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Ketone bodies as signaling metabolites

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Abstract

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

Keywords

acetylation; HDAC; calorie restriction; longevity; epigenetics

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) 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 SIRT3 is required for at least one of the benefits of CR in mice, prevention of age-related hearing loss [4].

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Intriguingly, the ketone body β -hydroxybutyrate (β OHB) 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, β OHB also possesses signaling activities, perhaps most intriguingly as an endogenous inhibitor of HDACs [5]. Thus, it 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.

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 is most intriguing. 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% [121]. Rpd3 deletion enhances ribosomal DNA (rDNA) silencing [121], similar to the mechanism by which overexpression of the sirtuin Sir2 enhances yeast replicative longevity [122]. However, co-deletion of Hda1, the yeast homolog of class II HDACs that partially overlaps Rpd3 function, actually increases yeast mortality—one example of a “Goldilocks” zone of HDAC function [121]. Another possible mechanism of increased longevity of yeast Rpd3 mutants is through increased autophagy, which is regulated by histone acetylation of specific genes [123].

In *Drosophila*, flies heterozygous for a null or hypomorphic Rpd3 allele show a 30–40% extension of lifespan, with no further increase with CR [124]. Both CR and reduced Rpd3 activity increase expression of Sir2 [124]. Conversely, mutations in Sir2 block lifespan extension by either CR or Rpd3 mutations [125]. Altogether, this indicates that in *Drosophila*, CR, Rpd3, and Sir2, all function in the same longevity pathway. Notably, while these modest reductions in Rpd3 activity enhance lifespan, strong hypomorphic alleles are embryonic lethal [126]. 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 [127]. 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 [128]. Valproic acid, another HDAC inhibitor, extends lifespan in *Caenorhabditis elegans*, although again high doses are toxic [129].

HDAC knockouts in mammals highlight their importance in longevity and age-related diseases. Although HDAC1 knockout is embryonic lethal [130], similar 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 [131]. Conditional knockouts in mouse embryonic fibroblasts and embryonic stem cells demonstrated roles for HDACs 1 and 2 in hematopoiesis [132] and stem cell differentiation [133]. 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.

Here we review the metabolism, regulation, and functions of ketone bodies, and how the newly discovered activity of β OHB as an endogenous HDAC inhibitor opens a broad new vista into its potential roles in 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 over 80% of the human body's stored energy [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 β OHB are in the low micromolar range, but begin to rise to a few hundred micromolar after 12–16 hours 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 β OHB can be reached after 90 minutes 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 β OHB 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 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 β OHB. Most acetoacetate is further metabolized by β -hydroxybutyrate dehydrogenase (BDH1, EC 1.1.1.30) to β OHB. β OHB is the most abundant circulating ketone body and is less likely to spontaneously degrade into acetone than acetoacetate. Once taken up by a target tissue, β OHB 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 HMG-CoA synthase. The differing enzymatic routes of synthesis and utilization prevent a futile cycle of β OHB synthesis and utilization in the liver since OXCT1 is not expressed in the liver [21]. Acetoacetyl-CoA can then be converted to two acetyl-CoA and fed into the TCA cycle for oxidation and ATP production [22].

Transcriptional and post-translational regulation of β OHB metabolism

The rate-limiting step of ketone body synthesis is the condensation of acetyl-CoA and acetoacetyl-CoA into HMG-CoA by mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2) [23]. HMGCS2, and therefore production of ketone bodies, is transcriptionally regulated 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], while glucagon activates FOXA2 via p300 acetylation [26]. FOXA2 deacetylation is controlled by yet another nutrient-responsive enzyme, SIRT1, working in cooperation with class I or II HDACs [26]. The second pathway of HMGCS2 transcriptional regulation involves mTORC1, peroxisome proliferator-activated receptor alpha (PPAR α), and finally fibroblast growth factor (FGF21) [23, 27–29]. Both PPAR α and its target gene FGF21 are dramatically up-regulated in liver after fasting or by ketogenic diet, and mice lacking either one have reduced levels of ketogenesis [27, 28]. The mTORC1 complex suppresses PPAR α , thus inhibition of mTORC1 is required for the induction of PPAR α [29], and in turn PPAR α is required to induce FGF21 [27].

The activity of HMGCS2 is also post-translationally regulated by succinylation and acetylation. HMGCS2 is deacetylated and activated by the primary mitochondrial deacetylase SIRT3 [30]. SIRT3 regulates many pathways involved in fasting metabolism, and mice lacking SIRT3 have reduced levels of β OHB upon fasting [30]. Interestingly, all of the enzymes involved in the generation of ketone bodies from lipids are acetylated, many of them heavily, and contain at least one site for SIRT3 deacetylation [31, 32]. Similar to acetylation, succinylation of HMGCS2 reduces its activity [33]. The mechanism that drives succinylation is not known, but some degree of dependence on succinyl-CoA levels is suggested by the fact that both liver succinyl-CoA abundance and succinylation of HMGCS2 are reduced after treatment of rats with glucagon [33, 34]. Lysine succinylation is removed from other proteins by the mitochondrial desuccinylase SIRT5, which regulates a variety of mitochondrial pathways involved in fasting metabolism [35], though it is not yet known if HMGCS2 is a target of SIRT5 desuccinylation. By contrast, the interconversion of acetoacetate and β OHB by BDH1 appears to be readily reversible and is regulated primarily by the ratios of substrates and cofactors (NAD/NADH₂) [22]. BDH1 contains several SIRT3-regulated acetylation sites, though their functional significance is not yet known [31, 32]. OXCT1 activity may be inhibited by tyrosine nitration [36], but little else is known of its regulation.

