BIOENERGETICS

Letting off electrons to cope with metabolic stress

Whereas textbooks depict metabolism in perfect homeostasis, disturbances occur in real life. One particularly relevant disturbance, caused by excess food and alcohol consumption and exacerbated by genetics, is reductive stress. New work by Goodman et al. identifies a biomarker of reductive stress and uses a gene therapy solution in mice. This work suggests how exercise and an accessible nutritional technology can synergistically increase catabolism and relieve reductive stress.

Collin D. Heer and Charles Brenner

The nicotinamide adenine dinucleotide reduced/oxidized (NADH/NAD$^+$ redox) couple functions as a biochemical battery for short-term storage and allocation of energy in the form of high-energy electrons. In oxidative metabolism, high-energy electrons are harvested from fatty acids, carbohydrates, amino acid derivatives and intermediates in the citric acid cycle by dehydrogenases and are transferred to NAD$^+$, forming NADH. In turn, NADH is oxidized back to NAD$^+$ by enzymes such as lactate dehydrogenase (LDH) and complex I of the mitochondrial-membrane electron-transport chain. NADH is also reoxidized by donating electrons for gluconeogenesis, ketone-body formation and, by way of reduced nicotinamide adenine dinucleotide phosphate (NADPH), anabolic programs including the synthesis of lipids.

The driving force of catabolism and oxidative metabolism is the NAD$^+$/NADH ratio, which is normally maintained in a highly oxidized state[1] (~500:1), thus strongly influencing the directionality and magnitude of hundreds of NAD-dependent reactions. A shift in this ratio or the accumulation of NADH can impair these driving forces and result in metabolic dysfunction. Because oxygen is the ultimate electron acceptor, conditions such as hypoxia and mitochondrial dysfunction result in a backup of electrons on NADH, a condition termed reductive stress.

The global obesity epidemic is associated with high energy intake coupled with sedentary lifestyles, thereby producing metabolic states in which the NAD redox batteries are overtaxed. In a recent study published in *Nature*, Goodman et al. examine the effects of direct changes in the NADH/NAD$^+$ redox couple on metabolism[2]. To this end, they used *Lb*NOX, a bacterial enzyme that harvests electrons from cytosolic NADH onto oxygen, forming water and NAD$^+$ and alleviating reductive stress.

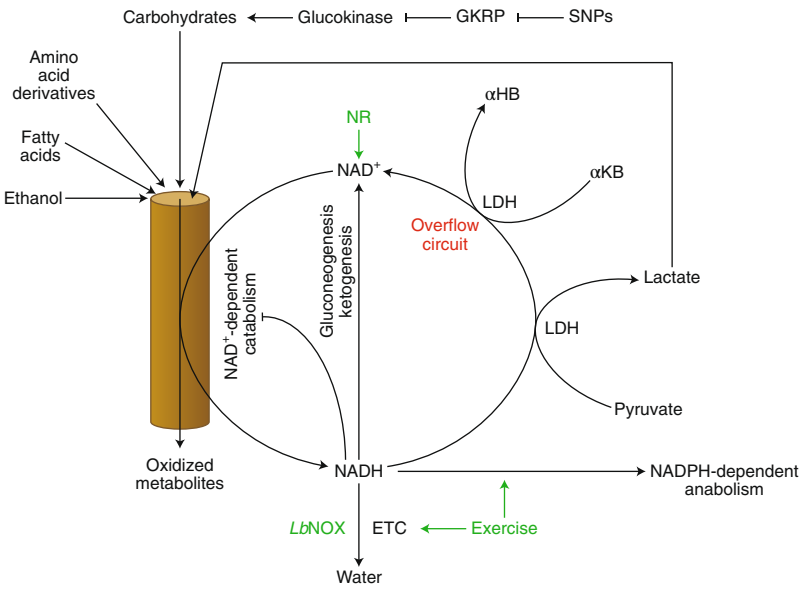

Fig. 1 | Fuel oxidation in excess of demand for NADH electrons drives reductive stress. In redox metabolism, NADH is formed with fuel oxidation and is reoxidized to NAD$^+$ by the actions of the electron-transport chain (ETC), gluconeogenesis, ketogenesis, NADPH-dependent anabolism and the activity of LDH. Overnutrition, ethanol and single-nucleotide polymorphisms (SNPs) that depress GKRP activity increase production of NADH, thereby producing αHB, a biomarker of reductive stress. *LbNOX* relieves reductive stress by pulling electrons from the bottom (exercise does this and more), whereas NR increases catabolism from the top.

In vitro expression of *Lb*NOX in the cytosol permitted identification of secreted metabolites whose abundance is tightly linked to the cytosolic NAD$^+$/NADH ratio. The most notable of these metabolites is α-hydroxybutyrate (αHB), which is generated from α-ketobutyrate (αKB) in an NADH-dependent reaction catalysed by cytosolic LDH[3]. The authors found that αHB secretion is diminished in *Lb*NOX-expressing cells. αKB had previously been identified as an electron acceptor capable of rescuing cells from growth arrest after respiratory-chain inhibition[3,4]. αHB is secreted from cells without being metabolized further, and thus the αKB–αHB system serves as a carrier that evicts electrons from cells under reductive-stress conditions. In fact, elevated circulating αHB is associated with impaired glucose tolerance and is a risk factor for insulin resistance and diabetes[5]. The finding that αHB levels are the most

sensitive to *Lb*NOX expression led the authors to conclude that αHB levels are both a function of and a marker for a depressed cellular NAD^+/NADH ratio. Further, these findings suggest that αHB levels might serve as a proverbial 'canary in the coal mine', indicating overnutrition and the resultant hepatic reductive stress that typically manifests in settings of metabolic disease and dysfunction.

