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Review Article

Extending life span by increasing oxidative stress

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abstract

Various nutritional, behavioral, and pharmacological interventions have been previously shown to extend life span in diverse model organisms, including *Saccharomyces cerevisiae, Caenorhabditis elegans, Drosophila melanogaster*, mice, and rats, as well as possibly monkeys and humans. This review aims to summarize published evidence that several longevity-promoting interventions may converge by causing an activation of mitochondrial oxygen consumption to promote increased formation of reactive oxygen species (ROS). These serve as molecular signals to exert downstream effects to ultimately induce endogenous defense mechanisms culminating in increased stress resistance and longevity, an adaptive response more specifically named mitochondrial hormesis or mitohormesis. Consistently, we here summarize findings that antioxidant supplements that prevent these ROS signals interfere with the health-promoting and life-span-extending capabilities of calorie restriction and physical exercise. Taken together and consistent with ample published evidence, the findings summarized here question Harman's Free Radical Theory of Aging and rather suggest that ROS act as essential signaling molecules to promote metabolic health and longevity.

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Calorie restriction

Calorie restriction (CR), i.e., a reduction in ad libitum calorie up- take by 10 to 50%, represents the most convincing intervention to retard aging and attenuate age-related disease in multiple species. Since 1935, when McCay initially described the influence of CR on life expectancy, it has been frequently demonstrated that CR is able to increase the median and maximal life span in a variety of organisms, suggesting a conserved underlying mechanism [1,2].

Although CR clearly reduces risk factors associated with aging in humans, including type 2 diabetes and cardiovascular diseases, it is still a matter of debate whether CR is capable of increasing life expectancy of humans [3–5]. A recent study in nonhuman primates found no significant effect of CR on overall mortality. However, arbitrarily defined "age-related mortality" (which moreover explained only 54% of deaths) was decreased in those monkeys. Most interestingly and contrasting with ad libitum-fed animals, monkeys on CR did not show any impairment in glucose homeostasis, strikingly reducing the prevalence of metabolic disorders such as type 2 diabetes [6]. Thus, it seems possible that CR is also sufficient to improve the life span of humans, which is also supported by additional findings [3–5,7,8].

The concept of CR was initially based on the assumption that lowering caloric intake would result in a subsequent reduction of the

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metabolic rate. Hence, it was postulated at the beginning of the 20th century that the maximum life span of an organism is inversely proportional to the nutritive energy metabolized [9]. Consequently, Pearl's Rate-of-Living Hypothesis, formulated soon after, suggests that increased metabolic rate results in decreased life span in eukaryotes [10].

A feasible molecular cause for this hypothesis was proposed in 1956 by Harman, who connected metabolic activity, especially that of respiratory enzymes, with the formation of potentially harmful reactive oxygen species (ROS) [11]. Accordingly, increased metabolic rate would promote ROS formation, which subsequently causes damages within the cell and beyond. The accumulation of these damages results in age-related decline of cellular functions and ultimately to death of the organism [11]. Up to now, this so-called Free Radical Theory of Aging (FRTA) has become a popular and frequently cited theory in aging research [12].

However, more recent findings regarding the question whether CR actually decreases metabolic rate are, at least in part, inconsistent with FRTA. Hence, it has been reported that CR increases metabolic rate (quantified by both oxygen consumption and heat production) in the nematode and well-established model organism for aging research, *Caenorhabditis elegans* [13]. Furthermore, a positive correlation between low metabolic rate and enhanced life span could also not be observed in the fruitfly *Drosophila melanogaster* [14].

