Review

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Feature Review

Ketone bodies as signaling metabolites

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Traditionally, the ketone body β-hydroxybutyrate (βOHB) has been looked upon as a carrier of energy from liver to peripheral tissues during fasting or exercise. However, βOHB also signals via extracellular receptors and acts as an endogenous inhibitor of histone deacetylases (HDACs). These recent findings support a model in which βOHB functions to link the environment, in this case the diet, and gene expression via chromatin modifications. We review the regulation and functions of ketone bodies, the relationship between ketone bodies and calorie restriction, and the implications of HDAC inhibition by the ketone body βOHB in the modulation of metabolism and in diseases of aging.

Metabolites in aging pathways

The past two decades have witnessed an explosion of knowledge of the genetic and metabolic factors that affect aging and lifespan. Calorie restriction (CR; see Glossary) remains the surest path to increased longevity and resilience to diseases of aging across many organisms, from yeast to monkeys and perhaps humans [1]. Many of the beneficial effects of CR appear to be due to modification of specific nutrient-responsive pathways such as the insulin/insulin-like growth factor (IGF-1) pathway, the target of rapamycin (TOR) signaling pathway, and the NAD^+-dependent deacetylases sirtuins. For example, genetic modulation of any one step in the IGF-1 signaling pathway, from ligand to receptor, to downstream kinase cascades and target transcription factors, enhances lifespan in worms and mice [2]. Rapamycin, the first small molecule found to extend lifespan in mammals, works by inhibiting the nutrient-responsive TOR pathway [3]. Finally, the mitochondrial NAD^+-dependent protein deacetylase sirtuin 3 (SIRT3) is required for at least one of the benefits of CR in mice – prevention of age-related hearing loss [4].

Intriguingly, the ketone body βOHB might also be a metabolic intermediary of the benefits of CR and fasting. Long viewed as a simple carrier of energy from the liver to peripheral tissues during prolonged fasting or exercise, βOHB also possesses signaling activities, perhaps most excitingly as an endogenous inhibitor of HDACs [5]. It therefore joins a small but growing list of metabolic intermediaries that affect gene expression via chromatin modifications [6]. These intermediaries may be key links between variations in the cellular environment and the epigenetic changes associated with increased healthspan and lifespan. Environmental factors such as nutrition dramatically alter cellular metabolism, and many also alter the epigenetic regulation of gene expression. Overall, energy balance controls the NAD/NADH ratio, which affects the activity of sirtuins [7]. Lipid-burning states, such as fasting, increase both acetyl-CoA production and levels of histone acetylation [5]. Intake of threonine affects the levels of the methyl donor *S*-adenosylmethionine, which in turn promotes histone methylation [8]. As discussed below, the activity of HDACs has already been linked to the regulation of lifespan (Box 1) and to diseases of aging such as diabetes and cancer.

Here we review the metabolism, regulation, and functions of ketone bodies, and how the newly discovered activity of βOHB as an endogenous HDAC inhibitor opens a broad new vista into its potential roles in the regulation of lifespan and diseases of aging.

Metabolism, regulation, and function of ketone bodies

Ketone bodies are small lipid-derived molecules that serve as a circulating energy source for tissues in times of fasting or prolonged exercise. Fatty acids in adipose tissue contain