β OHB transport, utilization and conservation

β OHB transport is relatively less well understood than its synthesis and utilization. A small, polar molecule, β OHB is readily soluble in water and blood [9]. Several monocarboxylic acid transporters, including MCT1 and MCT2, carry it across the blood-brain barrier [37]. Of interest, up-regulation of MCT1 in particular is associated with high utilization of ketone bodies in the neonatal period and on a ketogenic diet [38]. Recently, the monocarboxylate transporter SLC16A6 was identified as the key transporter for exporting β OHB from the liver, in model organisms; zebrafish lacking Slc16a6a develop fatty liver during fasting mainly due to the diversion of acetyl-CoA to lipid synthesis rather than ketone bodies [39].

Interestingly, the use of β OHB as a fasting energy source is evolutionarily ancient. Many species of bacteria synthesize polymers of β OHB to store energy [12], a rather interesting reaction that is utilized in the production of biopolymers as a plastic substitute [40]. A complete “suite” of ancestral β OHB biosynthetic enzymes, from HMG-CoA synthase through β OHB dehydrogenase, emerged early in eukarya and is present even in plants. This deep conservation likely reflects important roles in cholesterol biosynthesis, as these cytoplasmic enzymes are not known to participate in ketogenesis *in vivo*. Specialization for ketone body metabolism, along with mitochondria- and tissue-localization, emerged more recently and gradually. Mitochondrial HMGCS2 was the latest enzyme involved in ketone body metabolism to diverge from its cytoplasmic counterpart, and is conserved throughout amniota (including birds and humans) [41].

Signaling functions of β OHB

Although β OHB has long been known to be a circulating source of energy in the fasting state, its signaling functions have been recognized much more recently. Besides its predictable effects on cellular energy balance and metabolites, β OHB acts through at least two cell surface receptors and as an endogenous inhibitor of HDACs.

β OHB receptors

β OHB is a ligand for at least two G-protein coupled receptors (GPCRs) that bind short-chain fatty acids. HCAR2 (also known as PUMA-G or Gpr109), a $G_{i/o}$ -coupled GPCR, first identified as a nicotinic acid receptor [42], was recently shown to bind and get activated by β OHB [43]. HCAR2 activation by β OHB reduces lipolysis in adipocytes, perhaps as a feedback mechanism to regulate availability of the fatty acid precursors of ketone body metabolism [43, 44]. β OHB also binds and antagonizes free fatty acid receptor 3 (FFAR3, also known as GPR41), another $G_{i/o}$ -protein coupled receptor that is present in sympathetic ganglions, thereby suppressing sympathetic activity and, in turn, overall metabolic rate in mice [45]. Thus, through its actions on HCAR2 and FFAR3, β OHB may reduce lipolysis, reduce sympathetic tone, and lower metabolic rate (Figure 3) [43–45]. These receptors are part of a growing family of GPCRs, many with fatty acid ligands that have important roles in metabolism and metabolic disease [46, 47].

β OHB binds to and inhibits class I HDACs

It was recently discovered that β OHB inhibits class I HDACs [48], a family of proteins that suppress gene expression by deacetylating lysine residues on histone and non-histone proteins (reviewed in [49–51]); histone hyperacetylation is generally associated with activation of gene expression. Although histones were the first known targets, many non-histone proteins are also subject to HDAC-mediated deacetylation including p53, c-Myc, MyoD, and others [52]. HDACs belong in four separate classes: Class I HDACs (e.g., HDACs 1, 2, 3 and 8) are short primarily nuclear proteins that consist of mainly the deacetylase domain and are usually found in large regulatory multi-protein complexes; Class IIa HDACs (e.g., HDAC4, 5, 7 and 9) are larger proteins with extensive regulatory domains in their N-terminus, that shuttle between the nucleus and cytoplasm; Class IIb HDACs (e.g., HDAC6 and 10) are primarily cytoplasmic proteins containing tandem deacetylase domains; Class III HDACs, the sirtuins, are a structurally distinct group of NAD-dependent deacylases; and Class IV, a single, poorly-understood representative (HDAC11) [49].

The short-chain fatty acid butyrate, differing structurally from β OHB by only a hydroxyl group, was the first HDAC inhibitor to be identified [53]. Since then, at least four major structurally distinct classes of HDAC inhibitors have been discovered [54]. Crystal structures of the human class I HDAC8 bound to several hydroxamic acid inhibitors [55, 56], as well as modeling of other inhibitors, show that a carbonic or hydroxamic acid group of an inhibitor is commonly bound to the catalytic zinc at the bottom of the hydrophobic active site channel of the HDAC [57]. The kinetics of butyrate suggest that it is a competitive inhibitor [58], supporting the notion that both the carboxylic acid groups of butyrate and β OHB chelate zinc in a similar manner, with the beta-hydroxyl group of β OHB sufficiently inconspicuous to fit within the deep hydrophobic channel. Notably, butyrate, which is aliphatic beyond the carbonic acid moiety, is more potent than β OHB; and acetoacetate, with a second carbonyl group instead of β OHB's hydroxyl group, is much less potent than β OHB [58].