Despite the fact that CR has been extensively investigated in a broad range of species, the underlying mechanisms are still elusive. As mentioned above, it is commonly accepted that CR is able to retard the onset of a variety of diseases related to aging, including cardiovascular diseases, type 2 diabetes, and cancer. Therefore, CR-mediated prevention of chronic and ultimately life-threatening disorders that reduce longevity could be the reason for the life-span-extending effects of CR. Additionally, it has been shown that CR itself stimulates molecular processes that diminish age-associated disease as well as improving life expectancy. Accordingly, it was frequently reported that CR induces defense mechanisms, especially those that are involved in ROS detoxification such as radical-scavenging enzymes [15–22] and possibly beyond, including phase II response enzymes. This association of CR on the one hand and increased antioxidant defense on the other has been commonly misinterpreted as being caused by a primarily decreased ROS production in states of CR. Conversely, and as explained in more detail below, more recent investigations suggest that adaptive response mechanisms seem to be the cause of the aforementioned beneficial alterations unquestionably initiated by CR [23–27].

Reduction of specific macronutrients

Macronutrients are represented by carbohydrates, triglycerides, and proteins, which, after experiencing enzymatic breakdown, are ultimately metabolized as monosaccharides (such as glucose), fatty acids, and amino acids, respectively. They provide the bulk of energy required by the organism. In this regard it should be noted, however, that only glucose can be metabolized in the absence of oxygen. In contrast, ATP generation using fatty acids and some amino acids requires mitochondrial oxidative phosphorylation (OxPhos) and therefore oxygen. Inversely, only metabolism of glucose can generate ATP independent of mitochondrial organelles and hence without promoting ROS production.

So far, only a few studies have investigated the question whether restricting a single macronutrient can cause a response comparable to that seen in states of general CR. Whereas restriction of triglyceride uptake in invertebrates has not been examined yet, restriction of lipids in mice without CR does not influence life span [28].

The influence of dietary protein levels on life span has been investigated primarily in *D. melanogaster* and rodents. Accordingly, it was shown that reduction of nutritive protein content results in extension of life expectancy in mice [29–31]. Similarly, casein restriction prolongs life span in *D. melanogaster* [32]. On the other hand, supplementation of essential amino acids, especially methionine, abolishes the life-span-extending effect of CR in flies [33]. Interestingly, methionine restriction in rodents has been shown to exert antiaging properties and improves tissue-specific mitochondrial biogenesis as well as aerobic capacity [34–36], whereas high protein intake results in increased lipid peroxidation and reduced superoxide dismutase activity [37]. Consistently, impaired peptide transport extents life span in *C. elegans* [38].

In apparent contrast to the above-mentioned fact that ATP generation from glucose is capable of avoiding ROS production, glucose restriction has been found to be beneficial in various lower organisms as well as in rodents. In *D. melanogaster*, for instance, restriction of sugar reduces mortality and extends life span [39]. The same applies for the model organism *Saccharomyces cerevisiae*, in which depletion of glucose results in life-span extension dependent on induction of respiration as well as on sirtuins [40,41]. However, whether sirtuins are involved is still a matter of debate [42–45]. Accordingly, sirtuin-independent pathways have been discussed [22,46].

Although it is generally difficult to restrict dietary glucose in eukaryotic organisms such as *C. elegans* or rodents, the use of 2-deoxyglucose (DOG) is frequently reported to achieve depletion of glucose metabolism [47]. DOG is a synthetic glucose analogue that inhibits glycolysis in a competitive manner due to its inability to be further metabolized after conversion into deoxyglucose 6-phosphate [48]. Application of DOG was shown to mimic a ketogenic diet (very low carbohydrate diet) as well as metabolic hallmarks of CR in rodents [49–51]. It is therefore commonly accepted that DOG represent a powerful CR-mimetic compound [52–55].

