Critical Review

Ketone Bodies Mimic the Life Span Extending Properties of Caloric Restriction

Richard L. Veech[1]*
Patrick C. Bradshaw[2]
Kieran Clarke[3]
William Curtis[1]
Robert Pawlosky[1]
M. Todd King[1]

[1]*Lab of Metabolic Control, NIH/NIAAA, Rockville, MD, USA*
[2]*East Tennessee State University College of Medicine, Johnson City, TN, USA*
[3]*Dept. of Physiology, Anatomy & Genetics, Oxford, UK*

Abstract

The extension of life span by caloric restriction has been studied across species from yeast and *C*aenorhabditis *elegans* to primates. No generally accepted theory has been proposed to explain these observations. Here, we propose that the life span extension produced by caloric restriction can be duplicated by the metabolic changes induced by ketosis. From nematodes to mice, extension of life span results from decreased signaling through the insulin/insulin-like growth factor receptor signaling (IIS) pathway. Decreased IIS diminishes phosphatidylinositol (3,4,5) triphosphate (PIP_3) production, leading to reduced PI3K and AKT kinase activity and decreased forkhead box O transcription factor (FOXO) phosphorylation, allowing FOXO proteins to remain in the nucleus. In the nucleus, FOXO proteins increase the transcription of genes encoding antioxidant enzymes, including superoxide dismutase 2, catalase, glutathione peroxidase, and hundreds of other genes. An effective method for combating free radical damage occurs through the metabolism of ketone bodies, ketosis being the characteristic physiological change brought about by caloric restriction from fruit flies to primates. A dietary ketone ester also decreases circulating glucose and insulin leading to decreased IIS. The ketone body, D-β-hydroxybutyrate (D-βHB), is a natural inhibitor of class I and IIa histone deacetylases that repress transcription of the *FOXO3a* gene. Therefore, ketosis results in transcription of the enzymes of the antioxidant pathways. In addition, the metabolism of ketone bodies results in a more negative redox potential of the NADP antioxidant system, which is a terminal destructor of oxygen free radicals. Addition of D-βHB to cultures of *C. elegans* extends life span. We hypothesize that increasing the levels of ketone bodies will also extend the life span of humans and that calorie restriction extends life span at least in part through increasing the levels of ketone bodies. An exogenous ketone ester provides a new tool for mimicking the effects of caloric restriction that can be used in future research. The ability to power mitochondria in aged individuals that have limited ability to oxidize glucose metabolites due to pyruvate dehydrogenase inhibition suggests new lines of research for preventative measures and treatments for aging and aging-related disorders. © 2017 The Authors IUBMB Life published by Wiley Periodicals, Inc. on behalf of International Union of Biochemistry and Molecular Biology, 69(5):305–314, 2017

Abbreviations: *D-βHB, D-β-hydroxybutyrate; HIF-1α, hypoxia-inducible factor 1-alpha; IGF, insulin/insulin-like growth factor; FOXO, forkhead box transcription factor; HDAC, histone deacetylase; IIS, insulin/insulin-like growth factor receptor signaling pathway; ILP, insulin like protein; IST-1, insulin receptor substrate-1; Nrf2, Nuclear factor (erythroid-derived 2)-like 2; PGC1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PDH, pyruvate dehydrogenase; PDK1,4, pyruvate dehydrogenase kinase isozyme 1 or 4; PDPK1, phosphoinositide-dependent kinase-1; PI3K, phosphoinositide 3 kinase; PIP2, phosphatidylinositol (4,5)-bisphosphate; PIP3, phosphatidylinositol (3,4,5) triphosphate; PTEN, phosphatase and tensin homolog; RNS, reactive nitrogen species; ROS, reactive oxygen species; SASP, senescence-associated secretory phenotype; SIRT1, sirtuin 1; SOD, superoxide dismutase*

Volume 69, Number 5, May 2017, Pages 305–314

**Address correspondence to: Richard L. Veech, Laboratory of Metabolic Control, NIH/NIAAA, 5625 Fishers Lane, Rockville, MD 20852, USA. Tel: +301-443-4620. Fax: +301-443-0939.*
E-mail: rveech@mail.nih.gov

Received 8 February 2017; Revised 10 March 2017;
DOI 10.1002/iub.1627
Published online 3 April 2017 in Wiley Online Library (wileyonlinelibrary.com)

Keywords: *ketone bodies; aging; lifespan;* Caenorhabditis elegans*; NADPH; FOXO3a; reactive oxygen species*

Ketone Bodies and Extension of Life Span

In 1935, McCay et al. showed that caloric restriction of 30% to 50% increased the average life span of rats from 500 to 820 days (1). Since that time, caloric or dietary restriction has been shown to increase life span in a wide variety of species, from yeast (2) to nematodes (3) to fruit flies (4) to mice (5) and primates. In studies of primates, calorie restriction was shown to extend lifespan by one group (6), but an earlier study using a slightly different calorie restriction protocol did not find an effect on lifespan (7). A number of proposed mechanisms for the phenomena have been suggested including: retardation of growth, decreased fat content, reduced inflammation, reduced oxidative damage, body temperature, and insulin signaling, and increase in physical activity and autophagy (8). However, no coherent mechanistic explanation has been generally accepted for this widely observed phenomenon that caloric restriction extends life span across the species. Yet, an obvious metabolic change associated with caloric restriction is ketosis. Increased ketone body concentrations occur during caloric restriction in widely different species ranging from *Caenorhabditis elegans* (9) to *Drosophila* (4) to man where ketone bodies are produced in liver from free fatty acids released from adipose tissue (10).

Ketone bodies were first found in the urine of subjects with diabetes (11) creating in physicians the thought that their presence was pathological. However, Cahill showed that ketone bodies were the normal result from fasting in man (12), where they could be used in man in most extrahepatic tissue including brain (13). The ketone bodies, D-β-hydroxybutyrate (D-βHB) and its redox partner acetoacetate are increased during fasting (14), exercise (15), or by a low carbohydrate diet (16). Originally ketone bodies were thought to be produced by a reversal of the β-oxidation pathway of fatty acids. However, it was definitively and elegantly shown by Lehninger and Greville that the β-hydroxybutyrate of the β oxidation pathway was of the L form while that produced during ketogenesis was the D form (17). This fundamental difference in the metabolism of the D and L form of ketone bodies has profound metabolic effects.

The metabolism of the D-form results in oxidation of the mitochondrial co-enzyme Q couple (18) and an increase in the redox span between the mitochondrial NAD and Q couples with a resultant increase in the $\Delta G'$ of adenosine triphosphate (ATP). The L-form of β-hydroxybutyrate is activated by conversion of ATP to adenosine monophosphate (AMP), a more energetically costly process than the activation by succinyl-CoA. In contrast to the metabolism of D-βHB, which produces only NADH, the further metabolism of the L-form is metabolized by the fatty acid β-oxidation system, which results in the reduction of one mitochondrial NAD and one co-enzyme Q with no increase in redox span between the two couples and therefore no increase in the $\Delta G'$ of ATP hydrolysis. When catabolized for the synthesis of ATP in mitochondria, D-βHB produces more ATP per oxygen molecule consumed than many other respiratory substrates due to this unique nature of D-βHB metabolism (18,19).

Blood ketone bodies can also be elevated, without elevation of blood free fatty acids by ingestion of ketone body esters such as D-βHB-*R* 1,3 butanediol monoester (20). This ketone ester has been evaluated for toxicity (21) and is recognized as generally recognized as safe (GRAS) by the Food and Drug Administration.

Recently, it was shown that administration of D-βHB to *C. elegans* caused an extension of life span resulting in that ketone body to be presciently labeled as "an anti-aging ketone body" (9). In the same experiment, L-β-hydroxybutyrate failed to extend life span. If it is accepted that the ketone body, D-βHB is an "anti-aging" compound, this could account for the widespread observation that caloric restriction, and its resultant ketosis, leads to life span extension. Many of the signaling pathways mediating extension of life span have been determined by geneticists largely by work using the short-lived nematode *C. elegans*.