Exciting new data shows that β OHB inhibits HDACs 1, 3 and 4 (class I and IIa) *in vitro* with an IC_{50} of 2–5 mM. Treating cultured cells with β OHB induces dose-dependent histone hyperacetylation, particularly at histone H3 lysines 9 and 14. Interestingly, fasting also induces prominent histone hyperacetylation in many mouse tissues and especially the kidney. Along these lines, treating mice with β OHB via osmotic pump causes kidney histone hyperacetylation that is associated with specific changes in gene expression, including induction of forkhead box O3 (Foxo3a), the mammalian ortholog of the stress-responsive transcriptional factor DAF16 that regulates lifespan in worms [2]. Induction of Foxo3a appears to be a direct effect of HDAC inhibition, as HDAC1 and HDAC2 are found on its promoter, knockdown of both relieves the HDAC mediated Foxo3a repression, and β OHB causes hyperacetylation of histones at the Foxo3a promoter that results in increased Foxo3a expression [5].

β OHB indirectly promotes protein hyperacetylation

β OHB may also promote protein hyperacetylation more indirectly, by increasing the intracellular pools of acetyl-CoA (Figure 3). Metabolism of β OHB into acetyl-CoA should

raise intracellular acetyl-CoA levels, providing additional substrate for both enzymatic and non-enzymatic protein acetylation thus driving the reaction equilibria towards acetylation. For example, CR, fasting and high-fat diets, all states associated with increased lipid utilization and therefore high acetyl-CoA production, cause increased mitochondrial protein acetylation, even though the HDACs that are inhibited by β OHB are not known to enter the mitochondria [35]. Mitochondrial acetylcarnitine is known to be a source of acetyl-CoA for histone acetylation [59]. Export of acetyl-CoA from the mitochondria is an active process primarily mediated by citrate synthase and ATP citrate lyase [60]. ATP citrate lyase is a key enzyme in fatty acid biosynthesis, but its role in facilitating acetyl-CoA export from mitochondria is also required for the increase in histone acetylation that occurs with growth factor stimulation [60]. An alternative pathway for acetyl-CoA export from mitochondria is via the enzymes carnitine acetyltransferase (CAT) and carnitine/acylcarnitine translocase [61]. Indeed, a muscle-specific knockout of CAT in mice compromises glucose tolerance and decreases metabolic flexibility [61], demonstrating the importance of intracellular acetyl-CoA transport to overall metabolic health.

Ketone bodies, fasting metabolism and the ketogenic diet

Energy-restricted metabolic states, such as CR or intermittent fasting (every other day), extend lifespan in animals [1]. All such states in vertebrates are necessarily associated with elevations in ketone bodies, whether consistent and modest as in CR or periodic and substantial as in intermittent fasting (see above). Surprisingly, the health benefits of intermittent fasting do not require overall reduced caloric intake. Mice fed every-other-day have increased longevity [62], and mice fed only during 8 hours at night are resistant to diet-induced obesity [63], both without altering overall calorie intake. With our growing understanding of the non-energy functions of β OHB, β OHB might be an intermediary of some of the benefits of energy-restricted states. As described below, many of the data on the metabolic effects of ketone bodies come from studies of ketogenic diets, particularly in rodents. Ketogenic diets in rodents are not a restricted-energy state, but phenocopy some of the biochemical characteristics of fasting, including several that are associated with longevity. In particular, ketogenic diets are associated with low insulin levels [64–68], reduced IGF signaling [69–71] and Foxo3 induction [5], AMP-activated protein kinase (AMPK) activation [71, 72], mTOR repression [71], and induction of antioxidant genes [5] (Table 1).

Impact of ketogenic diet on energy homeostasis

Apart from inducing metabolic changes characteristics of fasting, ketogenic diets represent a high-fat, high-energy state and are also in some ways similar to non-ketogenic high-fat or Western diets. A ketogenic diet increases fasting leptin [67] and consistently causes hyperglycemia and insulin resistance, although basal insulin levels are lower [64–68]. It also promotes liver endoplasmic reticulum (ER) stress (based on the expression levels of genes involved in unfolded protein response), a phenotype associated with increased gluconeogenesis and insulin resistance in diabetic mice [68]. Similar to high-fat diets, there is strong induction of hepatic genes involved in fatty acid oxidation, including acyl coA dehydrogenases and trifunctional enzyme components [68, 72, 73].

Isolated studies have found ketogenic diets to be obesogenic in mice [65, 67], though the majority of studies have not [68, 69, 72–75]. Even among the same strain (C57BL/6), mice on a ketogenic diet have been reported to both gain weight [67] and lose weight [72], relative to controls, despite ingesting equal calories. This discrepancy may be due to differences in diet composition or genetic background. For example, in certain rodent genetic backgrounds, ketone bodies can suppress appetite through central effects in the hypothalamus (reviewed in [76]). The details of diet formulation are important as well. Two studies which found ketogenic diets to be obesogenic both used diets containing >20% calories from protein, similar to typical control diets [65, 67]. Meanwhile, the most popular ketogenic diet (Bioserv F3666), often found to be non-obesogenic, contains a very low 5% of calories from protein [68, 72–75] as well as low methionine content (0.22% vs. >0.4% for a typical control diet) [77]. Both protein restriction and methionine restriction extend lifespan in rodents [78, 79], and methionine restriction also reduces obesity from a high-fat diet [80].

