

findings to humans is potentially of great clinical significance for both maternal health and childhood development. NR has been shown to be well tolerated and is generally considered safe as a dietary supplement in humans [9,10]. However, future clinical trials are needed to determine the potentially exciting benefits of NR at the maternal–offspring interface.

Acknowledgments

Y.W. is Lawrence Raisz Professor in Bone Cell Metabolism and a Virginia Murchison Linthicum Scholar in Medical Research. This work was in part supported by The Welch Foundation (I-1751, Y.W.), NIH (R01CA229487, R01CA236802, Y.W.), CPRIT (RP180047, Y.W.), DOD (W81XWH-18-1-0014, Y.W.) and UTSW Endowed Scholar Startup Fund (Y.W.). The authors declare that they have no financial conflict of interest.

¹Department of Pharmacology, The University of Texas Southwestern Medical Center, Dallas, Texas 75390, USA

*Correspondence:

yihong.wan@utsouthwestern.edu (Y. Wan).

<https://doi.org/10.1016/j.tem.2019.02.004>

© 2019 Elsevier Ltd. All rights reserved.



References

- Imai, S.-I. and Guarente, L. (2014) NAD⁺ and sirtuins in aging and disease. *Trends Cell Biol.* 24, 464–471
- Belenky, P. et al. (2007) NAD⁺ metabolism in health and disease. *Trends Biochem. Sci.* 32, 12–19
- Katsyuba, E. and Auwerx, J. (2017) Modulating NAD⁺ metabolism, from bench to bedside. *EMBO J.* 36, 2670–2683
- Rajman, L. et al. (2018) Therapeutic potential of NAD-boosting molecules: the *in vivo* evidence. *Cell Metab.* 27, 529–547
- Ear, P.H. et al. (2019) Maternal nicotinamide riboside enhances postpartum weight loss, juvenile offspring development, and neurogenesis of adult offspring. *Cell Rep.* 26, 969–983
- Bieganski, P. and Brenner, C. (2004) Discoveries of nicotinamide riboside as a nutrient and conserved NRK genes establish a Preiss-Handler independent route to NAD⁺ in fungi and humans. *Cell* 117, 495–502
- Belenky, P. et al. (2007) Nicotinamide riboside promotes Sir2 silencing and extends lifespan via Nrk and Urh1/Pnp1/Meu1 pathways to NAD⁺. *Cell* 129, 473–484
- Ratajczak, J. et al. (2016) NRK1 controls nicotinamide mononucleotide and nicotinamide riboside metabolism in mammalian cells. *Nat. Commun.* 7, 13103
- Tammell, S.A.J. et al. (2016) Nicotinamide riboside is uniquely and orally bioavailable in mice and humans. *Nat. Commun.* 7, 12948
- Martens, C.R. et al. (2018) Chronic nicotinamide riboside supplementation is well-tolerated and elevates NAD⁺ in healthy middle-aged and older adults. *Nat. Commun.* 9, 1286

Spotlight

Anticatabolic Effects of Ketone Bodies in Skeletal Muscle

Andrew P. Koutnik,¹
Dominic P. D'Agostino,¹ and
Brendan Egan^{1,2,*}

The ketone bodies acetoacetate (AcAc) and β -hydroxybutyrate (β HB) are the subject of renewed interest given recently established pleiotropic effects regulating inflammation, oxidative stress, and gene expression. Anticatabolic effects of β -hydroxybutyrate have recently been demonstrated in human skeletal muscle under inflammatory insult, thereby expanding upon the wide-ranging therapeutic applications of nutritional ketosis.

AcAc, β HB, and acetone are lipid-derived ketone bodies whose production is amplified through ketogenesis during fasting, starvation, and carbohydrate restriction [1]. Metabolic effects of AcAc and β HB in many organs are well established, including anticatabolic processes such as attenuation of glycolysis, hepatic glucose output, and adipose tissue lipolysis [1]. Among more recent discoveries are the pleiotropic effects of β HB as a signaling metabolite regulating oxidative stress, inflammation, and gene expression [2]. Renewed scientific interest into the

therapeutic and performance potential of ketone bodies has emerged with the development of ingestible exogenous ketone supplements [3]. These compounds rapidly induce nutritional and/or therapeutic ketosis without dietary restriction.

A growing area of interest is the anabolic and anticatabolic potential of ketone bodies in skeletal muscle provided by therapeutic ketosis. Thomsen and colleagues [4] have recently made a significant contribution to this paradigm by examining both the anticatabolic and anabolic potential of β HB in human skeletal muscle under an acute inflammatory insult provided by lipopolysaccharide (LPS) endotoxin. This is salient because with aging, cancer, HIV/AIDS, chronic heart and renal failure, chronic obstructive pulmonary disease, and rheumatoid and osteoarthritis, dramatic losses in muscle mass and function are often observed. Additionally, a pathological decline in skeletal muscle health is not only a strong predictor of mortality, but also predicts a reduced ability to receive, tolerate, and respond to disease burden and/or standard of care therapies [5]. However, with minimal to no anticatabolic therapeutic options, novel mitigation strategies are critical for altering disease course and improving patient outcomes.