Glossary

β-Hydroxybutyrate (βOHB): a molecule that can be used as an energy source by the brain when blood glucose is low. It is one of three metabolically related molecules known collectively as ketone bodies but is itself technically a carboxylic acid. It can also be used for the synthesis of biodegradable plastics, such as poly(3-hydroxybutyrate).
Calorie restriction (CR): is defined as reduced calorie intake. CR without malnutrition slows the aging process, resulting in increased lifespan in a variety of species including yeast, flies, and rodents.
Ketogenic diet: a diet high in fat, low in carbohydrate, and with adequate but often variable amounts of protein. The ketogenic diet has been used extensively to treat epilepsy in children. Owing to its low amount of carbohydrates, the body switches to fatty acid oxidation as energy source that also results in the formation of ketone bodies. Elevated levels of ketone bodies in the blood, a state known as ketosis, have been shown to lead to a reduction in the frequency of epileptic seizures.
Ketone bodies: refers to three distinct molecules, acetone, acetoacetic acid, and βOHB, that are byproducts of fatty acid oxidation in the liver under fasting conditions.
Histone deacetylases (HDACs): a class of enzyme that removes acetyl groups from lysine residues residing on histones, as well as on non-histone proteins, often resulting in transcriptional repression.
Histone deacetylase inhibitors (HDIs): a group of compounds that inhibit the action of histone deacetylases. Some common HDAC inhibitors are valproic acid, sodium butyrate, and trichostatin A. HDIs are being investigated as possible treatments for cancers and inflammatory diseases.
Rapamycin: an immunosuppressant drug and inhibitor of mTOR, the first compound found to extend lifespan in healthy mammals.

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Box 1. HDACs in longevity and aging: lessons from model organisms

The association of class I HDACs with the regulation of lifespan in model organisms suggests that βOHB might regulate longevity as well. Deletion of Rpd3, the yeast and fly homolog of mammalian class I HDACs (e.g., HDACs 1 and 2), extends replicative lifespan in yeast by 40–50% [128]. Rpd3 deletion enhances ribosomal DNA (rDNA) silencing [128], similar to the mechanism by which overexpression of the sirtuin Sir2 enhances yeast replicative longevity [129]. However, co-deletion of Hda1, the yeast homolog of class II HDACs that partially overlaps with Rpd3 function, actually increases yeast mortality – one example of a 'Goldilocks' zone of HDAC function [128]. Another possible mechanism of increased longevity of yeast Rpd3 mutants is through increased autophagy, which is regulated by histone acetylation of specific genes [130].

In *Drosophila*, flies heterozygous for a null or hypomorphic *Rpd3* allele show a 30–40% extension of lifespan, with no further increase with CR [131]. Both CR and reduced Rpd3 activity increase expression of *Sir2* [131]. Conversely, mutations in *Sir2* block lifespan extension by either CR or *Rpd3* mutations [132]. Together, this indicates that CR, Rpd3, and Sir2 all function in the same longevity pathway in *Drosophila*. Notably, although these modest reductions in Rpd3 activity enhance lifespan, strong hypomorphic alleles are embryonic lethal [133]. The small molecule HDAC inhibitors trichostatin A and butyrate also extend lifespan in *Drosophila*, perhaps via increased expression of heat-shock proteins hsp22 and hsp70 [134]. Feeding 4-phenylbutyrate throughout adulthood increases lifespan in *Drosophila*, although high doses are toxic. Interestingly, it also increases stress resistance and climbing ability, and works even when given later in adult life [135]. Valproic acid, another HDAC inhibitor, extends lifespan in *Caenorhabditis elegans*, although again high doses are toxic [136].

HDAC knockouts in mammals highlight their importance in longevity and age-related diseases. Although HDAC1 knockout in mouse is embryonic lethal [137], similarly to fly Rpd3 knockout, HDAC2 knockout mice are viable but 25% smaller than normal, with impaired IGF-1 signaling and reduced tumor formation when crossed with oncogenic adenomatous polyposis coli (*Apc*) gene knockout mouse models [138]. Conditional knockouts in mouse embryonic fibroblasts and embryonic stem cells demonstrated roles for HDACs 1 and 2 in hematopoiesis [139] and stem cell differentiation [140]. Lifespan has not been rigorously reported for class I HDAC mutant mice, nor for HDAC inhibitor treatment in mammals. By analogy with yeast and fly studies, a positive effect might require careful calibration of gene dosage or function, or inhibitor concentration.