Genetic Mechanisms of Life Span Extension

There are marked heritable differences in median life span between species: from less than 3 weeks in *C. elegans,* between 2 and 3 years for the mouse or rat, 10 to 15 years for the dog, around 70 years for humans, and up to over 400 years in a bivalve mollusk *Artica islandica*. This observation makes it clear that life span has a heritable component.

The first genetically induced increase in life span in *C. elegans* was reported for a mutation in *age-1*, encoding a catalytic subunit of the of phosphatidylinositol 3-OH kinase of the insulin/insulin-like growth factor (IGF) receptor signaling (IIS) pathway (22) (23). The Kenyon laboratory insightfully took experimental advantage of the short life span of *C. elegans* to identify mutations in the abnormal dauer formation-2 (*daf-2*) insulin/IGF-1 receptor gene of the IIS pathway that led to a twofold increase in *C. elegans* life span (24). This life span extension was found to be predominately caused by the decreased phosphorylation and nuclear translocation of the DAF-16/FOXO transcriptional regulator leading to expression of over 200 genes including those involved in metabolism, proteostasis, and antioxidant defenses (25,26) (Fig. 1). Activation of other factors such as the SKN-1/Nrf2 transcriptional regulator also contributes to the longevity effects of reduced IIS under certain experimental conditions (27). The increased life

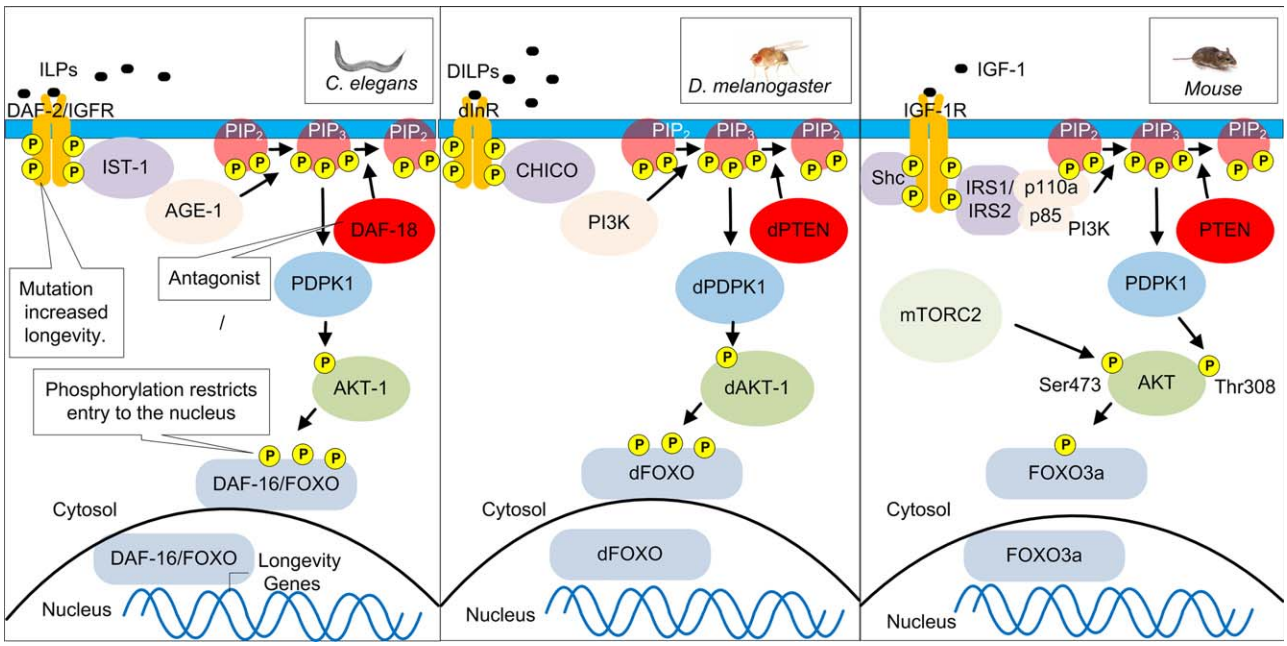

FIG 1

The longevity effect from mutation of genes in the IIS pathway was discovered in C. elegans*. This longevity effect was also later found in fruit flies and mice. In C.* elegans*, the normal signaling pathway results in phosphorylation and sequestration in the cytosol of the forkhead box transcription factor DAF-16. IGF-1 receptor, IGF-1R, is a tyrosine kinase receptor. Insulin-like peptides or IGFs are ligands for IGF-1R (DAF-2 in C.* elegans *and dInR in Drosophila) stimulating its autophosphorylation and recruitment of adaptor proteins (insulin receptor substrate-1 (IST-1), AGE-1, CHICO, Shc (Src homology and collagen protein), Insulin receptor substrate-1 (IRS-1), and Insulin receptor substrate- 2 (IRS-2)), which activate a class I phosphoinositide 3-kinase. This leads to an increased concentration of PIP3 in the plasm membrane. PTEN and the orthologue dPTEN and DAF-18 are antagonists reducing the concentration of PIP3. High membrane concentrations of PIP3 stimulate the signaling cascade by inducing PDPK1 phosphorylation of AKT-1, which in turn phosphorylates the various FOXO transcription factors. Abbreviations: AGE-1 is a phosphoinositide 3-kinase (PI3K), PIP3 stands for phosphatidylinositol (3,4,5)- triphosphate, PIP2 is the abbreviation for phosphatidylinositol (4,5)-bisphosphate, PTEN is phosphatase and TENsin homolog, PDPK1 is phosphoinositide-dependent kinase-1, AKT is named for the Ak mouse strain, and the t stands for thymoma. Dauer is German meaning endurance or duration, indicating a suspended developmental stage. DAF is the family of proteins that were investigated as dauer factors that regulate entrance into the dauer state, CHICO means small boy in Spanish referring to the small body size of the mutant flies. mTORC2 is a complex containing mechanistic target of rapamycin (mTOR). It is made up of mTOR, Rictor, mLST8, Protor1/2, Sin1, and Deptor. FOXO indicates a forkhead box transcription factor. ILP is the abbreviation for insulin-like peptides. IGF-1 stands for insulin-like growth factor-1.*

span of the *daf-2* mutant could be further increased by dietary restriction indicating at least partially distinct mechanisms of action (28).

It was at first puzzling why a mutation or decreased expression of the *daf-2* insulin/IGF-1 receptor gene in *C. elegans* was associated with extension of life span. After all, IGF causes proliferation in cells that are not postmitotic and hypertrophy in myocytes. It is ironic, given the attraction to sweet taste, that consumption of glucose with resultant stimulation of the insulin receptor should be a signal for shortening life span. Decreased signaling through this pathway likely evolved as a mechanism to delay reproduction until food was more abundant and the delay in reproduction carried with it an increase in survival. More understandable is the finding that an increase in the activity of the *DAF-16*/FOXO protein, controlling expression of several antioxidant enzymes and heat shock proteins should play a key role in life span extension. However, as animals have evolved longer life spans, FOXO proteins have evolved additional more complex roles in regulating cellular function and aging including stimulating apoptosis (29) that likely helps prevent tumorigenicity (30). FOXO proteins are modified post-translationally by acetylation and phosphorylation, which are regulated by many factors including metabolism, inflammation, and oxidative stress (29). The complex roles and signaling mechanisms thought to control the expression and activity of various FOXO proteins have recently been reviewed (31). An allele of *FOXO3a*, one of four human FOXO genes, is associated with extreme longevity in humans (32). Longevity may be regulated through other specific signaling pathways and transcriptional regulators such as mechanistic target of rapamycin (mTOR), 5′ AMP-activated protein kinase (AMPK), sirtuin 1 (SIRT1), sirtuin 3 (SIRT3), nuclear factor (erythroid-derived 2)-like 2 (Nrf2), and peroxisome proliferator-activated receptor gamma coactivator 1-α (PGC-1α) (33), although nematode orthologs of PGC-1α and SIRT3 appear to be absent.