In addition to serving as a tool for manipulating the NADH/NAD^+ couple, *Lb*NOX exhibited therapeutically interesting effects in mice: a liver-targeted viral construct improved glucose tolerance in mice fed a high-fat diet. The authors also sought to identify human genetic determinants of hepatic reductive stress by using αHB as a marker. They found that the top three human single-nucleotide polymorphisms associated with αHB levels are present in the *GCKR* gene. *GCKR* encodes glucokinase regulatory protein (GKRP), which negatively regulates the activity of glucokinase and thus exerts control over hepatic glycolysis, a primary source of cytosolic NADH. The authors found that an associated variant encodes a GKRP with impaired activity, and cells expressing this variant exhibit enhanced glycolytic flux, αHB production and lactate/pyruvate ratios, changes indicative of NADH-mediated reductive stress.

Although the authors concluded that αHB levels are a function of the NAD^+/NADH ratio, the absolute levels of NAD metabolites come under attack during various conditions of metabolic stress[6,7]. Moreover, the balance between the demand and disposal of reducing equivalents can be strikingly stimulated by exercise and other factors. Thus, models of reductive stress built on snapshots of overnutrition may need adjustment to account for levels of NAD metabolites being in flux. In addition, elevated αHB may signal levels of NADH that exceed an absolute threshold episodically, owing to imbalances between energy intake and expenditure.

Although the authors interpreted αHB levels as a strict function of the NAD^+/NADH ratio, the data in their study are more consistent with the view that cellular levels of NADH per se drive the production of αHB. When the authors examined the effects of *Lb*NOX expression and treatment with the NAD^+ precursor nicotinamide riboside (NR)[8], they found that NR supplementation increased cellular NAD^+ and the NAD^+/NADH ratio. Whereas *Lb*NOX decreased markers of reductive stress and rescued cells from complex I inhibition by virtue of NADH reoxidation, NR treatment increased the production of lactate and αHB from glucose and did not rescue cells from complex I inhibition despite increasing NAD^+/NADH. The combination of *Lb*NOX expression and NR treatment was also informative, producing cells with the high catabolic capacity of NAD-boosted cells without reductive stress[2].

Drawing conclusions from the data in Goodman et al., we propose a model (Fig. 1) in which we argue that *Lb*NOX pulls electrons out of the system by oxidizing NADH to form water, thereby decreasing the number of electrons evicted through formation of αHB and lactate. In contrast, NR, overnutrition, ethanol and low-activity *GCKR* variants push catabolism-derived electrons into the system, thus resulting in greater rates of NADH production. In the absence of increased aerobic exercise or biosynthetic processes to dispose of electrons, enhanced NADH production under these conditions exceeds the capacity of NADH consumption by biosynthetic processes and respiration. Consequently, electrons are transferred to lactate and αHB, which diffuse into circulation and serve as biomarkers of reductive stress. Although the NADH-dependent reduction of αKB to αHB was proposed to be driven by the NADH/NAD^+ ratio, the data are more consistent with the view that the αKB–αHB system is an overflow circuit sensitive to the absolute level of NADH.

Although ectopic expression of the intracellular enzyme *Lb*NOX is a nice medicine for mice, this system is unlikely to achieve clinical translation. However, examination of the effects of *Lb*NOX alone and in combination with NR suggests that *Lb*NOX functions as a reductive-stress reliever in the same manner as exercise. We eschew the term 'exercise mimetic' because *Lb*NOX-dependent relief of reductive stress cannot provide all the benefits of exercise, in part because of a lack of an anabolic component that would build lean mass and aerobic capacity. However, the data suggest that, in the same way that *Lb*NOX and NR are synergistic, NAD-boosting strategies are likely to be synergistic with exercise to keep the electrons flowing—a concept that has also emerged from three completed clinical trials of NR in obese and/or older people[9].

Mechanistically, future studies using *Lb*NOX have the potential to uncover markers of reductive stress in other tissues and could be expanded to the NADPH/$NADP^+$ couple[10]. In addition, the Mootha laboratory has developed LOXCAT, a fusion of lactate oxidase and catalase, to dispose of electrons on circulating lactate. As an extracellularly targeted gene therapy, LOXCAT has substantial translational potential for individuals with mitochondrial diseases[11].

Imbalances in cellular and systemic metabolism are notoriously difficult to control. As a result of these new insights into the push and pull of electrons, there are new evidence-based approaches to potentially address conditions of metabolic stress[9,11]. ❐

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Competing interests

C.B. owns stock in, and is chief scientific adviser to, ChromaDex, Inc. C.D.H. declares no competing interests.