suggests that βHB is a more potent anticatabolic stimulus than hyperinsulinemia in this model, and supports the hypothesis that βHB could exert anticatabolic effects in inflammation-driven muscle atrophy.

However, contrary to the previous observation of anabolic effects of βHB on skeletal muscle, Thomsen *et al*. observed that βHB may, in fact, attenuate MPS under this inflammatory insult. However, an attenuation of MPS is perhaps unsurprising given the association of inflammation and elevations in inflammatory cytokines with anabolic resistance in skeletal muscle [8]. The elevation in βHB concentrations produced by Thomsen *et al*. [4] failed to suppress the stimulation of cytokine production including interleukin (IL)-1β, which the authors hypothesized based on the recently described anti-inflammatory effect of βHB [9]. In that work, LPS-mediated activation of the NLRP3 inflammasome and IL-1β production by macrophages was attenuated by βHB. The model employed by Thomsen *et al*. is a rather different *in vivo* physiological context in which βHB infusion was unable to sufficiently mitigate the inflammatory response. In particular, the use of acipimox may have introduced competition for the HCAR2 receptor through which βHB also acts. Additionally, endotoxemia results in nitration and inactivation of succinyl-CoA:3-oxoacid CoA transferase (OXCT1) [10], the rate limiting enzyme in ketolysis and subsequent ketone utilization in extrahepatic tissues. Combined, these two mechanisms may have reduced the activity, and therefore metabolic effects, of βHB in selected tissues.

Ultimately, muscle atrophy results from either a decline in the rate of MPS, an increase in the rate of MPB, or a simultaneous decline in MPS in combination with an increase in MPB. Overall, muscle atrophy requires that MPS is repressed relative to MPB. Thomsen *et al*. [4] highlighted an anticatabolic effect of βHB, which, even in the presence of a degree of anabolic resistance consequent to inflammatory insult, elicited a net positive protein balance in skeletal muscle under these catabolic conditions. Looking forward, it will be intriguing to discover if the implications of these findings reach beyond LPS-mediated inflammation into subclinical (e.g., disuse atrophy, sarcopenia) and/or overt (e.g., cachexia) inflammation-induced skeletal muscle atrophy pathologies. Considering the increasing evidence for therapeutic ketosis and the emergent ability to easily and safely administer exogenous ketones, benefits in tissues beyond skeletal muscle atrophy pathologies may also be realized.

Disclaimer Statement

B.E. declares no competing interests. D.D. is an inventor on a patent entitled 'Composition and methods of elevating and sustaining ketosis' United States Patent and Trademark Office (USPTO)# 20170266148. This invention was made with government support under Grant number N00014-13-1-0062 awarded by the Department of Defense, Office of Naval Research. A.K. and D.D. are inventors on provisional patents 'Compositions and methods for weight loss maintenance' and 'Prevention of muscle wasting with Ketone supplementation'. At the time of this publication, provision patents were still under review. However, should provisional patents become accepted and royalties ever accrue, A.K. and D.D. will receive a share under the terms prescribed by the University of South Florida. D.D. is an owner of Ketone Technologies LLC.

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Spotlight

ACMSD: A Novel Target for Modulating NAD^+ Homeostasis

Jun Yoshino[1,*]

NAD^+ has a pivotal role in regulating many biological processes. A recent study (Palzer *et al*., *Cell Rep*. 2018, 25;1359–1370) demonstrated that alpha-amino-beta-carboxy-muconate-semialdehyde decarboxylase (ACMSD) is a key regulator of NAD^+ metabolism and overexpression of human ACMSD leads to niacin dependency for NAD^+ biosynthesis in mice, providing important insights into human diseases associated with niacin/NAD^+ deficiency.

