex I inhibition the mitochondrial NADH/NAD^+ ratio increased 77-fold [35], supporting the demonstrated particular cellular vulnerability of the mitochondrial NAD^+ pool [30]. This points to a sophisticated interplay of NAD regulation between cellular compartments.

4. Control of NAD by cellular oxygen availability and HIF

Beside the regulation of enzymatic activities related to NAD metabolism by oxygen, hypoxia substantially influences the NADH/NAD^+ ratio and cellular redox homeostasis. Acute hypoxia is initially associated with a hypoxic-dose-dependent reduction in cellular NAD^+, and a relative increase in NADH [2] due to reduced TCA activity and associated lower oxidation of NADH by type I NADH dehydrogenase (complex I) (Fig. 3), which favors an increased glycolysis: oxidative phosphorylation ratio, similar to the effects of HIF-1 activation [36]. The increased relative NADH levels result in reductive stress and reflect a higher cellular reducing capacity than pro-oxidant drive, perturbing physiological ROS-signaling [37]. Increased NADH levels likely play an important role in the downregulation of cellular energy expenditure in conditions of energy depletion (as during hypoxia), e.g., by inhibiting intracellular transport [38]. In interaction with other hypoxia response systems it may also be required to adjust cells to conditions of reduced oxygen availability. A successful response to the hypoxic stress may be associated with cellular adaptations enabling an improved control of redox and metabolic homeostasis [1]. NAD^+ depletion as occurs in hypoxia impairs a wide array of NAD^+ dependent enzymatic reactions, such as those involving sirtuins, PARP1, and several metabolic processes including fatty acid synthesis, as demonstrated in cancer cell lines [39].

Prolonged severe hypoxia/anoxia, especially together with repeated reoxygenation phases, can lead to an absolute decrease of NAD and a relative depletion of NADH to NAD^+, termed NADH (or mitochondrial) hyperoxidation, and thought to be associated with permanent cellular damage [2].

In the initial phase (up to several minutes) of hypoxia no alterations in carbon inputs into the TCA appear to occur, while in prolonged hypoxia – orchestrated partially by HIF-1 – carbon supply into the TCA, as well as oxidative phosphorylation are downregulated (reviewed in Ref. [40]). The HIF-1-mediated upregulation of pyruvate dehydrogenase kinase 1 [41,42] is likely instrumental for this effect; pyruvate dehydrogenase kinase 1 phosphorylates and thereby inhibits pyruvate dehydrogenase 1, resulting in reduced pyruvate conversion into acetyl-CoA, reduced acetyl-CoA availability for the TCA and thus the downregulation of TCA activity. This slows down the activity of the mitochondrial NADH-shuttles, the malate-aspartate shuttle and the glycerol-3-phosphate shuttle and of oxidative phosphorylation (Fig. 3). Although glycolysis requires NAD^+, in conditions of saturated mitochondrial NADH-shuttles, proliferating cells increasingly rely on glycolysis [43]. Therefore, compensatory mechanisms, such as lactate generation or increased reductive carboxylation of glutamine [44] may provide the necessary NAD^+ to drive glycolysis, for example in hypoxia. In addition, in hypoxic conditions, some of the catalytic reactions of the TCA can occur reversely (e.g., succinate dehydrogenase [45]) and enzymes – like mitochondrial malate dehydrogenase – will in this case generate NAD^+. Hypoxia thus may spur substrate-level phosphorylation linked to the TCA [46], e.g. by succinate thiokinase, with succinyl-CoA as the substrate for ATP or GTP production without the need for oxygen [47]. Thus, a transient relative surplus of NAD^+ may allow for increased glycolytic rates. The reverse mode of mitochondrial malate dehydrogenase in hypoxia has also been suggested to provide NAD^+ that may drive α-ketoglutarate dehydrogenase activity, favoring the accumulation of succinate in hypoxia [46]. Accumulated succinate inhibits PHDs

[48], stabilizing HIFs and therefore representing a potential forward-feeding loop of HIF-1 activity.

In hypoxia, HIF-1 activation leads to glutamate accumulation partly due to HIF-1 and HIF-2-dependent upregulation of glutamine carriers (SLC38A2 and SLC1A5) [49]. Glutamine that is taken up by cells can be converted by glutaminases into glutamate. Glutamate then can be converted into α-ketoglutarate (glutaminolysis), replenishing the TCA, or precursors of the antioxidant enzyme glutathione [49]. The glutamate accumulation in hypoxia may be particularly pronounced in mitochondria as suggested by fluxomics analyses [50].