Therapeutic strategies for skeletal muscle health primarily address muscle protein turnover; the continuous process of synthesis and degradation of skeletal muscle proteins. The balance between the rates of muscle protein synthesis (MPS) and degradation (muscle protein breakdown; MPB) determines changes in skeletal muscle mass in both acute and long-term scenarios. Inflammation is a primary factor and/or underlying characteristic of many atrophy-related conditions, including those impacting on skeletal muscle, likely through augmenting MPB, and/or attenuating MPS (Figure 1).

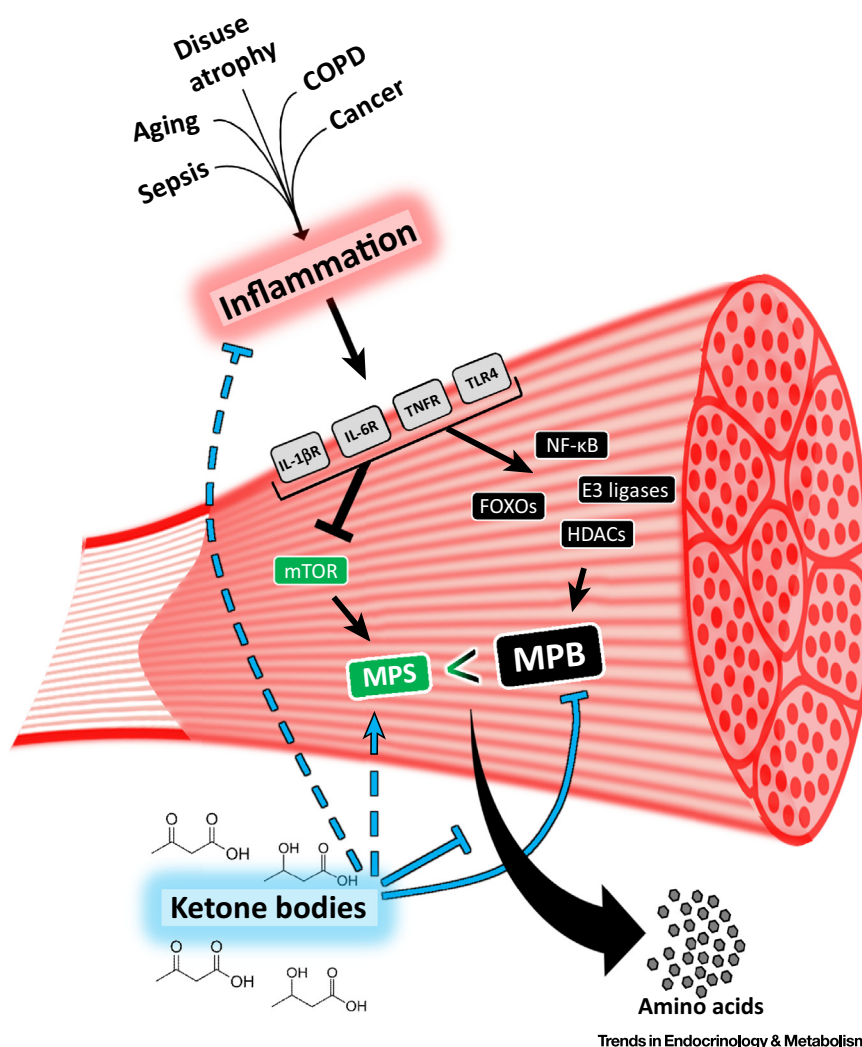


Figure 1. Pathways of Inflammation-Mediated Skeletal Muscle Atrophy and Their Potential Amelioration by Ketone Bodies. Mechanistically, the effects of inflammation on skeletal muscle protein turnover and loss of tissue mass are likely to manifest directly through activation of established catabolic pathways involving E3 ligases of the ubiquitin proteasome system resulting in elevated rates of MPB, and/or through inhibition of canonical mTOR signaling resulting in anabolic resistance and reduced rates of MPS [8]. Abbreviations: FOXOs, forkhead transcription factors; HDAC, histone deacetylase; IL-1 β R, interleukin 1 β receptor; IL-6R, interleukin 6 receptor; MPB, muscle protein breakdown; MPS, muscle protein synthesis; mTOR, mechanistic target of rapamycin; NF- κ B, nuclear factor κ B; TLR4, Toll-like receptor 4; TNFR, tumor necrosis factor α receptor.

Ketone bodies were long-hypothesized to be protein sparing, or anticatabolic, because experimentally elevating ketone bodies improved nitrogen balance (a proxy for muscle protein turnover) under catabolic conditions including post-surgery, skeletal trauma, severe burns, and

sepsis [6]. Additionally, the anabolic potential of β HB, at least in healthy men, was demonstrated by the infusion of sodium β HB to ~ 2 mM which attenuated leucine oxidation and increased MPS by $\sim 10\%$ [7], with analogous anabolic effects reported in post-

exercise feeding in humans, in myotubes *in vitro*, and muscle injury models in rodents [3].