DOG exposure results in decreased glucose availability and life-span extension in *C. elegans* [23], whereas it does not extend life span in rats [56]. Notably, and similar to the above-mentioned findings in *S. cerevisiae*, glucose restriction in *C. elegans* not only promotes life span but also increases oxygen consumption [23]. However, and in contrast to yeast, in nematodes sirtuins seem not to be involved [23]. It was suggested instead that the underlying mechanism in regard to life-span prolongation is dependent on AMP-activated kinase (AMPK) [23]. AMPK is assumed to be a central key regulator of energy metabolism within the cell [57]. Functionally similar AMPK orthologues have been found in lower organisms such as worms and flies, suggesting a highly conserved mechanism [58–60]. Metabolic stress, e.g., cellular lack of energy, activates AMPK, which in turn up-regulates energy-producing processes such as mitochondrial biogenesis leading to neutralization of the energy deficit, possibly with additional health-promoting implications [57]. Consistently, applying metformin, a long-standing antidiabetic drug, to *C. elegans* activates AMPK and subsequently promotes adaptive processes involved in CR and oxidative stress response, culminating in extended life span [61]. As an alternative approach to influencing intracellular glucose concentrations in mammals, mice with impaired GLUT-4 transporters in muscle and adipose tissue were established. These mice show typical metabolic switches such as fasting hyperglycemia, glucose intolerance, increased fatty acid turnover, and utilization. However, life span (examined up to 18 months of age) was not affected [62]. Increased cellular glucose availability due to overexpression of GLUT-4, on the other hand, was also shown to lack any effect regarding extension of life span [63]. In addition, increased glucose abundance in *C. elegans*, examined in three independent studies, reduces life span significantly [23,64,65].

In humans, varying the relative amounts of macronutrients within diets has been postulated to be health beneficial in regard to obesity and cardiovascular disease prevention. Although low-carbohydrate/high-protein diets are as efficient as low-fat/high-carbohydrate diets in regard to weight loss, serum parameters known to determine

cardiovascular risk were shown to be positively influenced by a reduction in dietary carbohydrate consumption [66–68]. Very low carbohydrate diet has been also demonstrated to reduce several inflammation markers in overweight men and women with athero- genic dyslipidemia [69]. However, more research, especially long- term studies, is needed to evaluate the putative effect of low-carbo- hydrate diets on human health.

Impaired insulin/IGF-1 signaling

In mammals, insulin and IGF-1 represent peptide hormones produced in pancreatic β-cells and liver, respectively. Insulin is a regulator of the peripheral glucose metabolism, most notably glucose uptake. In addition, insulin is also involved in other metabolic processes such as fat metabolism. IGF-1 is produced as a consequence of growth hormone (GH) (also called somatotropin) release from the pituitary gland, which stimulates subsequently IGF-1 production in the liver. IGF-1 is therefore a mediator for some of the GH functions, thus involved in growth and anabolism. Insulin, IGF-1, and GH mediate their effects by binding at specific and distinct receptors in mammals.

Mice with reduced GH and/or IGF-1 signaling exhibit dwarfism with a phenotype that is comparable to those of mice exposed to CR [70]. As shown for CR, those mice are also long-lived [71]. Conversely, increasing GH availability leads to improved body size and diminishes life expectancy [72,73]. Furthermore, heterozygote impairment of the IGF-1 receptor signaling in the entire animal, as well as impairment of the IGF-1 receptor in neurons, results in life-span extension in mice by preventing neurodegenerative processes [74,75]. Conversely, long- term IGF-1 exposure leads to mitochondrial dysfunction and reduced cell viability in human cell culture [76].

Down-regulation of insulin receptor activity in humans is assumed to be a cause for insulin resistance. This state is defined as an inappropriate reduction in the intracellular response to extracellular insulin [77]. Consequently, a reduction in GLUT-4-mediated glucose uptake, which represents a key insulin response, occurs. Therefore, intracel- lular glucose availability is reduced in subjects suffering from insulin resistance [78].

However, despite the fact that global disturbance of the insulin receptor in mice results in a prenatally lethal phenotype, muscle- specific knockout mice experience neither hyperglycemia nor diabetes. Instead, a remarkable rise in fatty acid turnover has been observed [79]. Although life-span data on these mice are unavailable, disruption of the insulin receptor in adipose tissue only causes prolongation of life span [80]. Moreover, disruption of the insulin receptor substrate 1 (IRS-1), which is localized downstream of both the insulin and the IGF-1 receptors, is associated with murine lon- gevity as well as knockouts of neuronal IRS-2 and heterozygous global IRS-2 [81,82].