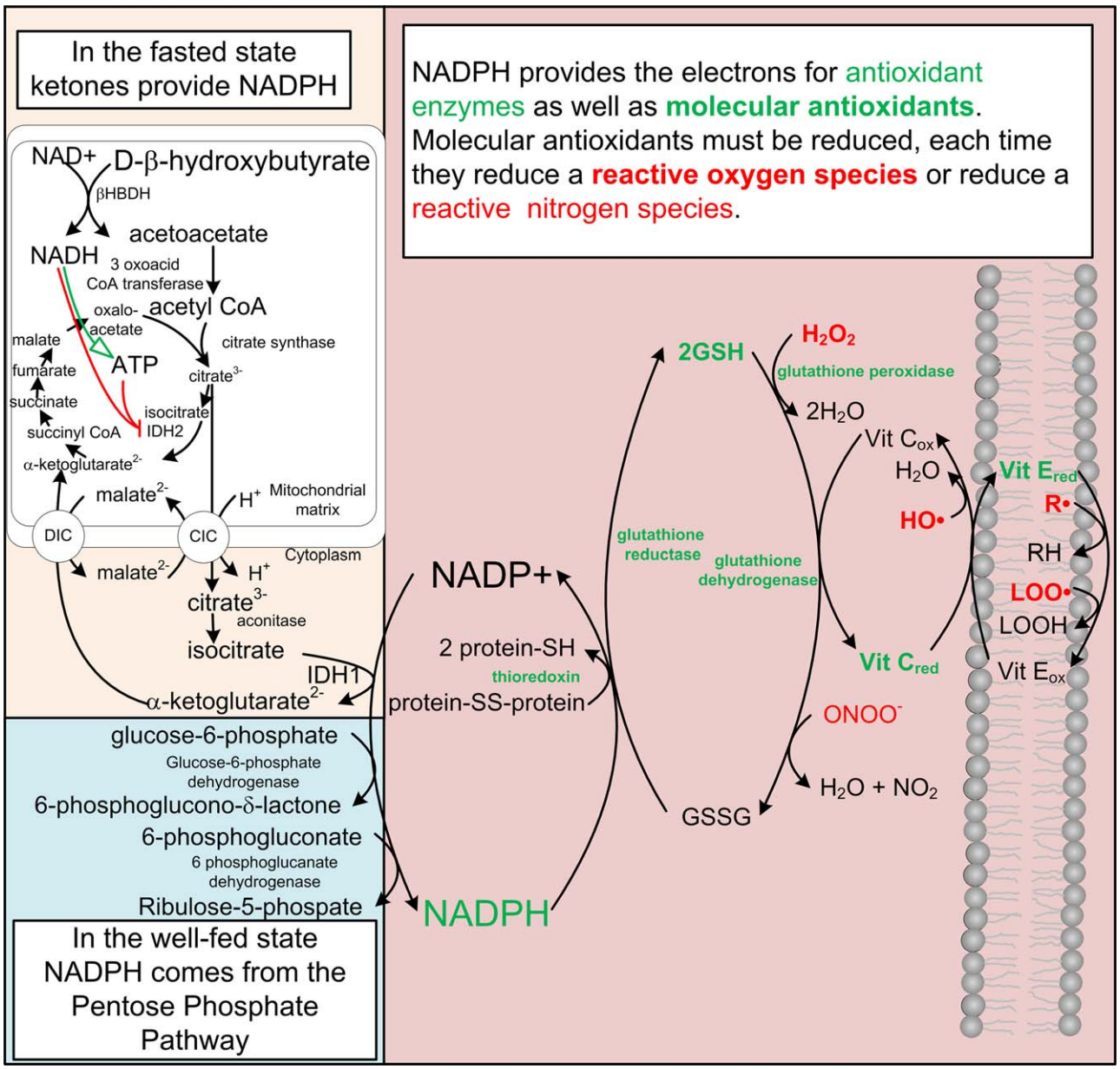

FIG 2

The NADPH system of antioxidant enzymes and NADPH-dependent molecular antioxidants. The two primary pathways providing sufficient electron donors for the reduction of oxidized species in the cytosol, organelles, and membranes are shown. This is accomplished, in part, through NADPH-dependent reduction of glutathione (GSH), vitamin C (Vit C), and vitamin E (Vit E). The redox potential of these secondary systems are all set by the redox potential of the free cytosolic [$NADP^+$]/[NADPH] system to which they are linked enzymatically.

Aging, Oxidative Stress, and the NADPH-Linked Antioxidant System

In the 1950s, Harman postulated that the toxicity of reactive oxygen species (ROS) was central to the mechanism of aging (34) as it was to radiation toxicity (35). There has been accumulating evidence since then that the mechanism limiting the life span results from ROS damage (36,37). Later, data (37) greatly support the mitochondrial free radical theory of aging. The first is the strong inverse correlation between mitochondrial ROS production and longevity, and the second is the strong inverse correlation between the degree of fatty acid unsaturation in tissue membranes and longevity among related species. No other parameters measured corresponded with life span as well as these indicators, which likely evolved to minimize ROS-mediated damage.

While ROS and reactive nitrogen species (RNS) are necessary for certain signaling pathways, their unregulated production is destructive leading to pathology. However, in the last 10 years evidence from *C. elegans* and other model organisms

has demonstrated that ROS-mediated signaling is required for some mechanisms of life span extension.

The toxicity of ROS/RNS is ameliorated by the NADPH system (Fig. 2), the redox potential of which is made more negative by the metabolism of ketone bodies (19,38,39). The redox potential of the free cytosolic [$NADP^+$]/[NADPH] system is about −0.42 V, about the same redox potential as hydrogen and is the most negative redox potential in the body (38). Other reducing agents, such as the ascorbic acid couple, are linked enzymatically to the [$NADP^+$]/[NADPH] couple (19). In peripheral tissues in the fed state, NADPH is primarily produced from glucose metabolism by the hexose monophosphate pathway (40). During fasting when glucose is limiting, NADPH is produced from the metabolism of ketone bodies in the Krebs cycle (18,39) mainly through the action of NADP-dependent isocitrate dehydrogenase. During calorie restriction, mitochondrial SIRT3 deacetylates and activates the NADP-dependent isocitrate dehydrogenase IDH2 leading to increased NADPH production and an increased ratio of reduced-to-oxidized glutathione in mitochondria (41). Both FOXO1 and FOXO3a induce expression of IDH1 (42), a cytoplasmic form of NADP-dependent isocitrate dehydrogenase. Citrate or isocitrate formed from ketone body catabolism in mitochondria can be exported by the citrate–isocitrate carrier to the cytoplasm for the production of NADPH by IDH1 (Fig. 2).

Addition of reducing agents such as ascorbic acid did not uniformly increase the life span of model organisms, perhaps because these treatments had both pro and antioxidant effects or that reducing agents block ROS signals required for life span extension. Extravagant claims for the beneficial effects of high doses of ascorbic acid made by Linus Pauling were largely unsubstantiated. It was through the work of Krebs and Veech that the control of redox states in the cell and the dominant role of the free [$NADP^+$]/[NADPH] was appreciated (38). The detoxification of free radicals is dependent upon the multiple redox couples which are linked to and whose redox potential is set by the most negative NADP system (19). In the absence of malnutrition, there is little or no reason to take antioxidant supplements because the ability of these compounds to function as antioxidants is largely determined by the [$NADP^+$]/[NADPH]. As aluded to above, this ratio is regulated by the flux of substrates through enzymes that generate or consume $NADP^+$ and NADPH, the reduction of which is brought about by the metabolism of ketone bodies. Therefore, consuming increased amounts of antioxidants has little effect on the [$NADP^+$]/[NADPH].