These promising data, in concert with the aforementioned effects of ketone bodies reducing inflammation and oxidative stress, leads to the hypothesis that ketone bodies may have anticatabolic and/or anabolic effects during inflammation-related muscle atrophy. To test this hypothesis, Thomsen *et al.* [4] used LPS as an acute inflammatory stimulus to ten healthy, overnight-fasted, men. LPS rapidly induces weight loss in both skeletal muscle and adipose tissue compartments via established disease-induced catabolic pathways. However, Thomsen *et al.* focused on the first 6 h after insult using labeled tracers and muscle biopsy sampling to measure protein kinetics and intracellular signaling pathways under separate occasions of infusion of (i) saline, (ii) lipids to elevate free fatty acid concentrations, and (iii) sodium D/L- β HB, which produced circulating β HB concentrations of ~ 3.5 mM. These effects were investigated under fasting (basal; 4 h), and insulin-stimulated (hyperinsulinemic–euglycemic clamp; 2 h) conditions. Uniquely, acipimox, a niacin derivative that inhibits lipolysis via hydroxycarboxylic acid receptor 2 (HCAR2) signaling, was provided in all conditions in order to control the influence of altered free fatty acid concentrations as a confounding factor between conditions.

Under these conditions, β HB produced a robust anticatabolic response, as indicated by a 70% reduction in phenylalanine efflux from muscle (and confirmed by other tracer kinetic measurements), even though systemic inflammation was higher in this condition compared to controls. The anticatabolic effect of β HB was only slightly enhanced under hyperinsulinemia, and the absence of β HB was unable to reduce phenylalanine efflux with hyperinsulinemia alone. This

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

However, contrary to the previous observation of anabolic effects of β HB on skeletal muscle, Thomsen *et al.* observed that β HB 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 β HB concentrations produced by Thomsen *et al.* [4] failed to suppress the stimulation of cytokine production including interleukin (IL)-1 β , which the authors hypothesized based on the recently described anti-inflammatory effect of β HB [9]. In that work, LPS-mediated activation of the NLRP3 inflammasome and IL-1 β production by macrophages was attenuated by β HB. The model employed by Thomsen *et al.* is a rather different *in vivo* physiological context in which β HB 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 β HB 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 β HB 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 β HB, 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.

¹Department of Molecular Pharmacology and Physiology, Morsani College of Medicine, University of South Florida, Tampa, FL, USA

²National Institute for Cellular Biotechnology, School of Health and Human Performance, Dublin City University, Glasnevin, Dublin 9, Ireland

*Correspondence: brendan.egan@dcu.ie (B. Egan).
<https://doi.org/10.1016/j.tem.2019.01.006>

© 2019 Elsevier Ltd. All rights reserved.

References

1. Robinson, A.M. and Williamson, D.H. (1980) Physiological roles of ketone bodies as substrates and signals in mammalian tissues. *Physiol. Rev.* 60, 143–187

2. Newman, J.C. and Verdin, E. (2017) β -Hydroxybutyrate: a signaling metabolite. *Annu. Rev. Nutr.* 37, 51–76
3. Evans, M. *et al.* (2017) Metabolism of ketone bodies during exercise and training: physiological basis for exogenous supplementation. *J. Physiol.* 595, 2857–2871
4. Thomsen, H.H. *et al.* (2018) Effects of 3-hydroxybutyrate and free fatty acids on muscle protein kinetics and signaling during LPS-induced inflammation in humans: anticatabolic impact of ketone bodies. *Am. J. Clin. Nutr.* 108, 857–867
5. Aapro, M. *et al.* (2014) Early recognition of malnutrition and cachexia in the cancer patient: a position paper of a European School of Oncology Task Force. *Ann. Oncol.* 25, 1492–1499
6. Thompson, J.R. and Wu, G. (1991) The effect of ketone bodies on nitrogen metabolism in skeletal muscle. *Comp. Biochem. Physiol. B* 100, 209–216
7. Nair, K.S. *et al.* (1988) Effect of beta-hydroxybutyrate on whole-body leucine kinetics and fractional mixed skeletal muscle protein synthesis in humans. *J. Clin. Invest.* 82, 198–205
8. Gordon, B.S. *et al.* (2013) Regulation of muscle protein synthesis and the effects of catabolic states. *Int. J. Biochem. Cell Biol.* 45, 2147–2157
9. Youm, Y.H. *et al.* (2015) The ketone metabolite beta-hydroxybutyrate blocks NLRP3 inflammasome-mediated inflammatory disease. *Nat. Med.* 21, 263–269
10. Marcondes, S. *et al.* (2001) Nitration of succinyl-CoA:3-oxoacid CoA-transferase in rats after endotoxin administration. *Proc. Natl. Acad. Sci. U. S. A.* 98, 7146–7151

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