Moreover, and as initially published more than 20 years ago, impaired insulin/IGF-1 signaling strikingly prevents aging in inverte- brates. Whereas in mammals insulin and IGF-1 bind to specific and distinct receptors, in *C. elegans* and *D. melanogaster* insulin and IGF-1 signaling is limited to one receptor. Hence, mutations in the corre- sponding receptor orthologues as well as in downstream components were shown to be life-span extending in worms and flies in a manner even more pronounced than in mammals [83–87]. *C. elegans* daf-2 mutants, which show impaired activity of the orthologue of the mammalian insulin/IGF-1 receptor, live twice as long as wild-type nematodes [84]. Although it is not known whether glucose uptake or intracellular glucose availability is affected in this regard, a very recent work on daf-2 mutants indicates that the age-associated decline in mitochondrial activity, e.g., mitochondrial protein content and energy supply, is delayed in comparison to wild-type animals [88].

In summary, it seems that reduction of the insulin receptor as well as insulin receptor substrate below a certain threshold contributes to longevity in a variety of organisms, including worms, flies, and mice. This may be also relevant to humans because mutations of insulin/ IGF-1 signaling have been linked to regulation of life expectancy in various cohorts [89,90].

Whether reduced insulin/IGF-1/GH signaling lengthens life span in the same manner as CR is an ongoing matter of debate. Although several studies have demonstrated independent mechanisms, others have proposed that similar pathways and processes are initiated by both interventions [59,91–101]. Based on the assumption that mutations associated with impaired insulin/IGF-1 signaling cause reduced intracellular glucose availability, it seems likely that subsequent effects are comparable to those seen in glucose-restricted model organisms, at least in regard to metabolic shifts and also possibly life-span-extending mechanisms. Although to date direct evidence is missing, some studies provide support for this hypothesis [102–106].

Induction of mitochondrial metabolism by calorie/glucose restriction

In general, mitochondria are cellular organelles that provide the bulk of energy within the cell. ATP generation due to mitochondrial OxPhos is considerably more efficient in comparison to nonoxidative metabolism of glucose and some amino acids. Whereas glycolytic breakdown of 1 mol of glucose generates 4 mol of ATP, its oxidative metabolism produces 30 mol of ATP. Mitochondria also produce ROS as a by-product of OxPhos. Thus, being the main producer of cellular energy as well as a source of potentially harmful ROS, mitochondria appear to exert a central role in physiological and pathophysiological processes.

Accordingly, mitochondrial dysfunction is associated with the onset of age-related diseases such as diabetes, cancer, and neurode- generation [107–110]. Furthermore, impairment of mitochondrial activity is assumed to be a main cause of the aging process [111,112]. Whether this decrease in mitochondrial capacity is linked to altered production of mitochondrial ROS seems questionable.

Although a few studies suggested that overall net calorie uptake during the lifetime is unaltered in CR [39,113], it is commonly accepted and agreed upon that by definition calorie/glucose restric- tion causes a reduction in available nutritive energy. This short-term energy deficit has been proposed to induce mitochondrial activity to counteract the energy depletion. Accordingly, calorie/glucose restric- tion causes an increase in mitochondrial respiration in yeast and worms [23–25,40]. Enhanced mitochondrial activity is, as shown in these studies, associated with life-span extension [23–25,40]. Fur- thermore, CR promotes mitochondria biogenesis and OxPhos in rodents as well as enhancement of respiratory capacity in mammalian cells [114,115]. These results are in line with the observation that energy expenditure as a function of body mass is unexpectedly increased in calorie-restricted rats [116]. Moreover, as mentioned before, reduced insulin/IGF-1/GH signaling stimulates mitochondria metabolism in rodents [102,104–106]. In addition, an abundant supply of branched-chain amino acids increases mitochondrial biogenesis and promotes longevity in yeast and mice [117,118]. Finally, further interventions that induce mitochondrial activity, such as pharmacological treatments and physical exercise, are capable of improving life span [119–123].