However, the ketogenic diet is unusual in that it simultaneously activates “fasting” pathways despite being a high-energy state. For example, a high-fat, non-ketogenic diet induces not only fatty acid oxidation enzymes but also enzymes involved in fatty acid synthesis. Knockdown in liver of the fatty acid synthesis enzyme stearoyl-CoA desaturase-1 (SCD1), ameliorates the development of metabolic syndrome in SIRT3 knockout mice fed a high-fat diet [81]. In contrast, ketogenic diets suppress fatty acid synthesis enzymes including SCD1 [72, 73]. Instead, PPAR α , a regulator of ketogenic genes, is strongly induced [73], as is one of its crucial downstream targets, FGF21 [68, 73, 74], resulting in increased transcription of ketogenic enzymes, such as HMGCS2 [68, 72, 73]. A ketogenic diet also increases expression of peroxisome proliferator-activated receptor gamma co-activator 1-alpha (PGC1 α), a master regulator of mitochondrial function [74, 75], in mouse liver and brown adipose tissue, which may explain how the ketogenic diet promotes mitochondrial biogenesis in a mouse myopathy model [82]. Finally, one study also reported increased expression of SIRT1 [75], a mammalian homolog of the yeast *Sir2* deacetylase that is increased during calorie restriction and regulates a variety of aging-related pathways (reviewed in [83]).

Mouse knockout models have shown that these “fasting” adaptations are driven by the actions of specific nutrient-regulated genes, including leptin, PPAR α , and FGF21. For example, leptin-deficient *ob/ob* mice have a defective response to the ketogenic diet: they have elevated hepatic PPAR α at baseline but do not increase hepatic FGF21 [73]. PPAR α knockout mice on a ketogenic diet show reduced ketonemia, as well as fatty liver and lipemia, and suppressed hepatic fatty acid oxidation and ketogenic gene expression [27]. Similarly, FGF21 knockout mice on a ketogenic diet have less ketosis, higher levels of insulin and leptin, and more weight gain, along with reduced PGC1 α and lipolytic gene expression, in adipocytes [28]. Interestingly, some of these genes respond differently to fasting and a ketogenic diet, for example hepatic FGF21 is induced by fasting but not by ketogenic diet in *ob/ob* mice [73], while PPAR α -knockout mice induce FGF21 on a ketogenic diet but not fasting [27].

Ketogenic diet and longevity

Ketogenic diets alter other nutrient-sensitive pathways that are implicated in longevity. For example, a ketogenic diet is associated with high activity of AMPK in mouse muscle and liver [71, 72] and inhibition of the mTOR pathway including reduced phosphorylation of S6 kinase in rat liver and hippocampus [71], although the latter could be due in part to the low protein content of the ketogenic diet used. A ketogenic diet also lowers the serum ratio of IGF to insulin-like growth factor-binding protein 3 (IGFBP3) in mice [69, 70], that has been associated with metabolic syndrome and cancer, and reduces pAkt in rat liver and in mouse prostate tumor xenografts [70, 71]. Altogether, these crucial differences between a ketogenic and non-ketogenic high-fat diet may explain the beneficial metabolic effects of a ketogenic diet and have potentially important implications for longevity.

Benefits of ketogenic diet

Both obese mice and obese humans show improved metabolic measures when placed on a ketogenic diet. In contrast to the effects of a ketogenic diet on lean mice, obese or diabetic mice show improved glucose tolerance and insulin sensitivity [69, 73, 84]. A ketogenic diet can even reverse diabetic nephropathy in mice [84]. Nevertheless, ample caution must be exercised when extending laboratory rodent findings to humans. Ketogenic diets in adult humans should only be used under physician supervision, or in the context of a clinical trial. Still, such trials to date have had suggestive results; intermittent severe energy restriction, presumably ketogenic, is more effective than daily energy restriction at improving insulin sensitivity and promoting weight loss [85] and a recent clinical trial of a ketogenic diet found that obese, diabetic humans lost more weight with greater improvement in glucose control than a lower-calorie low-fat diet [86], a result echoing the findings of a recent meta-analysis [87].

Ketone bodies are neuroprotective and cytoprotective

Fasting has been used as an anticonvulsive therapy since ancient times, while the ketogenic diet has been in clinical use for over a century. It continues to be an effective therapy, particularly for certain childhood epilepsies that are resistant to anticonvulsant medications [88]. Ketogenic diets are clinically beneficial in mouse models of several common human neurodegenerative diseases, with promising early data from limited human clinical trials. In the 3xTgAD mouse model of Alzheimer's disease, β OHB delivered via the diet in the form of a synthetic ester suppresses beta-amyloid pathology and improves learning and memory [89]. Two small clinical trials of C8 medium chain triglycerides improved cognitive function in patients with mild-moderate Alzheimer's disease [90, 91]. β OHB infusion ameliorates the phenotype of drug-induced Parkinsonism in mice [92], and a preliminary uncontrolled study of ketogenic diet in humans with Parkinson's disease showed improvement in disease severity scale [93]. Ketogenic diets improve motor performance and motor neuron number in a mouse model of amyotrophic lateral sclerosis [94] and are neuroprotective in mouse models of chronic epilepsy [95]. They also improve motor function and clinical measures in mouse models of genetic and drug-induced Huntington's disease [96], a disease where aberrant histone hypoacetylation may be crucial to pathogenesis [97].