Data in apparent conflict with the free radical theory of aging has led to its increased scrutiny. In *C. elegans*, one study showed that increasing free radicals by knocking out the superoxide dismutase (SOD) genes one at a time did not shorten life. Knockout of *sod-4*, an extracellular protein, even had the counter-intuitive effect of extending life span (43). Another group showed that mice engineered to overexpress SOD and catalase did not live longer than normal (44). However, overexpressing mitochondrial-targeted catalase in mice did lead to life span extension (45). SOD and catalase are both dismutases that are not linked to the NADPH system of clearing ROS/RNS that we hypothesize to be the most important driving force for life span extension.

There is substantial data supporting the ability of decreased [$NADP^+$]/[NADPH] to extend life span. Glucose-6-phosphate dehydrogenase overexpression increased median life span in *Drosophila* (46) and female mice (47). Longer-lived strains of *Drosophila* were shown to possess higher glucose-6-phosphate dehydrogenase activity than shorter lived strains (48). By increasing flux through NADP-dependent forms of the enzyme, knocking out or knocking down NAD-dependent isocitrate dehydrogenase increased life span in yeast (49) and *C. elegans* (50). Finally, overexpression of NADP-dependent malic enzyme extended life span in *Drosophila* (51). Studies should now be performed to more directly test the hypothesis that increased NADPH levels extend lifespan through bolstering the NADPH antioxidant system.

Decreased Pyruvate Dehydrogenase Activity in Aged Tissues is Bypassed by Ketone Body Metabolism

Aging has been shown to lead to decreased mitochondrial pyruvate dehydrogenase (PDH) complex activity. In heart, this decreased activity was not due to lower PDH complex levels, but due to increased phosphorylation that inhibits complex activity (52). PDH phosphatases, which are able to increase PDH activity, have been shown to be stimulated by insulin (53), and in skeletal muscle, this stimulation was shown to decline with age, but be restored by exercise (54). Decreased PDH activity has been found in specific regions of aged brain such as in the striatum and brain stem as a result of increased PDH kinase activity (55). This finding could result from increased ROS production from mitochondria during aging that increases hypoxia-inducible factor 1-alpha (HIF-1α) activity (56) and the expression of the pyruvate dehydrogenase kinase isoform 4 (PDK4) (57). Consistent with it playing a role in the aging process, the PDH complex has also been shown to regulate cellular senescence (58). However, too much PDH activity may have pro-aging effects through a hyperstimulation of mitochondrial metabolism resulting in increased ROS production. Therefore, FOXO proteins have evolved to induce expression of PDH kinases such as PDK4 to negatively regulate PDH activity and ROS production (59). During fasting and caloric restriction, the FOXO-mediated expression of PDK enzymes may also serve an important role in the shunting of pyruvate and/or lactate to cell types or tissues with the highest energy needs, such as neurons that cannot oxidize fatty acids.

Studies in *C. elegans* also support a role for PDH activity in the regulation of longevity. Inhibiting PDH kinase activity with dichloroacetate extended life span (60), while overexpression of the PDH phosphatase, PDP-1, also increased longevity through increased DAF-16/FOXO nuclear translocation (61). It is important to determine whether PDH activity regulates the activity of FOXO proteins in humans. This could

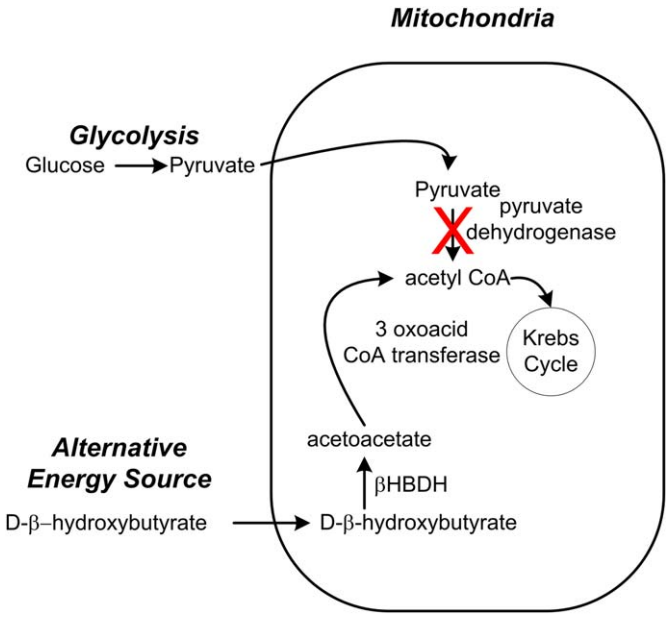

FIG 3 *Ketone body metabolism bypasses decreased PDH activity in aged tissues.*

potentially occur through nuclear localized PDH providing acetyl-CoA for histone acetylation (62) of FOXO promoters. If PDH activity stimulates FOXO expression, declining PDH activity during aging may lead to a downstream loss of FOXO-mediated transcriptional events and increased oxidative stress. As metabolism of ketone bodies bypasses PDH activity as shown in Fig. 3, ketone or ketone ester supplementation may be able to mitigate metabolic and transcriptional alterations resulting from decreased PDH activity to promote longevity. Calorie restriction was shown to stimulate skeletal muscle mitochondrial pyruvate metabolism by increasing expression of the mitochondrial pyruvate carrier and decreasing expression of the lactate dehydrogenase A gene (63). The longevity effects of caloric restriction in mammals require the *FOXO3a* gene (64).

Telomere Shortening is Linked to Cellular Redox Status and Metabolism

The work of Hayflick and Moorhead (65) pointed out that shortening of the telomeres set a limit to the number of divisions cells in culture could undergo before senescence occurs. Expression of the telomerase enzyme in certain germ and progenitor cells provides a solution to replicate the ends of linear chromosomes, so that the chromosomes do not become shorter with each new round of DNA replication. Telomeres are lengthened by starvation (66) and shortened by ROS damage (59). These observations are consistent with aging being a function of reactive oxygen and its reversal a function of the increasing redox potential of the NADPH system brought about by caloric restriction. The FOXO protein FOXO1 was shown to be essential for the calorie restriction-mediated increase in telomerase subunit expression (67). As cells approach their Hayflick limit, the expression of the FOXO genes *FOXO1* and *FOXO4* have also been shown to decline (68), which would lead to decreased *SOD2* and catalase expression. Senescent cells and tissues not only show decreased function but also acquire a senescence-associated secretory phenotype (SASP), a pro-inflammatory, pro-aging state. Mitochondrial dysfunction that increases ROS/RNS production also induces a cellular senescence program with a modified SASP (67).

Other Potential Anti-Aging Mechanisms of Ketone Bodies

Decreased insulin signaling activates mammalian FOXO proteins such as FOXO1 and FOXO3, which stimulates expression of many genes involved in autophagy (69). In addition, decreased insulin signaling or nutrient deprivation inactivates mTOR kinase to stimulate autophagy, which is required for dietary restriction-mediated life span extension in *C. elegans* (70). Consistent with these effects, D-βHB treatment has been shown to stimulate autophagy in cultured cortical neurons (71) and increased rates of autophagy are likely one of the several molecular mechanisms that contribute to the life span extending effects exerted by ketone bodies. One mechanism through which D-βHB may decrease IIS to activate FOXO proteins and autophagy is through a direct inhibition of AKT (72). This inhibition may also be responsible for the fasting-induced insulin resistance observed in muscle, heart, and other peripheral tissues that preserves glucose use for the brain (73,74).