In contrast, and as mentioned before, reduced mitochondrial activity has been shown to decrease life span in various organisms such as *S. cerevisiae, C. elegans*, and rodents [124–126].

In regard to proposed mechanisms involved in the activation of mitochondrial metabolism some key cellular regulators have been frequently reported, including the previously mentioned sirtuins and AMPK. Activation of these proteins is associated with increased mitochondria activity. In contrast, impairment of another nutrient-sensing pathway, mTOR (mammalian target of rapamycin), was

shown to extend life span in *S. cerevisiae* by inducing mitochondrial metabolism [127,128]. Consistently, the translational inhibitor 4E-BP, which is repressed by TOR, regulates mitochondrial activity in CR flies [129]. Furthermore, TOR signaling has been shown to be regulated by AMPK, suggesting that both nutrient-sensing pathways are located upstream of mitochondria function, thereby representing key regulators of mitochondrial metabolism [130].

Taken together, there are numerous studies linking mitochondrial activity with prolongation of life expectancy, suggesting that a metabolic switch to oxidative metabolism seems to be beneficial in regard to delay aging and the onset of age-related diseases.

Oxidative stress and mitochondrial hormesis (mitohormesis)

Increased ROS formation as a consequence of increased metabolic rate has been postulated to be the major determinant of life span [11]. Because mitochondria are an intracellular source of ROS, the theory was extended to the mitochondrial free radical theory [131], without the knowledge that meanwhile the fact that increased metabolic rate does not necessarily result in increased ROS formation had been established. Thus, significant research has been done to prove this hypothesis with inconsistent and partly contradictive results [132]. However, a considerable number of findings in various organisms suggest that reduction of oxidative stress is associated with prolongation of life expectancy [133–147]. Consequently, ROS-lowering interventions were widely proposed as an antiaging strategy in humans. Antioxidants, a group of synthetic or naturally occurring substances, which are capable of scavenging free radicals, were extensively examined in that regard. Unexpectedly and in contrast to some of the above-mentioned work in lower organisms, several prospective clinical intervention studies were unable to show a positive association between supplementation with antioxidants and health-beneficial effects. Whereas most studies found a lack of effect in regards to health promotion in humans [148–162], other reports even suggest that antioxidants may promote cancer growth [163–168]. Moreover, supplementation with antioxidants has been linked to increased incidence of a number of diseases with adverse effects on human longevity [169–175].

Not surprisingly, these findings question Harman's FRTA and require a different point of view concerning the role of mitochondrial ROS formation. Accordingly, numerous findings have emerged in recent years indicating that ROS may evoke cellular signaling that promotes metabolic health and longevity. It has been assumed that they serve as essential signaling molecules delivering messages from the mitochondria to other cellular compartments in response to physiological or pathophysiological changes [23,176–190]. Moreover, and given the increased levels of oxidative damage during increasing age, intrinsic aging may be considered an insufficient ability to respond to endogenous ROS signals.

Interestingly, exposure of *C. elegans* to hyperbaric conditions results in stress resistance and prolongation of life expectancy, whereas such conditions cause an increase in mitochondrial ROS formation [191–194]. Hypothermia, a state that is associated with extend life span in mice and *C. elegans* [195,196], has been recently shown to induce mitochondrial ROS production as well [197]. Moreover, it was shown that CR also induces low-level stress leading to the same adaptive processes, such as increased stress resistance and longevity [21,26,198–200].