Neuroprotective actions of β OHB

β OHB appears to have broadly neuroprotective effects in these and other neurodegenerative disease models, but its mechanism of action has not been established. *In vitro*, β OHB protects cultured neurons from MPP (a chemical used to induce Parkinsonism in mice) and β -amyloid ($A\beta$ 42) induced toxicity (the peptide that accumulates in Alzheimer's amyloid plaques) [98]. β OHB infusions or ketogenic diets protect against neuronal death in several animal models of brain injury. In rat models of traumatic brain injury, ketogenic diets reduce neuronal apoptosis, reduce brain edema, and improve sensorimotor and cognitive outcomes [99, 100]. Similarly, in rat models of stroke by either cerebral artery occlusion [101] or cardiac arrest [102], a ketogenic diet reduces neuronal loss and infarct size. *In vitro*, β OHB reduces apoptosis after hypoxia in rat hippocampal neuron cultures [103] and protects hippocampal cultures from a variety of insults including hypoglycemia, hypoxia and *N*-methyl-D-aspartate-induced excitotoxicity [104]. Both β OHB and acetoacetate also inhibit uptake of glutamate into synaptic vesicles by competing with chloride, an allosteric activator of vesicular glutamate transporters [105]. Acetoacetate is 10-fold more potent in this effect and reduces both glutamate release and seizure severity in a mouse model of epilepsy [105]. *In vitro*, β OHB enhances survival of cultured cortical neurons from exposure to hydrogen peroxide, both under no glucose (high cell death) and normal glucose (low cell death) conditions [84].

Resistance to cellular stressors by β OHB

One hallmark of longevity-enhancing pathways in model organisms is the induction of resistance to multiple forms of cellular stress [106]. In the roundworm *Caenorhabditis elegans*, for example, genetic mutations that confer enhanced longevity activate an array of cytoprotective pathways that are required for longevity extension [76]. Although a longevity effect for β OHB has not been established, the resistance provided to multiple cellular stressors by β OHB is reminiscent of longevity factors, and is not restricted to neurons. β OHB in Ringer's solution reduces lung injury after hemorrhage and fluid resuscitation in rat models [107–110], as does β OHB alone [111, 112]. β OHB also reduces myocardial damage in a rat model of cardiac ischemia [113]. Administration of β OHB via osmotic pump enhances resistance of mouse kidney to oxidative stress from paraquat, with reduced accumulation of lipid peroxides and protein carbonylation. This resistance to oxidative stress may be due to induction of antioxidant genes, including Foxo3, metallothionein 2 (Mt2), superoxide dismutase 1 (Sod1) and catalase. Mt2 and Foxo3 in particular are both up-regulated by β OHB via its effect on HDAC inhibition and promoter hyperacetylation [5]. The full suite of genes regulated by β OHB via HDAC inhibition is not yet known, but HDAC inhibition provides a possible mechanism by which multiple stress-response pathways could be activated by β OHB.

HDACs in longevity and diseases of aging

As discussed above, inhibition of HDACs by β OHB indicates that β OHB has specific regulatory effects in addition to its metabolic effects, particularly on HDACs and histone acetylation. Interestingly, reduced HDAC activity, either by genetic or pharmacologic means, also has beneficial metabolic and cytoprotective effects similar to β OHB. Moreover,

HDACs regulate a variety of pathways implicated in longevity, including autophagy and IGF signaling (Figure 4), and modulation of HDAC activity regulates lifespan in model organisms (Box 1).

HDACs have a key role in regulating metabolic disease, and loss or inhibition of class I HDAC function appears to phenocopy some of the benefits of a ketogenic diet. HDAC3 regulates expression of gluconeogenic genes [114] and HDAC3 knockout mice have lower fasting glucose and insulin [115–117]. In fact, chronic treatment with butyrate keeps mice essentially metabolically normal on a high-fat diet, with lower glucose and insulin, better glucose tolerance, prevention of weight gain, and improved respiratory efficiency; butyrate also provides some of these benefits even to mice already obese from being fed a high-fat diet [118]. One mechanism for this may be up-regulation of PGC1 α in liver, brown adipose tissue and muscle by butyrate [118], as seen in ketogenic diets.

Also reminiscent of β OHB, HDAC inhibitors are cytoprotective in animal models of tissue injury. Butyrate improves overall survival in a rat sepsis model [119], as well as reducing lung injury after lipopolysaccharide infusion in mice [120].

There is a growing literature on the importance of epigenetic regulation in learning and memory, and specifically mouse models of dementia. In the severely affected CK-p25 mouse (an inducible mouse model for p25 accumulation, the cleaved isoform of cyclin-dependent kinase 5 activator 1) of neurodegeneration, environmental enrichment that improves learning and memory is associated with increased histone H3 and H4 acetylation in the cortex and hippocampus [134]. Treatment with the HDAC inhibitor butyrate also improves learning and memory [134]. Age-related impairments in learning and memory in wild-type mice are associated with alterations in histone acetylation [135], and treatment with HDAC inhibitors improves memory performance in both young and aged mice [135, 136]. HDAC2 appears to be the crucial mediator of these effects, as overexpression of HDAC2, but not HDAC1, impairs learning and memory in wild-type mice [136]. Conversely, HDAC2 knockout mice show improved memory formation, which is not further improved by HDAC inhibitors [136]. HDAC2 expression is increased in the brains of two mouse dementia models as well as the brains of humans with Alzheimer's disease, and knockdown of HDAC2 improves memory in the CK-p25 dementia mouse model [137].