D-βHB may also exert protective metabolic effects by binding at least two different G protein-coupled receptors, HCAR2/Gpr109/PUMA-G (first discovered to be a nicotinic acid receptor) and free fatty acid receptor 3 on the plasma membrane (75). As these genes evolved in chordates and are not present in invertebrates, they could not function in the evolutionarily conserved role of ketone bodies in life span extension. But activation of these receptors likely plays important metabolic signaling roles mediated by D-βHB. Finally, the gut microbiome plays an important role in providing substrates for ketone body synthesis (76) and could therefore effect the extent of ketone body synthesis during caloric restriction to influence the magnitude of life span extension, but a further discussion of this research topic is beyond the scope of this review.

Feeding Ketone Esters

One effect of feeding rats with the ketone ester, D-βHB-*R* 1,3 butanediol monoester, was a 1.7-fold decrease in blood glucose and over a twofold decrease in blood insulin (77). The same decreases in glucose and insulin are seen after feeding ketone esters to mice (78). These metabolic changes induced by feeding ketone esters mimic the decreased IIS induced by a longevity-inducing mutation in *daf-2* in the nematode (23,26).

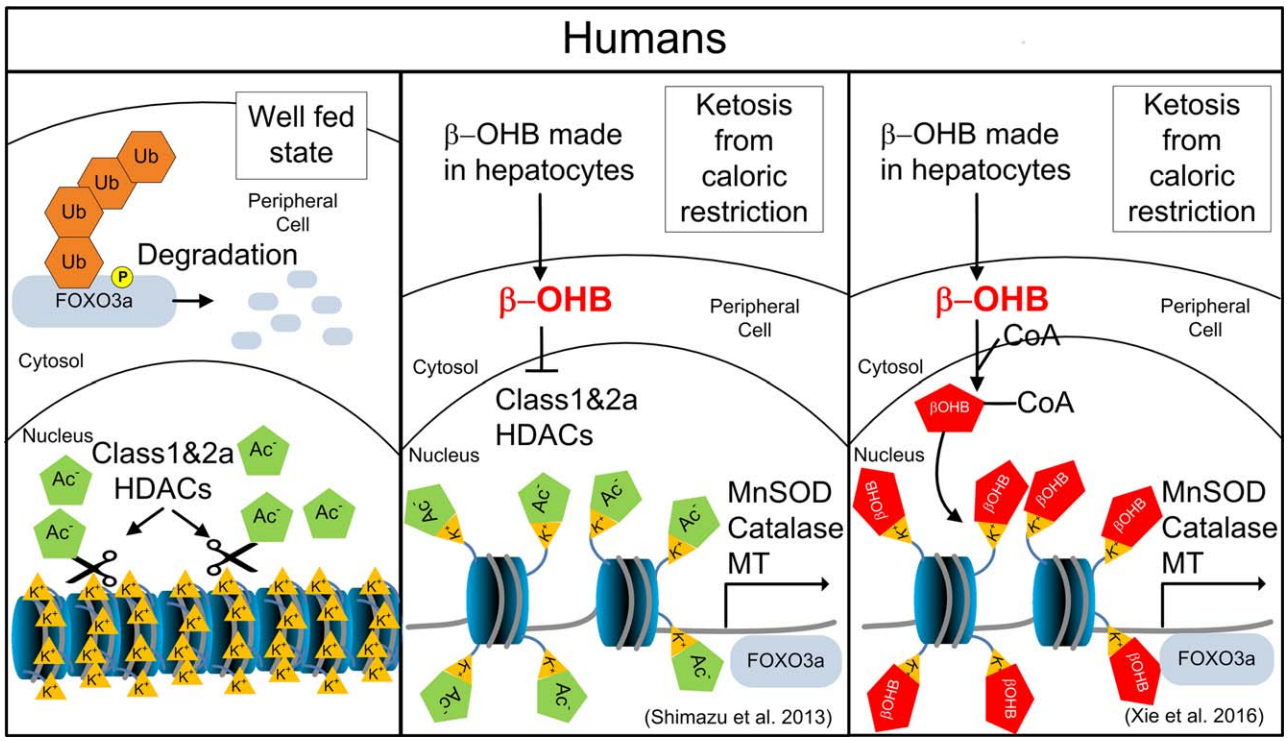

FIG 4 *In the well-fed state, the FOXO3a transcription factor is prevented from entering the nucleus by phosphorylation. FOXO3A is marked for degradation by ubiquitin (Ub). DNA with FOXO3a promoter remains out of reach due to lysine (K+) interacting with negative charges in DNAs phosphodiester backbone keeping histones in the condensed state. In a state of ketosis, HDAC is inhibited by D-β-hydroxybutyrate. The acetyl (Ac-) group neutralizes the charge on lysine opening the histone complex exposing the FOXO3a promoter and upregulating superoxide dismutase (MnSOD), catalase, and metallothionein MT. An alternative mechanism proposed by Xie et al. is shown in the third panel.*

In addition to mutations in *daf-2* that increase nuclear translocation and activity of DAF-16/FOXO, there are ways to increase the transcription of FOXO genes metabolically. For example, in mammals, the transcription of *FOXO3a* can be induced by inhibition of class I and IIa histone deacetylases (HDACs) by D-βHB (79) or possibly by β-hydroxybutyrylation (80) (Fig. 4). Inhibition of these HDACs by D-βHB induces the expression of other antioxidant and detoxification genes such as the metallothionein-1 (*MTL1*) that can lead to reduced ROS toxicity. In liver, fasting also increases FOXO1 activity through a mechanism where glucagon stimulates class IIa HDAC translocation to the nucleus to recruit the class I HDAC HDAC3 to deacetylate FOXO1 (81) These pathways also affect metabolism, phosphorylation potential, redox states, and the ability to clear ROS. In *C. elegans*, the life span extension induced by administration of the ketone body D-βHB required nematode homologs of AMPK, SIRT1, FOXO, and Nrf2. No additional increase in life span was observed for D-βHB treatment to a long-lived S6 kinase mutant of the target of rapamycin (TOR) signaling pathway suggesting that TOR inhibition also plays a role in the ketone body-mediated longevity effects (33).

As somewhat distinct from genetic manipulation of the IIS pathway, feeding ketone bodies results in the reduction of the free cytosolic [$NADP^+$]/[NADPH] ratio (39), which provides the thermodynamic force required to reduce glutathione and other antioxidant couples that destroy oxygen free radicals (19). The metabolism of ketone bodies, which lower both blood glucose and insulin, decrease the activity of the IIS pathway that in turn leads to an increase in the level and activity of the unphosphorylated FOXO transcription factors central for life span extension (26). Ketosis, which is a common consequence of caloric restriction, may provide an explanation for why caloric restriction leads to life span extension in most species. Here, we propose that the life span extension produced by caloric restriction can be duplicated by the metabolic changes induced by ketosis.

Conclusion

Aging in man is accompanied by deterioration of a number of systems. Most notable are a gradual increase in blood sugar and blood lipids, increased narrowing of blood vessels, an increase in the incidence of malignancies, the deterioration and loss of elasticity in skin, loss of muscular strength and physiological exercise performance, deterioration of memory and cognitive performance, and in males decreases in erectile function. Many aging-induced changes, such as the incidence of malignancies in mice (82), the increases in blood glucose and insulin caused by insulin resistance (39,78), and the muscular weakness have been shown to be decreased by the

metabolism of ketone bodies (18,83), a normal metabolite produced from fatty acids by liver during periods of prolonged fasting or caloric restriction (12).

The unique ability of ketone bodies to supply energy to brain during periods of impairment of glucose metabolism make ketosis an effective treatment for a number of neurological conditions which are currently without effective therapies. Impairment of cognitive function has also been shown to be improved by the metabolism of ketone bodies (84). Additionally, Alzheimer's disease, the major cause of which is aging (20) can be improved clinically by the induction of mild ketosis in a mouse model of the disease (85) and in humans (86). Ketosis also improves function in Parkinson's disease (87) which is thought to be largely caused by mitochondrial free radical damage (19,88). Ketone bodies are also useful in ameliorating the symptoms of amyotrophic lateral sclerosis (89). It is also recognized that ketosis could have important therapeutic applications in a wide variety of other diseases (90) including Glut 1 deficiency, type I diabetes (91), obesity (78,92), and insulin resistance (20,39,93), and diseases of diverse etiology (90).