NAD^+ is an important redox coenzyme found in all species. Recent studies

identified new functions for this old molecule in many biological processes, such as metabolism, circadian rhythms, aging, and inflammation. Importantly, defects in NAD^+ metabolism have a causative role in various diseases, such as type 2 diabetes, cardiovascular disease, cancer, and Alzheimer's disease [1–3]. In mammals, NAD^+ is dynamically regulated by multiple enzymatic reactions in a *de novo* biosynthetic pathway starting from tryptophan, Preiss-Handler and salvage biosynthetic pathways starting from niacin [nicotinic acid, nicotinamide, nicotinamide riboside (NR)], and a degradative pathway (Figure 1). Over the past decade, many studies have reported the importance of NAD^+ biosynthetic and degradative enzymes under physiological and pathophysiological conditions. For example, genetic deletion of nicotinamide phosphoribosyltransferase (NAMPT), a rate-limiting enzyme in the salvage NAD^+ biosynthetic pathway, causes severe metabolic dysfunction in a tissue-specific manner [2]. Increased activity of nicotinamide mononucleotide (NMN) adenylyltransferase (NMNAT), another salvage enzyme, prevents axon degeneration, whereas SARM1 activation promotes axon degeneration through NAD^+ decomposition [3]. Genetic ablation of quinolinate phosphoribosyltransferase (QPRT), a key *de novo* NAD^+ biosynthetic enzyme, results in increased susceptibility to ischemic renal injury [4]. In addition, genetic and pharmacological inhibition of the major NAD^+-degradative enzymes, such as CD38 and poly(ADP-ribose) polymerase (PARP), protects mice against metabolic stresses [1,3]. These discoveries shed light on the pathophysiological significance and therapeutic potential of NAD^+ biosynthetic and degradative enzymes.

A recent study by Palzer *et al*. [5] revealed a novel function of alpha-amino-beta-carboxy-muconate-semialdehyde decarboxylase (ACMSD) in NAD^+ metabolism and mouse physiology.

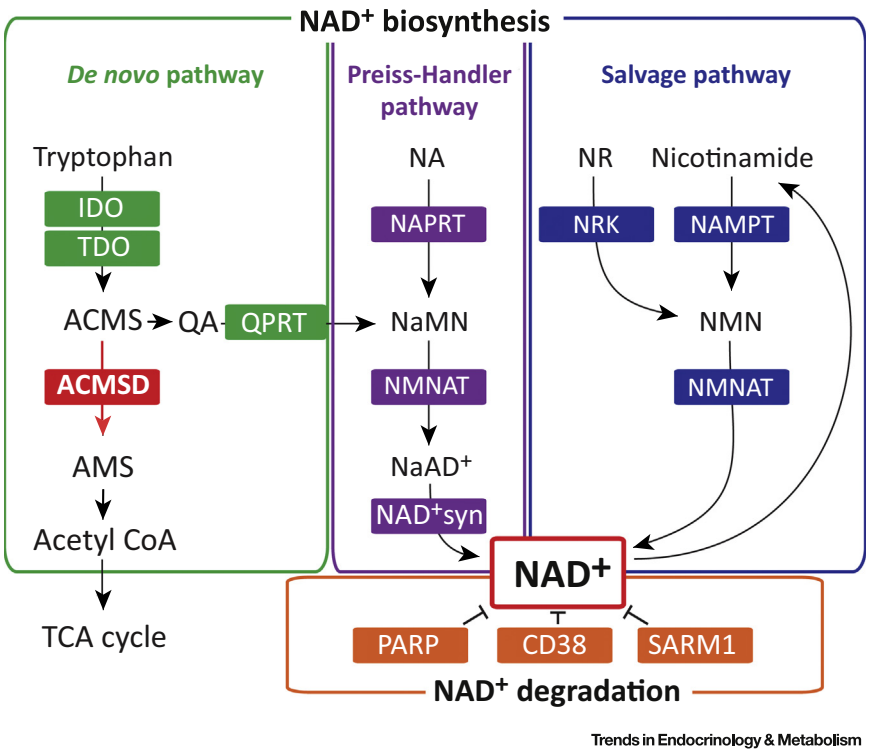

Figure 1. NAD^+ Biosynthetic and Degradative Enzymes. In mammals, NAD^+ homeostasis is regulated by multiple enzymes in a *de novo* biosynthetic pathway starting from tryptophan, Preiss-Handler and salvage biosynthetic pathways starting from niacin [nicotinamide, nicotinic acid (NA), and nicotinamide riboside (NR)], and a degradative pathway. A new study by Palzer *et al*. [5] identified alpha-amino-beta-carboxy-muconate-semialdehyde decarboxylase (ACMSD) as an important regulator of NAD^+ metabolism. ACMSD converts ACMS into aminomuconic semialdehyde (AMS) in a *de novo* NAD^+ biosynthetic pathway. Increasing ACMSD expression shifts the balance from *de novo* NAD^+ biosynthesis toward acetyl-CoA production, leading to the development of niacin dependency. Abbreviations: IDO, indoleamine-pyrrole 2,3-dioxygenase; $NaAD^+$, nicotinic acid adenine dinucleotide; NAD^+syn, NAD^+ synthase; NaMN, nicotinic acid mononucleotide; NAMPT, nicotinamide phosphoribosyltransferase; NAPRT, nicotinic acid phosphoribosyltransferase; NMN, nicotinamide mononucleotide; NMNAT, nicotinamide mononucleotide adenylyltransferase; NRK, nicotinamide riboside kinase; PARP, poly(ADP-ribose) polymerase; QA, quinolinic acid; QPRT, quinolinate phosphoribosyltransferase; SARM1, sterile alpha and TIR motif containing 1; TCA cycle, tricarboxylic acid cycle; TDO, tryptophan 2,3-dioxygenase.