These findings insinuate that so-called adaptive response processes may explain how increased ROS formation culminates in promotion of health and life span. Interestingly, low doses of ROS seem to exert such effects, whereas higher doses are unquestionably detrimental. Such biphasic responses to a potentially harmful compound are commonly named hormesis, a concept that was initially postulated in 1943 by Southam and Ehrlich and which was shown to have significant impact on aging with a variety of stressors described [201–205]. Later, this term was extended to mitochondrial hormesis or mitohormesis, with regard to mitochondrial ROS as a hypothetically sublethal stressor [206].

In agreement with this concept, it has been frequently reported that rodents exposed to CR exhibit elevated antioxidant defense capacities [15–20,207]. Furthermore, life-extending glucose restriction in yeast was shown to be accompanied by a decrease in ROS production, whereas respiration was enhanced [22]. On the other hand and in conflict with these data, it was also reported that the same intervention in the same model organism increases ROS production as well as respiration [23–25,43,208,209]. Moreover, antioxidant enzyme activity was found to be elevated as well [24,43,208,209], suggesting a potential involvement of increased respiration, enhanced ROS formation, and the induction of ROS defense mechanisms in regard to regulation of longevity.

Consistently, numerous studies using various model organisms were unable to find any evidence to support that lowering ROS is beneficial in regard to longevity, nor that increasing antioxidant capacity extends life span [210–227]. Moreover, life-span-extending mutations in *C. elegans* are commonly accompanied by increased stress resistance and sometimes paralleled by enhanced metabolic activity [228–233]. Furthermore, in the field of neuroprotective research, similar hormetic results were achieved with CR as well as DOG application in rodents [234]. Depletion of mitochondrial NADH kinase, an enzyme crucial for antioxidant defense, causes life-span extension and DNA stability due to adaptive mechanisms in *Podospora anserine* [235]. Finally, human subjects on a carbohydrate-depleted diet (i.e., a ketogenic diet) show improved ROS defense capacity presumably due to elevated oxidative metabolism [236].

Taken together, all these findings provide indirect evidences for the hypothesis that ROS production and subsequent induction of ROS defense are essential contributors to longevity. To prove this hypothesis, the previously described inhibitor of glycolysis, DOG, was applied to *C. elegans*, resulting in a decrease in glucose availability followed by a compensatory increase in respiration [23]. The increase in oxygen consumption was associated with an increase in ROS formation and a consequent induction of antioxidant enzyme activity, finally leading to life-span extension [23]. Most importantly, simultaneous treatment with various antioxidants completely abolished this life-span-extending effect of DOG, suggesting that an increase in ROS formation is essential for CR-induced promotion of longevity [23]. These findings were corroborated by very recent studies that examine the effect of CR in *S. cerevisiae* and *Schizosaccharomyces pombe* [24,25,27]. Correspondingly, an increased mitochondrial respiration and/or a subsequent enhanced ROS production after CR were observed [24,25,27]. Hence, similar to the above-mentioned observations in *C. elegans*, activation of stress response pathways as well as induction of defense mechanisms has been discussed as representing the underlying life-span-extending mechanisms [24,25,27,188–190]. It should be noted that endogenously produced ROS presumably not only induce ROS defense enzymes, but also increase activities of phase II response enzymes that protect from damage beyond ROS. On a hypothetical basis this would explain the clearly opposite effects of supplementation with exogenous antioxidants and/or genetic overexpression of antioxidant enzymes, on the one hand, and endogenous response to endogenous ROS production on the other hand. Future research will also have to investigate whether response mechanisms to stressors such as endogenous ROS may be less likely to be activated at higher age.