In addition to ameliorating a number of diseases associated with aging, the general deterioration of cellular systems independent of specific disease seems related to ROS toxicity and the inability to combat it. In contrast increases in life span occur across a number of species with a reduction in function of the IIS pathway and/or an activation of the FOXO transcription factors, inducing expression of the enzymes required for free radical detoxification (Figs. 1 and 2). In *C. elegans*, these results have been accomplished using RNA interference or mutant animals. Similar changes should be able to be achieved in higher animals, including humans, by the administration of D-βHB itself or its esters.

In summary, decreased signaling through the insulin/IGF-1 receptor pathway increases life span. Decreased insulin/IGF-1 receptor activation leads to a decrease in PIP_3, a decrease in the phosphorylation and activity of phosphoinositide-dependent protein kinase (PDPK1), a decrease in the phosphorylation and activity of AKT, and a subsequent decrease in the phosphorylation of FOXO transcription factors, allowing them to continue to reside in the nucleus and to increase the transcription of the enzymes of the antioxidant pathway.

In mammals, many of these changes can be brought about by the metabolism of ketone bodies. The metabolism of ketones lowers the blood glucose and insulin thus decreasing the activity of the IIS and its attendant changes in the pathway described above. However, in addition ketone bodies act as a natural inhibitor of class I HDACs, inducing FOXO gene expression stimulating the synthesis of antioxidant and metabolic enzymes. An added important factor is that the metabolism of ketone bodies in mammals increases the reducing power of the NADP system providing the thermodynamic drive to destroy oxygen free radicals which are a major cause of the aging process.

References

[1] McCay, C. M., Crowell, M. F., and Maynard, L. A. (1935) The effect of retarded growth upon the lenght of life span and upon the ultimate body size. J. Nutr. 10, 63–79.

[2] Lee, S. H. and Min, K. J. (2013) Caloric restriction and its mimetics. BMB Rep. 46, 181–187.

[3] Klass, M. R. (1977) Aging in the nematode *Caenorhabditis elegans*: major biological and environmental factors influencing life span. Mech. Age. Dev. 6, 413–429.

[4] Luong, N., Davies, C. R., Wessells, R. J., Graham, S. M., King, M. T., et al. (2006) Activated FOXO-mediated insulin resistance is blocked by reduction of TOR activity. Cell Metab. 4, 133–142.

[5] Fontana, L., Partridge, L., and Longo, V. D. (2010) Extending healthy life span – from yeast to humans. Science 328, 321–326.

[6] Colman, R. J., Beasley, T. M., Kemnitz, J. W., Johnson, S. C., Weindruch, R., et al. (2014) Caloric restriction reduces age-related and all-cause mortality in rhesus monkeys. Nat. Comm. 5, 3557.

[7] Mattison, J. A., Roth, G. S., Beasley, T. M., Tilmont, E. M., Handy, A. M., et al. (2012) Impact of caloric restriction on health and survival in rhesus monkeys from the NIA study. Nature 489, 318–321.

[8] Masoro, E. J. (2009) Caloric restriction-induced life extension of rats and mice: a critique of proposed mechanisms. Biochim. Biophys. Acta. 1790, 1040–1048.

[9] Edwards, C., Copes, N., and Bradshaw, P. C. (2015) D-ss-hydroxybutyrate: an anti-aging ketone body. Oncotarget 6, 3477–3478.

[10] Krebs, H. A. (1960) Biochemical aspects of ketosis. Proc. Roy. Soc. Med. 53, 71–80.

[11] Walter, F. (1877) Unteruchungen uber die Wirkung der Sauren auf den thierischen Organismus. Arch. F. Exper. Path. u Pharmakol. 7, 148.

[12] Cahill, G. F. Jr. (1970) Starvation in man. N. Engl. J. Med. 282, 668–675.

[13] Cahill G.F., Jr. and Aoki T.T. (1980) Alternate fuel utilization in brain. In: Cerebral Metabolism and Neural Function (Passonneau, J. V., Hawkins, R. A., Lust, W. D., and Welsh, F. A., eds.). pp. 234–242, Williams and Wilkins, Baltimore.

[14] Krebs, H. (1960) Biochemical aspects of ketosis. Proc. Royal Soc. Med. 53, 71–80.

[15] Johnson, R. H., Walton, J. L., Krebs, H. A., and Williamson, D. H. (1969) Metabolic fuels during and after severe exercise in athletes and non-athletes. Lancet 2, 452–455.

[16] Wilder, J. M. (1921) The effects of ketonemia on the course of epilepsy. Mayo Clinic. Proc. 2, 307–308.

[17] Lehninger, A. L. and Greville, G. D. (1953) The enzymatic oxidation of d and l b hydroxybutyrate. Biochem. Biophys. Acta. 12, 188–202.

[18] Sato, K., Kashiwaya, Y., Keon, C. A., Tsuchiya, N., King, M. T., et al. (1995) Insulin, ketone bodies, and mitochondrial energy transduction. FASEB J. 9, 651–658.

[19] Curtis, W., Kemper, M. L., Miller, A. L., Pawlosky, R., King, M. T., et al. (2017) Mitigation of damage from reactive oxygen species and ionizing radiation by ketone body esters. In: Ketogenic Diet and Metabolic Therapy (Masino, S. A., ed.). pp. 254–270, Oxford University Press, Oxford.

[20] Veech R.L. and King, M.T. (2017) Alzheimer's disease: causes and treatment. In: Ketogenic Diet and Metabolic Therapy (Masino, S. A., ed.). pp. 241–253, Oxford University Press, Oxford.

[21] Clarke K., Tchabanenko, K., Pawlosky, R., Carter, E., King, M. T., et al. (2012) Kinetics, safety and tolerability of (R)-3-hyroxygutyl (R)-3-hydroxybutyrate in healthy adult subjects. Regul. Toxicol. Pharmacol. 63, 401–408.

[22] Johnson, T. E. (1987) Aging can be genetically dissected into component processes using long-lived lines of *Caenorhabditis elegans*. Proc. Natl. Acad. Sci USA 84, 3777–3781.

[23] Johnson, T. E., Tedesco, P. M., and Lithgow, G. J. (1993) Comparing mutants, selective breeding, and transgenics in the dissection of aging processes of *Caenorhabditis elegans*. Genetica 91, 65–77.

[24] Dorman, J. B., Albinder, B., Shroyer, T., and Kenyon, C. (1995) The age-1 and daf-2 genes function in a common pathway to control the lifespan of *Caenorhabditis elegans*. Genetics 141, 1399–1406.