Although HDAC2 seems to have a memory-impairing role, HDAC1 – another class I HDAC – has been reported to be neuroprotective [138]. HDAC1 activity is important for the repair of double-stranded DNA breaks in neurons, and its own deacetylase activity is enhanced by SIRT1 via deacetylation [139]. This provides a fascinating example of how multiple metabolically-responsive pathways (e.g. SIRT1 and β OHB) might intersect to provide overlapping epigenetic regulation: while β OHB inhibition of HDAC2 may be broadly beneficial, the potentially detrimental inhibition of HDAC1 by β OHB is offset by SIRT1 activation. Such cross-talk may be common, where fasting-activated sirtuins provide tissue- or subcellular-specific fine-tuning of the broad effects of β OHB. Alternatively, different target specificities of class I and class III HDACs could provide ample opportunity for coordinated regulation or targets, even perhaps via different lysines on the same protein. It remains to be determined if inhibition of HDACs by β OHB has similar effects on learning

and memory in rodent models as chemical HDAC inhibition or genetic manipulation, and precisely how HDAC inhibition by β OHB intersects with other fasting-related mechanisms of epigenetic regulation.

Concluding Remarks and Future Perspectives

Ketone bodies are emerging as crucial regulators of metabolic health and longevity, via their ability to regulate HDAC activity and thereby epigenetic gene regulation. Ketogenic diets provide a partial phenocopy of CR, through their effects on insulin, IGF, Foxo3, fatty acid metabolism, AMPK and mTOR. The finding that β OHB is an inhibitor of HDACs, together with the coincidence of biological effects of ketone bodies and HDAC inhibition, suggests the fascinating possibility that β OHB could be an endogenous avenue to attain some of the benefits of lifespan extension seen with HDAC inhibition in model organisms. It will be of great interest to define the molecular targets of HDAC inhibition by β OHB in specific tissues and metabolic states, investigate whether β OHB regulates HDAC-targeted pathways like autophagy, and determine if these effects culminate in enhancement of longevity by β OHB in mammals.

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GLOSSARY

β-hydroxybutyrate (βOHB)	is 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 down the aging process, resulting in an increased lifespan in a variety of species, including yeast, flies, and rodents
Ketone bodies	refers to three distinct molecule, acetone, acetoacetic acid, and β OHB, by-products of fatty acids oxidation in the liver under fasting conditions
Ketogenic diet	a diet high in fat, low in carbohydrate and with adequate but often variable amounts of protein. Ketogenic diet has been used extensively to treat epilepsy in children. Due 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, has been shown to lead to a reduction in the frequency of epileptic seizures

Histone deacetylases (HDACs)	refers to a class of enzyme that removes acetyl groups from lysine residues residing on histones as well as 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	is an immunosuppressant drug and inhibitor of mTOR, the first compound found to extend lifespan in healthy mammals

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Box 2**Outstanding questions**

- What are the molecular targets of HDAC inhibition by β OHB in specific tissues and metabolic states?
- Does β OHB regulate HDAC-targeted pathways like autophagy?
- Do pathways downstream of β OHB actions increase longevity in mammals?
- Are mammalian sirtuins downstream targets of β OHB and HDACs?
- If fasting or ketogenic conditions promote inhibition of class I HDACs (via β OHB) but activation of class III HDACs (sirtuins), how are these potentially opposing activities coordinated?
- Is there an acetylation code that may trigger the metabolic reprogramming of dietary restriction?

Highlights

- Ketone bodies have signaling functions as well as being a mobile source of cellular energy
- Ketone bodies inhibit histone deacetylases and control gene transcription
- Histone deacetylase function is implicated in the regulation of aging
- Ketone bodies may link environmental cues such as diet to the regulation of aging