ACMSD is an important enzyme involved in regulating tryptophan degradation along the kynurenine pathway and converts aminocarboxymuconic semialdehyde (ACMS) into aminomuconic semialdehyde (AMS), which is utilized for generating acetyl-CoA in the tricarboxylic acid (TCA) cycle (Figure 1). Palzer *et al*. hypothesized that increasing ACMSD activity would inhibit conversion of ACMS into quinolinic acid (QA), a key intermediate in the *de novo* NAD^+ biosynthetic pathway, and lead to niacin dependency. To test this hypothesis, the authors generated a novel mouse model overexpressing the human ACMSD (hACMSD) gene under doxycycline (DOX) control, namely an 'acquired niacin-dependency (ANDY)' mouse. Control (water)- and DOX-treated ANDY mice were studied under three different dietary conditions: a niacin-free diet (ND1); an ND1 diet containing a moderate amount (30 mg/kg) of niacin (CD1); and a regular chow diet containing 63 mg/kg of niacin. They found that blood NAD^+ levels were decreased in DOX-treated ANDY mice on ND1 (ANDY/DOX/ND1) compared with DOX-treated or control ANDY mice on CD1 (ANDY/DOX/CD1 or ANDY/water/CD1, respectively), or on regular chow diet. ANDY/DOX/ND1 mice also showed marked decreases in NAD^+ levels in liver, kidney, spleen, and brain compared with ANDY/DOX/CD1 mice. In addition, NAD^+ phosphate ($NADP^+$) levels were reduced in ANDY/DOX/ND1 mice. Intriguingly, blood NAD^+ and $NADP^+$ levels were fully restored in ANDY/DOX/ND1 mice after switching the diet from niacin-free ND1 to niacin-replete CD1. As expected, overexpression of hACMSD enhanced hepatic acetyl-CoA production regardless of niacin intake. Taken together, these results are consistent with the hypothesis and demonstrate that ACMSD critically regulates tryptophan catabolism by shifting the balance from *de novo* NAD^+ biosynthesis toward acetyl-CoA production and, thus, ANDY mice overexpressing hACMSD become dependent on dietary niacin intake for NAD^+ biosynthesis.

Finally, the authors investigated *in vivo* metabolic phenotypes in ANDY mice. Interestingly, niacin-deficient ANDY/DOX/ND1 mice displayed significant decreases in body weight and adipose tissue mass independently of food intake compared with niacin-replete ANDY/DOX/CD1 mice. Consistent with these results, ANDY/DOX/ND1 mice also had pronounced decreases in hepatic lipid accumulation, pyruvate content, and NAD^+:NADH ratios, indicating impaired energy and redox metabolism. In addition, ANDY/DOX/ND1 mice showed lethargy-like behavior by progressively reducing voluntary ambulatory physical activity. Remarkably, similar phenotypes are often observed in humans with niacin deficiency. Given that humans use niacin more efficiently than tryptophan to synthesize NAD^+ [6], many aspects of ANDY mice could closely mimic NAD^+ metabolism in humans. Therefore, the study by Palzer *et al*. provides important groundwork for future studies that explore the molecular mechanisms of human diseases associated with niacin/NAD^+ deficiency.