[25] Lin, K., Dorman, J. B., Rodan, A., and Kenyon, C. (1997) Daf-16: an HNF-3/forkhead family member that can function to double the life-span of *Caenorhabditis elegans* [see comments]. Science 278, 1319–1322.
[26] Kenyon, C. (2011) The first long-lived mutants: discovery of the insulin/IGF-1 pathway for ageing. Philos. Trans. R. Soc. Lond. B Biol. Sci. 366, 9–16.
[27] Tullet, J. M., Hertweck, M., An, J. H., Baker, J., Hwang, J. Y., et al. (2008) Direct inhibition of the longevity-promoting factor SKN-1 by insulin-like signaling in *C. elegans*. Cell 132, 1025–1038.
[28] Kenyon, C., Chang, J., Gensch, E., Rudner, A., and Tabtiang, R. (1993) A *C. elegans* mutant that lives twice as long as wild type. Nature 366, 461–464.
[29] Wang, Y., Zhou, Y., and Graves, D. T. (2014) FOXO transcription factors: their clinical significance and regulation. Bio. Med. Res. Int. 2014, 925350.
[30] Greer, E. L. and Brunet, A. (2008) FOXO transcription factors in ageing and cancer. Acta. physiol. (Oxford, England) 192, 19–28.
[31] Eijkelenboom, A. and Burgering, B. M. (2013) FOXOs: signalling integrators for homeostasis maintenance. Nat. Rev. Mol. Cell Biol. 14, 83–97.
[32] Willcox, B. J., Donlon, T. A., He, Q., Chen, R., Grove, J. S., et al. (2008) FOXO3A genotype is strongly associated with human longevity. Proc. Natl. Acad. Sci. USA 105, 13987–13992.
[33] Edwards, C., Canfield, J., Copes, N., Rehan, M., Lipps, D., et al. (2014) D-beta-hydroxybutyrate extends lifespan in *C. elegans*. Aging 6, 621–644.
[34] Harman, D. (1956) Aging: a theory based on free radical and radiation chemistry. J. Gerontol. 11, 298–300.
[35] Gerschman, R., Gilbert, D. L., Nye, S. W., Dwyer, P., and Fenn, W. O. (1954) Oxygen poisoning and x-irradiation: a mechanism in common. Science 119, 623–626.
[36] Harman, D. (1981) The aging process. Proc. Natl. Acad. Sci. USA 78, 7124–7128.
[37] Barja, G. (2004) Free radicals and aging. Trends Neurosci. 27, 595–600.
[38] Krebs H.A. and Veech, R.L. (1969) Pyridine nucleotide interrelations. In: The Energy Level and Metabolic Conntrol in Mitochondria (Papa, S., Tager, J.M., Quagliariello, E., and Slater, E.C., ed.). pp. 329–384, Adriatica Editrice, Bari.
[39] Kashiwaya, Y., King, M. T., and Veech, R. L. (1997) Substrate signaling by insulin: a ketone bodies ratio mimics insulin action in heart. Am. J. Cardiol. 80, 50A–64A.
[40] Warburg, O., Christiansen, W., and Griiesen, A. (1935) Hydrogen transferring co-eenzyme its structure and function. Biochem. Z. 282, 157.
[41] Someya, S., Yu, W., Hallows, W. C., Xu, J., Vann, J. M., et al. (2010) Sirt3 mediates reduction of oxidative damage and prevention of age-related hearing loss under caloric restriction. Cell 143, 802–812.
[42] Charitou, P., Rodriguez-Colman, M., Gerrits, J., van Triest, M., Groot Koerkamp, M., et al. (2015) FOXOs support the metabolic requirements of normal and tumor cells by promoting IDH1 expression. EMBO Rep. 16, 456–466.
[43] Doonan, R., McElwee, J. J., Matthijssens, F., Walker, G. A., Houthoofd, K., et al. (2008) Against the oxidative damage theory of aging: superoxide dismutases protect against oxidative stress but have little or no effect on life span in *Caenorhabditis elegans*. Genes Dev. 22, 3236–3241.
[44] Perez, V. I., Van Remmen, H., Bokov, A., Epstein, C. J., Vijg, J., et al. (2009) The overexpression of major antioxidant enzymes does not extend the lifespan of mice. Aging Cell 8, 73–75.
[45] Schriner, S. E., Linford, N. J., Martin, G. M., Treuting, P., Ogburn, C. E., et al. (2005) Extension of murine life span by overexpression of catalase targeted to mitochondria. Science 308, 1909–1911.
[46] Legan, S. K., Rebrin, I., Mockett, R. J., Radyuk, S. N., Klichko, V. I., et al. (2008) Overexpression of glucose-6-phosphate dehydrogenase extends the life span of *Drosophila melanogaster*. J. Biol. Chem. 283, 32492–32499.
[47] Nobrega-Pereira, S., Fernandez-Marcos, P. J., Brioche, T., Gomez-Cabrera, M. C., Salvador-Pascual, A., et al. (2016) G6PD protects from oxidative damage and improves healthspan in mice. Nat. Comm. 7, 10894.
[48] Luckinbill, L. S., Riha, V., Rhine, S., and Grudzien, T. A. (1990) The role of glucose-6-phosphate dehydrogenase in the evolution of longevity in *Drosophila melanogaster*. Heredity 65, 29–38.
[49] Kaeberlein, M., Powers, R. W., 3rd, Steffen, K. K., Westman, E. A., Hu, D., et al. (2005) Regulation of yeast replicative life span by TOR and Sch9 in response to nutrients. Science 310, 1193–1196.
[50] Hamilton, B., Dong, Y., Shindo, M., Liu, W., Odell, I., et al. (2005) A systematic RNAi screen for longevity genes in *C. elegans*. Genes Dev. 19, 1544–1555.
[51] Kim, G. H., Lee, Y. E., Lee, G. H., Cho, Y. H., Lee, Y. N., et al. (2015) Overexpression of malic enzyme in the larval stage extends *Drosophila* lifespan. Biochem. Biophys. Res. Commun. 456, 676–682.
[52] Nakai, N., Sato, Y., Oshida, Y., Yoshimura, A., Fujitsuka, N., et al. (1997) Effects of aging on the activities of pyruvate dehydrogenase complex and its kinase in rat heart. Life Sci. 60, 2309–2314.
[53] Hutson, N. J., Kerbey, A. L., Randle, P. J., and Sugden, P. H. (1979) Regulation of pyruvate dehydrogenase by insulin action. Prog. Clin. Biol. Res. 31, 707–719.
[54] Consitt, L. A., Saxena, G., Saneda, A., and Houmard, J. A. (2016) Age-related impairments in skeletal muscle PDH phosphorylation and plasma lactate are indicative of metabolic inflexibility and the effects of exercise training. Am. J. Physiol. Endocrinol. Metab. 311, E145–E156.
[55] Pandya, J. D., Royland, J. E., MacPhail, R. C., Sullivan, P. G., and Kodavanti, P. R. (2016) Age- and brain region-specific differences in mitochondrial bioenergetics in Brown Norway rats. Neurobiol. Aging 42, 25–34.
[56] Wang, H., Wu, H., Guo, H., Zhang, G., Zhang, R., et al. (2012) Increased hypoxia-inducible factor 1alpha expression in rat brain tissues in response to aging. Neural. Regen. Res. 7, 778–782.
[57] Lee, J. H., Kim, E. J., Kim, D. K., Lee, J. M., Park, S. B., et al. (2012) Hypoxia induces PDK4 gene expression through induction of the orphan nuclear receptor ERRgamma. PLoS One 7, e46324.
[58] Kaplon, J., Zheng, L., Meissl, K., Chaneton, B., Selivanov, V. A., et al. (2013) A key role for mitochondrial gatekeeper pyruvate dehydrogenase in oncogene-induced senescence. Nature 498, 109–112.
[59] Kwon, H. S., Huang, B., Unterman, T. G., and Harris, R. A. (2004) Protein kinase B-alpha inhibits human pyruvate dehydrogenase kinase-4 gene induction by dexamethasone through inactivation of FOXO transcription factors. Diabetes 53, 899–910.
[60] Schaffer, S., Gruber, J., Ng, L. F., Fong, S., Wong, Y. T., et al. (2011) The effect of dichloroacetate on health- and lifespan in *C. elegans*. Biogerontology 12, 195–209.
[61] Narasimhan, S. D., Yen, K., Bansal, A., Kwon, E. S., Padmanabhan, S., et al. (2011) PDP-1 links the TGF-beta and IIS pathways to regulate longevity, development, and metabolism. PLoS Genet. 7, e1001377.
[62] Sutendra, G., Kinnaird, A., Dromparis, P., Paulin, R., Stenson, T. H., et al. (2014) A nuclear pyruvate dehydrogenase complex is important for the generation of acetyl-CoA and histone acetylation. Cell 158, 84–97.
[63] Chen, C. N., Lin, S. Y., Liao, Y. H., Li, Z. J., and Wong, A. M. (2015) Late-onset caloric restriction alters skeletal muscle metabolism by modulating pyruvate metabolism. Am. J. Physiol. Endocrinol. Metab. 308, E942–E949.
[64] Shimokawa, I., Komatsu, T., Hayashi, N., Kim, S. E., Kawata, T., et al. (2015) The life-extending effect of dietary restriction requires Foxo3 in mice. Aging Cell 14, 707–709.
[65] Hayflick, L. and Moorhead, P. S. (1961) The serial cultivation of human diploid cell strains. Exp. Cell Res. 25, 585–621.
[66] Kupiec, M. and Weisman, R. (2012) TOR links starvation responses to telomere length maintenance. Cell Cycle 11, 2268–2271.
[67] Makino, N., Oyama, J., Maeda, T., Koyanagi, M., Higuchi, Y., et al. (2016) FoxO1 signaling plays a pivotal role in the cardiac telomere biology responses to calorie restriction. Mol. Cell Biochem. 412, 119–130.
[68] Csiszar, A., Pacher, P., Kaley, G., and Ungvari, Z. (2005) Role of oxidative and nitrosative stress, longevity genes and poly(ADP-ribose) polymerase in cardiovascular dysfunction associated with aging. Curr. Vasc. Pharmacol. 3, 285–291.
[69] Liu, H. Y., Han, J., Cao, S. Y., Hong, T., Zhuo, D., et al. (2009) Hepatic autophagy is suppressed in the presence of insulin resistance and hyperinsulinemia: inhibition of FoxO1-dependent expression of key autophagy genes by insulin. J. Biol. Chem. 284, 31484–31492
[70] Hansen, M., Chandra, A., Mitic, L. L., Onken, B., Driscoll, M., et al. (2008) A role for autophagy in the extension of lifespan by dietary restriction in *C. elegans*. PLoS Genet. 4, e24.
[71] Camberos-Luna, L., Geronimo-Olvera, C., Montiel, T., Rincon-Heredia, R., and Massieu, L. (2016) The ketone body, beta-hydroxybutyrate stimulates