In hypoxic human cells, increased reductive carboxylation has been shown, converting glutamine-derived α-ketoglutarate, NADPH and CO_2 to isocitrate and NADP $^+$ via cytosolic isocitrate dehydrogenase-1 and then into acetyl CoA [51]. Glucose-derived carbons required for TCA and fatty acid synthesis are directed towards glycolytic metabolism in hypoxia, leading to reduced acetyl-CoA production. Therefore, glutamine-derived acetyl-CoA may be integral for *de novo* lipogenesis in hypoxia and could be partly reversed in cells by the restoration of hypoxia-mediated reduction of pyruvate dehydrogenase activity using dichloroacetate [51]. Reductive carboxylation also supports the biogenesis of nucleotides, attenuates mitochondrial ROS production [52] and – supported by reverse action of malate dehydrogenase 1 (reaction 6 in Fig. 1) – contributes to NAD^+ regeneration [44]. Von Hippel Lindau protein-deficient cells and cells with mitochondrial dysfunction predominantly rely on reductive glutamine metabolism for acetyl-CoA production even in normoxia [44,51].

While the HIF-system is a crucial cellular oxygen sensor, various systemic oxygen sensors exist as well. Among them, the peripheral chemosensors located in the carotid bodies appear to be the primary oxygen sensors mediating cardio-respiratory responses to changes in the partial pressure of oxygen in blood [53,54]. Hypoxia sensing in the glomus (type I) cells of the carotid body regulates ion fluxes across the membranes of these cells. Inhibition of potassium channels is thought to be a principal mechanism leading to the depolarization of glomus cells and initiating signaling to respiratory and autonomic brain stem centers that regulate major systemic responses to hypoxia [53,54]. The modulation of glomus cell potassium channels likely depends on hypoxia-induced inhibition of complex I, which results in elevated levels of NADH (see above) and mitochondrial ROS, both of which have been suggested to inhibit potassium channels at the glomus cell membrane [54,55]. Moreover, carotid bodies also sense increasing blood lactate levels (hypoxia increases lactate levels), which has recently been shown to rapidly increase the cytosolic NADH:NAD^+ ratio, resulting in cell depolarization through modulation of membrane cation channels [56]. The observation of a particularly high sensitivity of the NAD(P) redox system to hypoxia in the carotid body [57] corroborates its role in systemic oxygen sensing and hypoxia responses. Interestingly, the presumed primary molecular oxygen sensor in the carotid body, mitochondrial complex IV [54], appears to be particularly sensitive to hypoxia at least in part due to a HIF-regulated specific protein composition [58,59].

5. Bidirectional regulation between hypoxia and sirtuins

NAD^+ is consumed by various enzymes, such as enzymes of the ADP-ribosyltransferase diphtheria toxin-like family (ARTDs, including the nuclear poly(ADP-ribose) polymerase 1 (PARP1)), cyclic ADP-ribose (cADPR) synthases (CD38 and CD157) or by sterile alpha and TIR motif containing 1 (SARM1) and NAD-dependent deacetylases (sirtuins).