over 80% of the stored energy of the human body [9]. During fasting, muscle and liver stores of glycogen are depleted first. Then, fatty acids are mobilized from adipocytes and transported to the liver for conversion to ketone bodies. Ketone bodies are then distributed via the circulation to metabolically active tissues, such as muscle or brain, where they are converted to acetyl-CoA and used as a glucose-sparing energy source [9]. In humans, basal serum levels of βOHB are in the low micromolar range, but begin to rise to a few hundred micromolar after 12–16 h of fasting, reaching 1–2 mM after 2 days of fasting [10,11], and 6–8 mM with prolonged starvation [12]. Similar 1–2 mM levels of βOHB can be reached after 90 min of intense exercise [13]. Consistent levels above 2 mM are also reached with a ketogenic diet that is almost devoid of carbohydrates [14]. Children produce and utilize βOHB more efficiently than adults, a capability crucial in the days immediately after birth when the brain depends on ketone bodies as an energy source, and serum levels can reach 2–3 mM [12]. At the other end of life, the elderly generate ketone bodies after a fast or ketogenic meal to the same extent as younger adults [15,16].

Ketone body production and utilization

Most ketone body production occurs in the liver [9], although smaller amounts may be produced in other tissues through aberrant expression of ketogenic enzymes [17,18] or reversal of the ketolysis pathway [19,20]. In hepatic ketogenesis (Figure 1), fatty acids are first metabolized to acetyl-CoA via mitochondrial β-oxidation. Mitochondrial hydroxymethyl glutaryl (HMG)-CoA synthase (HMGCS2, EC 2.3.3.10) condenses acetyl-CoA with acetoacetyl-CoA to form HMG-CoA, from which acetoacetate is liberated by HMG-CoA lyase (HMGCL, EC 4.1.3.4) (Figure 1). Acetoacetate is the common precursor of the two other circulating ketone bodies, acetone and βOHB. Most acetoacetate is further metabolized by β-hydroxybutyrate dehydrogenase (BDH1, EC 1.1.1.30) to βOHB. βOHB is the most abundant circulating ketone body and is less likely to degrade spontaneously into acetone than acetoacetate. Once taken up by a target tissue, βOHB is converted back into acetoacetate by the same enzyme, but from there the pathway of ketone body utilization diverges from the synthetic pathway. Succinyl-CoA donates its CoA to acetoacetate to form acetoacetyl-CoA, a reaction catalyzed in most tissues by succinyl-CoA:3-ketoacid coenzyme A transferase (OXCT1, also known as SCOT, EC 2.8.3.5). This reaction bypasses the essentially irreversible reaction catalyzed by HMGCS2. The differing enzymatic routes of synthesis and utilization prevent a futile cycle of βOHB synthesis and utilization in the liver because OXCT1 is not expressed in the liver [21]. Acetoacetyl-CoA can then be converted to two acetyl-CoA and fed into the tricarboxylic acid cycle for oxidation and ATP production [22].

Transcriptional and post-translational regulation of βOHB metabolism

The rate-limiting step of ketone body synthesis is the condensation of acetyl-CoA and acetoacetyl-CoA into HMG-CoA by mitochondrial HMGCS2 [23]. HMGCS2, and therefore the production of ketone bodies, is transcriptionally regulated by at least two nutrient-responsive pathways (Figure 2). The first involves the forkhead box transcription factor FOXA2, which binds to the *Hmgcs2* promoter and activates transcription [24]. FOXA2 itself is regulated by dueling hormonal signals: insulin signaling leads to inactivation of FOXA2 via phosphorylation and nuclear export [25], whereas glucagon activates FOXA2 via p300 acetylation [26]. FOXA2 deacetylation is controlled by a further nutrient-responsive enzyme, SIRT1, working in cooperation with class I or II HDACs [26]. The second pathway of *Hmgcs2* transcriptional regulation involves mTORC1 (mammalian target of rapamycin complex 1), PPARα (peroxisome proliferator-activated receptor α), and finally FGF21 (fibroblast growth factor 21) [23,27–29]. Both PPARα and its target gene *Fgf21* are dramatically upregulated in liver after fasting or by ketogenic diet, and mice lacking either one have reduced levels of