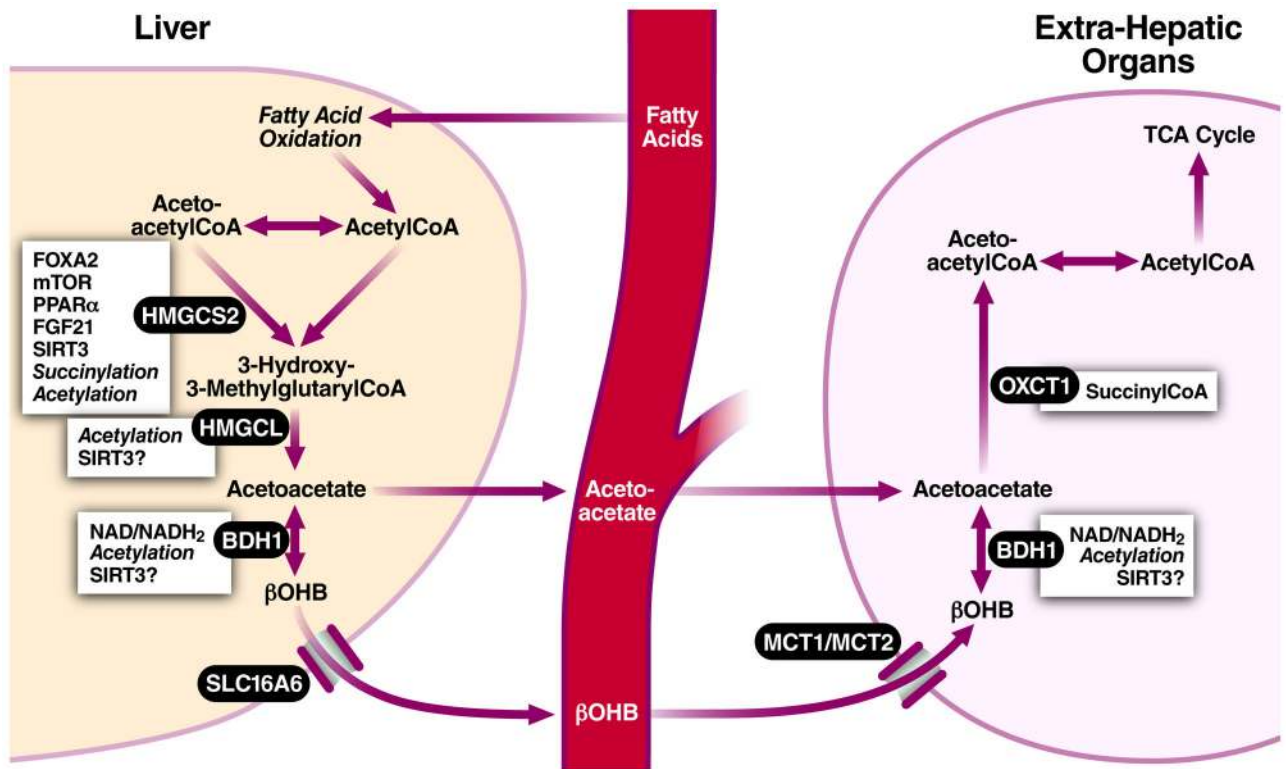


Figure 1. Outline of ketone body metabolism and regulation

The key irreversible step in ketogenesis is synthesis of 3-hydroxy-3-methylglutaryl-CoA by HMGCS2. Conversely, the rate limiting step in ketolysis is conversion of acetoacetate to acetoacetyl-CoA by OXCT1. HMGCS2 transcription is heavily regulated by FOXA2, mTOR, PPAR α , and FGF21. HMGCS2 activity is post-translationally regulated by succinylation and acetylation/SIRT3 deacetylation. Other enzymes are regulated by cofactor availability (e.g., NAD/NADH₂ ratio for BDH1). All enzymes involved in ketogenesis are acetylated and contain SIRT3 deacetylation targets, but the functional significance of this is unclear other than for HMGCS2. Although ketone bodies are thought to diffuse across most plasma membranes, the transporter SLC16A6 may be required for liver export, while several monocarboxylic acid transporters assist with transport across the blood-brain barrier.

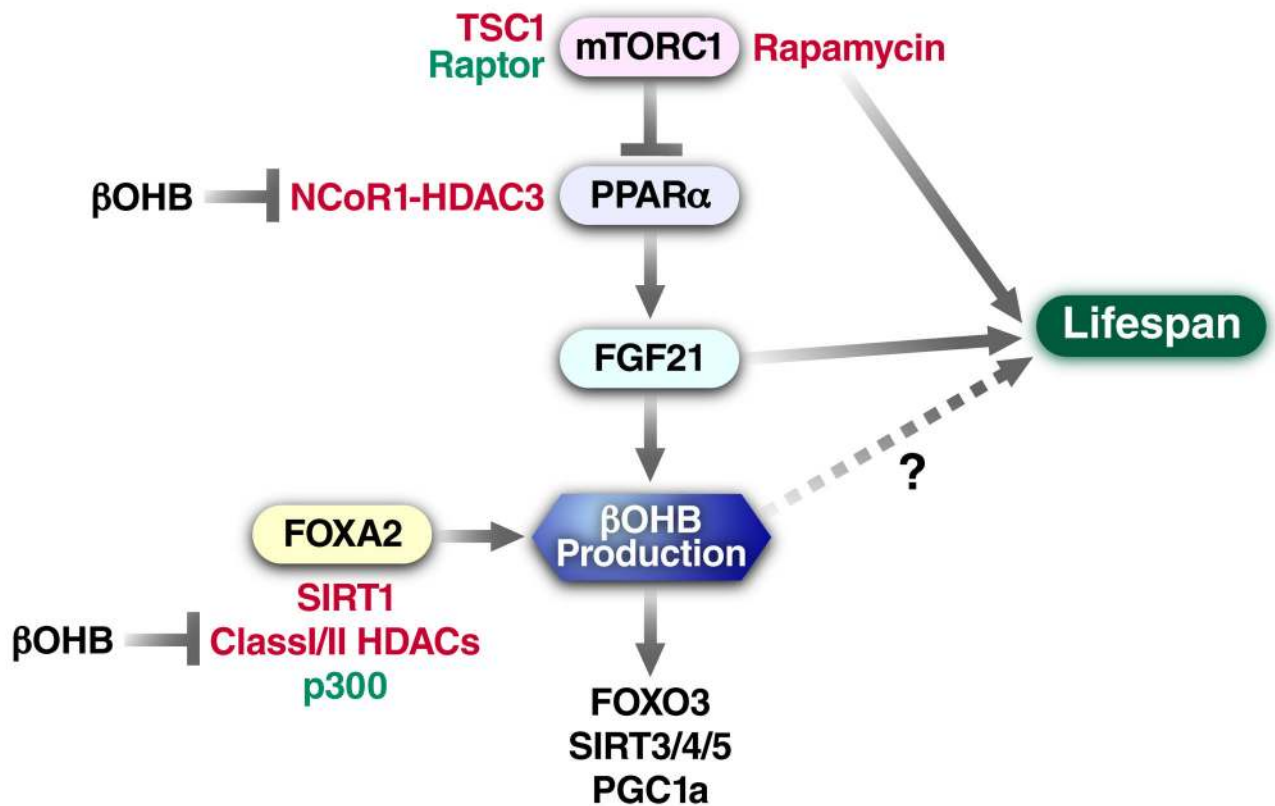


Figure 2. Intersection of longevity pathways and regulation of β OHB production

β OHB production is controlled by at least two nutrient-responsive pathways that are implicated in longevity and may be subject to regulation by β OHB via HDAC inhibition. Rapamycin and down-regulation of the mTOR pathway promote ketogenesis; rapamycin and FGF21 enhance mammalian longevity. FOXA2 also enhances ketogenesis, and its activation is regulated by both class III (sirtuins) and class I/II HDACs.