Sirtuins are crucial coordinators of metabolism and modulators of aging. They transfer acyl groups of substrate proteins to the ADP-ribose moiety of NAD^+, cleaving it and releasing nicotinamide and O-acetyl-ADP-ribose. By the deacetylation of transcription factors, including of HIFs, sirtuins coordinate their activity and thereby cellular physiology [60]. Sirtuin interactions with HIFs represent an important link between NAD-homeostasis and hypoxia. The stress-inducible sirtuin-1 deacetylases HIFs at lysine 674 in normoxia, which results in inactivation of HIF-1 gene transactivation *in cellulo* and in mouse tissues by inhibiting cofactor recruitment [61]. Decreased NAD^+ levels and/or increased NADH (e.g., during hypoxia) inhibit sirtuin 1 and thereby increase HIF-1 activity [61]. Accordingly, the upregulation of sirtuin 1 activity (dietary by resveratrol or genetically by overexpression of sirtuin 1) increased deacetylation of HIF-1α in hypoxic kidney cells and attenuated HIF-1α activity [62]. However, in the absence of pyruvate, high acetylation rates of HIF-1α due to NADH-mediated deactivation of sirtuin-1 also led to (proline-hydroxylation independent) HIF-1α degradation by the von Hippel-Lindau pathway [63]. Deacetylation of HIF-2α by sirtuin-1 has been shown to increase HIF-2 activity in cultured cells and mice [64]. However, in renal interstitial cells sirtuin-1 protected from renal fibrosis, possibly by inhibiting HIF-2 [65]. This – potentially tissue-specific – finetuning of the HIF-system by sirtuin-1 may be required for protective adaptations of hypoxia, as sirtuin-1 has been described as required for beneficial effects of hypoxia conditioning, for example in a mouse model of aneurysmal subarachnoid hemorrhage [66]. Sirtuin-1 is an important component of cellular responses to stress and is also involved in the regulation of mitochondrial biogenesis, e.g. by regulating peroxisome proliferator-activated receptor-γ coactivator 1α (PGC1α) and other factors [15]. Not only does hypoxia modulate NAD metabolism but NAD depletion and associated reduced sirtuin-1 activity has also been linked to the induction of a “pseudohypoxic” state, associated with HIF-1α stabilization and a downregulation of the expression of mitochondrially encoded components of the oxidative phosphorylation machinery [67]. Many of the reported health benefits of NAD^+ boosters have been attributed to enabling sirtuin actions [16]. The effect of decreased sirtuin-1-mediated deacetylation of HIF-1 due to reduced NAD^+ availability in hypoxia may be crucial to counteract negative consequences of excessive HIF-1 activity (which – *inter alia* – is associated with inflammation, cell death and fibrosis and is involved in the development of diseases like rheumatoid arthritis, chronic obstructive pulmonary disease and cancers [68,69]) and contributes to the HIF-switch, promoting transition from increased HIF-1α levels to dropping HIF-1α and rising HIF-2α [12]. Besides sirtuin-1 almost all other sirtuins have been demonstrated to regulate HIF-levels: sirtuin-2 [70], sirtuin-3 [71,72], sirtuin-4 [73], sirtuin-6 [72] and sirtuin-7 [74]. Importantly, while sirtuins are commonly considered protective, some reports suggest tumor-promoting effects of some sirtuins in specific cancers [75]. In the context of HIF-sirtuin interactions this is of particular interest, since HIFs are also involved in cancer progression [76] and cancer-related inflammation [77].

PARP1, a crucial regulator of DNA repair and replication, transcription, ribosome biogenesis, programmed cell death and other processes [78], also requires NAD^+ as a co-factor for the transfer of NAD’s ADP ribose moieties to nuclear proteins. PARP1 activities increase as a consequence of the DNA damage response in severe hypoxia leading to a competition for NAD^+ and this can result in decreased sirtuin activity [2]. PARP1 can activate both HIF-1 and HIF-2 [2].

6. Modulation of NAD metabolism in health and disease: oxygen regulation and NAD precursor supplementation

Many human pathologies are associated with hypoxia, either as a cause or consequence of an insult or disease, and the regulation of NAD is emerging as an important factor of the hypoxic response in such diseases. One method to target the hypoxic response is hypoxia conditioning, in which subjects are repeatedly exposed to mild/controlled environmental hypoxia with the goal to improve the hypoxic tolerance and cellular and systemic function by inducing endogenous adaptations to hypoxia [1]. It is important to differentiate hypoxia conditioning from severe/uncontrolled forms of intermittent hypoxia (e.g., as in obstructive sleep apnea) in which adaptive responses are not successful.

Intermittent hypoxia conditioning has been shown to increase NAD^+

Table 1
NAD supplementation strategies in hypoxia-related conditions.