The findings of Palzer *et al*. [5] have important implications for NAD^+ biology research and raise exciting new questions. For example, what are the molecular links between NAD^+ deficiency and metabolic and neurological disorders? Interestingly, recent studies have shown that the NAD^+-dependent protein deacetylase SIRT1 regulates adipogenesis, energy metabolism, and physical activity [7], suggesting that reduced SIRT1 activity is likely involved in functional defects in niacin-deficient ANDY mice. It is also possible that other NAD^+-dependent enzymes, such as CD38 and PARP, and other sirtuin(s) or redox species are downstream mediators. Another important question concerns the complex role of ACMSD in the brain. Data from studies conducted in ANDY mice suggest that increased ACMSD activity contributes to the development of neurological disorders [5]. However, previous studies found that ACMSD deficiency or mutation is also linked to neurological diseases, such as epilepsy and Parkinson's disease [8]. These apparently conflicting results could emphasize the importance of QA (Figure 1), a key NAD^+ intermediate that is known to cause neurotoxicity [9]. Future studies are warranted to investigate the functions of ACMSD and QPRT simultaneously and dissect the mechanisms regulating the balance between QA accumulation and NAD^+ biosynthesis from tryptophan. Lastly, the therapeutic potential of ACMSD remains to be explored. Strikingly, in line with the recent study by Palzer *et al*. [5], Auwerx's group recently reported that pharmacological inhibition of ACMSD enhanced NAD^+ levels in liver and kidney and protected mice from nonalcoholic fatty liver disease (NAFLD) and acute kidney injury (AKI) [10]. There is also evidence that NMN, a product of the NAMPT reaction (Figure 1) and a chemical inhibitor of CD38 can be used to treat NAFLD and AKI [1–3]. Therefore, it will be important to determine whether ACMSD inhibition and other NAD^+ boosters can synergistically increase NAD^+ levels and achieve therapeutic effects.

In conclusion, the findings of Palzer and colleagues [5] increase our understanding of the complex and sophisticated regulatory mechanisms of NAD^+ metabolism. In addition, a novel transgenic mouse model, the ANDY mouse, provides important insights into human diseases associated with niacin/NAD^+ deficiency. These findings will undoubtedly accelerate translation of basic

NAD^+ biology research into clinical application.

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Spotlight

'Et Tu, Leptin?'

Diego Perez-Tilve[1,*]

Leptin promotes adequate caloric intake and glycemia in healthy lean individuals, harnessing the benefits of the ideal therapy against metabolic syndrome. Yet, new evidence demonstrates an unexpected causal role for leptin in obesity-associated hyperglycemia. Like the betrayal of Julius Caesar by Brutus, insulin did not see that coming from leptin.

The hormones leptin and insulin are cornerstones of the control of energy homeostasis. Individually, leptin primarily regulates energy stores, whereas insulin maintains tissue fuel availability, and together they provide an intertwined contribution to optimal metabolic control. Their circulating levels are tightly regulated by changes in energy status, each dropping to a minimum during severe energy deprivation and each increasing during energy surplus. These synchronous increases enable insulin to maintain adequate fuel deposition and utilization as leptin reduces further energy intake. These activities are coordinated by the action of both hormones on discrete neurons of the brain, including hypothalamic arcuate neurons expressing agouti-related protein (AGRP) that integrate many aspects of the control of energy intake and whole-body glucose metabolism [1].

During unrestricted access to normal food, circulating levels of both insulin and leptin are modest, yet efficacious at maintaining a stable body weight. In contrast, in conditions favoring chronic energy surplus, such as having access to a highly palatable energy-dense diet, both adipose tissue mass and plasma glucose gradually increase concomitantly with an increase of circulating leptin and insulin and an apparent reduction in their ability to curb energy intake and maintain glucose control. The cause of the apparent reduction of leptin and insulin efficacy has stimulated intense research activity to discover novel targets for treating obesity and type 2 diabetes.

With this in mind, Balland *et al*. [2] tested the hypothesis that elevated circulating leptin is causally linked to the reduction of the ability of insulin to act in the brain to maintain glucose control in diet-induced obese (DIO) mice. They focused on the interplay between leptin and insulin action directly at AGRP neurons. To dissociate the effect of elevated brain leptin from other obesity-induced factors, they administered a leptin-receptor antagonist (LAN) locally into the brain prior to performing a euglycemic-hyperinsulinemic clamp to assess whole-body insulin action. Reducing leptin action from the brain of DIO mice resulted in an increase in the glucose-infusion rate during the clamp, associated with a suppression of hepatic glucose production, signifying improved efficacy of circulating insulin to regulate glucose metabolism (i.e., in the obese state, ongoing leptin signaling actively countered the ability of insulin to restrain hyperglycemia). Importantly, LAN was ineffective when coadministered with an insulin antagonist to blunt insulin signaling in the brain. Intracerebral coadministration of LAN with an inhibitor of protein-tyrosine phosphatase-1B (PTP1B), a negative regulator of both insulin- and leptin-receptor signaling, resulted in a comparably improved glucose infusion rate during the insulin clamp without additive benefits. Consistent with this, DIO mice lacking PTP1B expression uniquely in AGRP neurons had comparably improved glucose control, as occurred with intracerebral LAN administration. Based on these results, the authors propose that chronic brain exposure to increased leptin reduces insulin signaling in AGRP neurons in a PTP1B-dependent manner, compromising the ability of insulin to suppress hepatic glucose production.