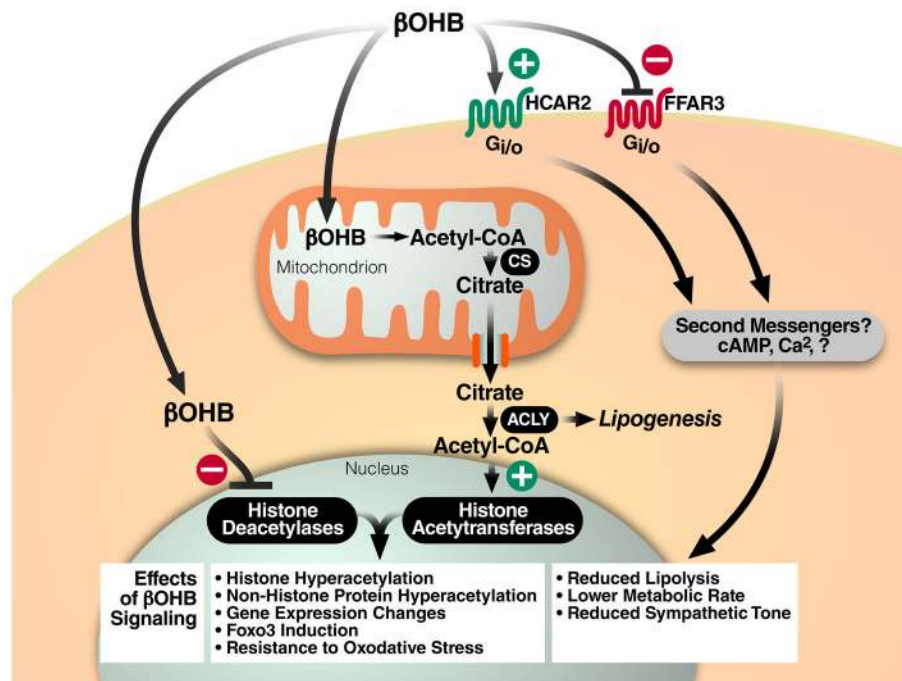


Figure 3. Cellular signaling mediated by β OHB

β OHB is a ligand for at least two cell-surface G-protein-coupled receptors that modulate lipolysis, sympathetic tone, and metabolic rate. In addition, β OHB alters protein acetylation through at least two mechanisms: increasing the cellular pool of acetyl-CoA that is a substrate for histone acetyltransferases, and directly inhibiting the activity of class I histone deacetylases. Abbreviations: CS, citrate synthase. ACLY, ATP-citrate lyase.

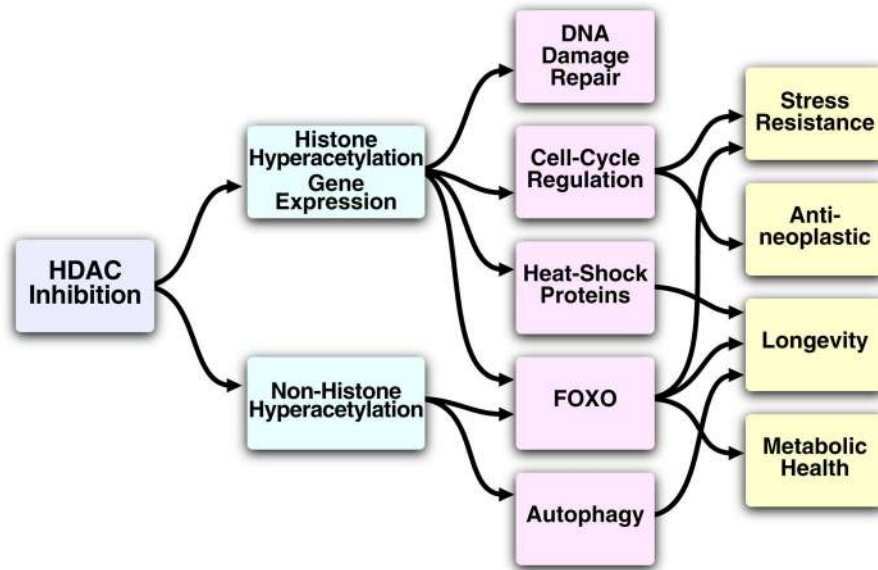


Figure 4. HDAC regulation of longevity pathways

HDACs deacetylate both histone and non-histone proteins, regulating gene transcription and the post-translational function of proteins. HDACs regulate a variety of pathways implicated in longevity and age-related disease, and modulation of HDAC activity regulates lifespan in model organisms.

Table 1

Comparison of longevity pathways regulated by ketogenic diets and CR

		Ketogenic Diet	Calorie Restriction	References
	Glucose content of diet	↓↓	—	[140]
	Energy content of diet	—	↓	[140]
	βOHB production	↑↑	↑	[140]
	Insulin levels	↓↓	↓	[2, 64–68]
	IGF signaling	↓	↓	[2, 69–71]
	AMPK activity	↑	↑	[2, 71, 72]
	mTOR activity	↓	↓	[2, 71]
βOHB	Foxo3	↑	↑	[5]
	Protein acetylation	↑	↑	[5, 141]
	Stress resistance	↑	↑	[5, 84, 98–113]
	Longevity	?	↑	[1]