Species/model	NAD intervention	Outcomes	Ref
Mice, model of high-altitude blood-retinal barrier injury	100 mg/kg NMN per day, five days before hypoxia exposure, oral	Hypoxia increased retinal cell apoptosis and significant phosphorylation of VE-cadherin. NMN treatment prevented endothelial cell DNA oxidative damage and protected the endothelial barrier	[87]
Mice, neonatal HI, and HT22 neuronal cells, OGD-R	Mice: 5 mg/kg NAD^+ per day, i.p., before and after HI, cells: NAD^+: 100 μM for 12 h	NAD^+ improved morphological damages and neurobehavioral defects, increased seizure thresholds. NAD^+ enhanced cell viability and prevented ferroptosis in HT22 cells after OGD-R	[88]
Rats, PH model	8.5 mg/100 g nicotinic acid, and 40 mg/kg honokiol (daily gavage for 6 weeks)	Sugen/Hypoxia (three weeks) induced PH, lowered SIRT3 levels and caused exercise intolerance. Honokiol and Nicotinic acid increased SIRT3 levels in the gastrocnemius and soleus muscles and enhanced exercise capacity	[89]

Notes. *HI, hypoxic-ischemic injury; i.p., intraperitoneal injection; NMN, nicotinamide mononucleotide; OGD-R, oxygen glucose deprivation and reoxygenation; PH, pulmonary hypertension; SIRT3, sirtuin 3.*

levels [79] and improve glucose metabolism in mice [79]. Reversely, supplementation of β-cells with NMN prevented their impaired HIF-signaling when exposed to excessive free fatty acids [80]. Hypoxia conditioning has also been demonstrated to be beneficial in models of acute kidney injury, at least partially due to the effects of hypoxia on NAD^+ levels (summarized in Ref. [2]). Moreover, hypoxia conditioning has been shown to increase serum kynurenine levels and preserve NAD^+ in the kidney after subsequent renal ischemia reperfusion injury [81]. Low cerebral NAD^+ levels in a mouse model of Leigh syndrome could be prevented by keeping the mice continuously in an environment with 11 % oxygen [82]. Additionally, the combination of very mild hypoxia (17 % oxygen) with nicotinic acid supplementation improved pathology and survival in this model [82]. In contrast, in cancer, NAD-metabolism (e.g., NAMPT activity) is commonly upregulated [83], likely due to cancers high jacking hypoxia-response mechanisms. It is possible that ambient hypoxia promotes cancer-related NAD metabolism upregulation and HIF- and NAD-metabolism-related responses to stress may interact to increase cancer-cell resistance [84]. However, hypoxia conditioning may also improve NAD homeostasis, preventing excessive NAD production in tumor environments, a possibility that requires future investigation.

Some evidence indicates that the interaction of NAD and HIF can be used therapeutically in pathological hypoxia by boosting cellular NAD^+ levels. Exposure of mice to severe, prolonged hypoxia resulted in HIF-1-mediated adipose tissue fibrosis [85]. Dietary supplementation with NMN prevented excessive HIF-1 activation and reduced fibrosis [85]. There is also increasing evidence that NAD^+ supplementation efficiently protects from myocardial ischemia/reperfusion injury, as recently reviewed [2]. Similarly, restoration of NAD^+ has been shown to be required for bioenergetic recovery of cardiomyocytes after oxygen–glucose deprivation injury [86].

We recently summarized the published reports, how hypoxia conditioning can be used to modulate NAD levels and how NAD supplementation has been shown to protect from hypoxic injury or related diseases [2]. Since then, some new reports have been published that further corroborate these interactions. These new findings are summarized in Table 1.

7. Concluding remarks and perspectives

Reduced oxygen availability as well as impaired NAD homeostasis are warning signs for cellular damage and cause physiological stress responses to counteract pending danger. Interactions of hypoxia responses with the NAD system are instrumental for successful hypoxic stress responses and possibly can be harnessed for targeting cellular metabolism, redox regulation and management of hypoxia (Fig. 4). In addition, modulating NAD availability (e.g., by supplementation with NAD^+ precursors) alters the cellular resistance to hypoxia and may influence the efficiency of physiological adaptations, as recently reviewed [2]. This opens up exciting new scientific and clinical perspectives, since most diseases and normal ageing are associated with dysregulations in