the autophagic flux and prevents neuronal death induced by glucose deprivation in cortical cultured neurons. Neurochem. Res. 41, 600–609.
[72] Yamada, T., Zhang, S. J., Westerblad, H., and Katz, A. (2010) {beta}-Hydroxybutyrate inhibits insulin-mediated glucose transport in mouse oxidative muscle. Am. J. Physiol. Endocrinol. Metab. 299, E364–E373.
[73] van der Crabben, S. N., Allick, G., Ackermans, M. T., Endert, E., Romijn, J. A., et al. (2008) Prolonged fasting induces peripheral insulin resistance, which is not ameliorated by high-dose salicylate. J. Clin. Endocrinol. Metab. 93, 638–641.
[74] Rojas-Morales, P., Tapia, E., and Pedraza-Chaverri, J. (2016) beta-Hydroxybutyrate: a signaling metabolite in starvation response? Cell. Signal. 28, 917–923.
[75] Newman, J. C. and Verdin, E. (2014) Ketone bodies as signaling metabolites. Trends Endocrinol. Metab. 25, 42–52.
[76] Crawford, P. A., Crowley, J. R., Sambandam, N., Muegge, B. D., Costello, E. K., et al. (2009) Regulation of myocardial ketone body metabolism by the gut microbiota during nutrient deprivation. Proc, Natl. Acad. Sci. USA 106, 11276–11281.
[77] Kashiwaya, Y., Pawlosky, R., Markis, W., King, M. T., Bergman, C., et al. (2010) A ketone ester diet increases brain malonyl-CoA and Uncoupling proteins 4 and 5 while decreasing food intake in the normal Wistar Rat. J. Biol. Chem. 285, 25950–25956.
[78] Srivastava, S., Kashiwaya, Y., King, M. T., Baxa, U., Tam, J., et al. (2012) Mitochondrial biogenesis and increased uncoupling protein 1 in brown adipose tissue of mice fed a ketone ester diet. FASEB J. 26, 2351–2362.
[79] Shimazu, T., Hirschey, M. D., Newman, J., He, W., Shirakawa, K., et al. (2013) Suppression of oxidative stress by beta-hydroxybutyrate, an endogenous histone deacetylase inhibitor. Science 339, 211–214.
[80] Xie, Z., Zhang, D., Chung, D., Tang, Z., Huang, H., et al. (2016) Metabolic Regulation of Gene Expression by Histone Lysine beta-Hydroxybutyrylation. Mol. Cell 62, 194–206.
[81] Mihaylova, M. M., Vasquez, D. S., Ravnskjaer, K., Denechaud, P. D., Yu, R. T., et al. (2011) Class IIa histone deacetylases are hormone-activated regulators of FOXO and mammalian glucose homeostasis. Cell 145, 607–621.
[82] Weindruch, R. and Walford, R. L. (1982) Dietary restriction in mice beginning at 1 year of age: effect on life-span and spontaneous cancer incidence. Science 215, 1415–1418.
[83] Cox, P. J., Kirk, T., Ashmore, T., Willerton, K., Evans, R., et al. (2016) Nutritional Ketosis Alters Fuel Preference and Thereby Endurance Performance in Athletes. Cell Metab. 24, 256–268.
[84] Murray, A. J., Knight, N. S., Cole, M. A., Cochlin, L. E., Carter, E., et al. (2016) Novel ketone diet enhances physical and cognitive performance. FASEB J 30, 4021–4032.
[85] Kashiwaya, Y., Bergman, C., Lee, J. H., Wan, R., Todd King, M., et al. (2013) A ketone ester diet exhibits anxiolytic and cognition-sparing properties, and lessens amyloid and tau pathologies in a mouse model of Alzheimer's disease. Neurobiol. Aging 34, 1530–1539.
[86] Newport, M. T., VanItallie, T. B., Kashiwaya, Y., King, M. T., and Veech, R. L. (2015) A new way to produce hyperketonemia: use of ketone ester in a case of Alzheimer's disease. Alzheimers Dement. 11, 99–103.
[87] VanItallie, T. B., Nonas, C., Di Rocco, A., Boyar, K., Hyams, K., et al. (2005) Treatment of Parkinson disease with diet-induced hyperketonemia: a feasibility study. Neurology 64, 728–730.
[88] Kashiwaya, Y., Takeshima, T., Mori, N., Nakashima, K., Clarke, K., et al. (2000) D-beta -Hydroxybutyrate protects neurons in models of Alzheimer's and Parkinson's disease. Proc. Natl. Acad. Sci. USA 97, 5440–5444.
[89] Siva, N. (2006) Can ketogenic diet slow progression of ALS? Lancet Neurol. 5, 476.
[90] Veech, R. L., Chance, B., Kashiwaya, Y., Lardy, H. A., and Cahill, G. F. Jr. (2001) Ketone bodies, potential therapeutic uses. IUBMB Life 51, 241–247.
[91] Cahill, G. F., Jr. and Veech, R. L. (2003) Ketoacids? Good medicine? Trans. Am. Clin. Climatol. Assoc. 114, 149–161. discussion 162 – 163, 149 – 161.
[92] Kashiwaya, Y., Pawlosky, R., Markis, W., King, M. T., Bergman, C., et al. (2010) A ketone ester diet increased brain malonyl CoA and uncoupling protein 4 and 5 while decreasing food intake in the normal Wistar rat. J. Biol. Chem. 285, 25950–25956.
[93] Veech, R. L. (2013) Ketone esters increase brown fat in mice and overcome insulin resistance in other tissues in the rat. Ann. NY Acad Sci. 1302, 42–48.