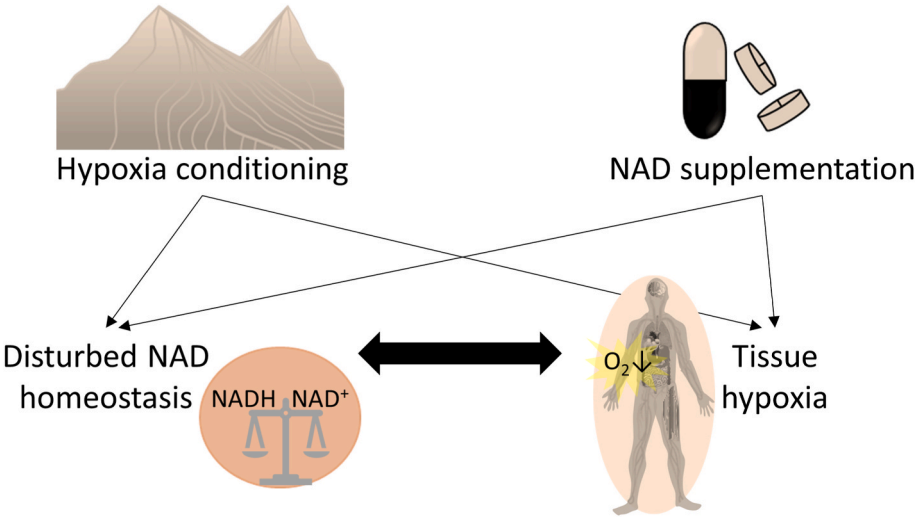

Fig. 4. Potentially complementary effects of strengthening the hypoxic response and NAD supplementation. Most diseases are associated with both disturbances in NAD metabolism and with tissue hypoxia. While treating dysregulated NAD homeostasis with NAD supplements and hypoxia with approaches to improve adaptations to hypoxia (e.g., hypoxia conditioning) are obvious applications, the close interplay of hypoxia responses and changes in NAD metabolism suggests that also targeting NAD homeostasis with strategies to improve hypoxia and reducing hypoxic injury by strengthening NAD homeostasis can represent complementary approaches.

Questions for future research

- How does systemic hypoxia regulate cellular uptake and distribution of NAD metabolites and NAD^+ precursors?
- Can hypoxia conditioning effects be mimicked by pharmacological manipulation of NAD metabolism?
- Does hypoxia conditioning induce long-lasting improvements of the control of NAD homeostasis or merely temporarily perturb NAD metabolism?
- What are the absolute concentrations of NAD^+ and NADH in different organelles and how cell-specific is such cellular compartmentalization?
- Which concentrations of NAD^+ precursors are optimal to increase the resilience to hypoxic stress?
- What are the dynamics (spatial and temporal) of NAD metabolism changes in response to hypoxia?
- How inter-individually different is the response of NAD metabolism to hypoxia?
- How does the interaction of NAD metabolism and hypoxic response pathways (e.g., HIF pathways) differ between organs and species?
- How do different approaches to modulate NAD^+ availability (different types of NAD^+ supplementation, hypoxia, exercise) interact?

oxygen transport or utilization and with impaired NAD metabolism and redox control. To optimally harness this potential, several questions remain to be answered (see box "Questions for future research"). A better understanding of the dynamics of NAD alterations depending on the severity of hypoxia, time (acute, prolonged), combination with reoxygenation phases, metabolic/nutritional status and cell-type and organ-specific responses will be important for optimal strategies to maintain NAD homeostasis or modulate components of the NAD system to protect from hypoxia. Emerging technologies, such as *in vivo* imaging of NAD levels in different tissues (e.g., Ref. [90]) will allow addressing some of these questions also in humans, including how NAD supplementation can be used to protect e.g. from risks due to ambient hypoxia (i.e., altitude illnesses).

In addition, the precise control of NAD-homeostasis and its interaction with hypoxic response components relies on the spatial separation of NAD metabolic pathways within subcellular compartments [4,91]. Although NAD metabolism has been extensively studied at the levels of whole cells and tissues, in order to modulate its metabolism, it is of pivotal importance to understand how cells maintain organellar metabolic NAD homeostasis, and how oxygen availability is involved in intracellular NAD pool regulation.

Moreover, to modulate NAD metabolism, it is crucial to have quantitative information on NAD fluxes and NADH/NAD^+ imbalances for each organelle.

CRediT authorship contribution statement

Johannes Burtscher: Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Conceptualization. **Tobias Dünnwald:** Writing – review & editing, Conceptualization. **Giuseppe Paglia:** Writing – review & editing, Conceptualization.

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Declaration of Competing interests

The authors declare no competing interests.

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