Physical exercise

Consistent with the concept of mitohormesis, glucose restriction leads to an increase in mitochondrial activity accompanied by an increase in respiration-derived ROS formation that serves as a mild stressor (Fig. 1). This ROS signal is able to induce conserved downstream processes (such as activation of specific oxidative stress-

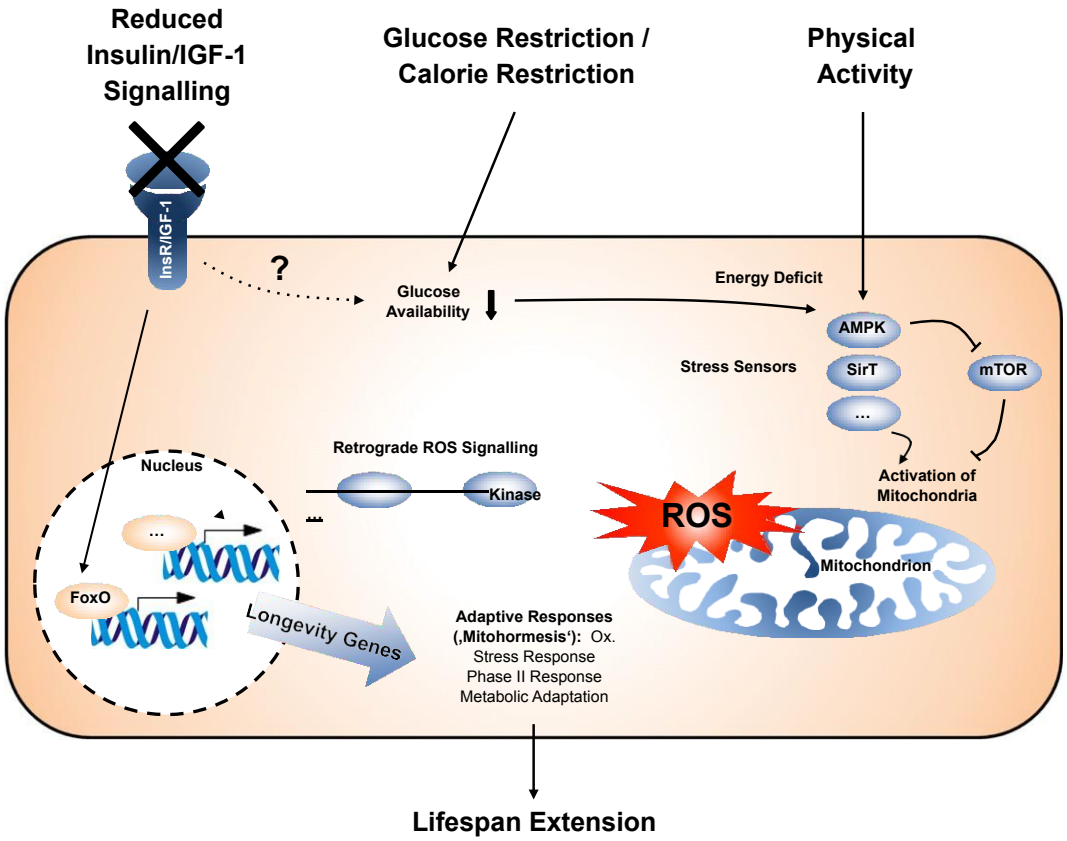

Fig. 1. Life-span-extending interventions generate mitochondrial ROS signals to activate longevity-promoting genes. For calorie and especially glucose restriction, but also for physical exercise, evidence exists that these interventions extend life span in various model organisms, but also increase mitochondrial metabolism. This activation promotes formation of mitochondrial ROS signals that cause an adaptive response (mitohormesis) in the nucleus to promote longevity. The possible link to impaired insulin/IGF-1 signaling, however, remains to be experimentally shown.

sensitive MAP-kinase cascades and redox-sensitive transcription factors) that culminate in an overall adaptive response, represented by an improvement in antioxidant capacity and finally longevity. Cotreatment with antioxidants inhibits ROS signal transduction and prevents the adaptive response. Thus, glucose-restriction-mediated longevity is abolished.

Therefore, interventions that induce mitochondrial function seem to be promising in regard to regulation of life expectancy. Accordingly, moderate physical activity, an intervention that is known to be health beneficial in a broad spectrum [120,121,237–239], is assumed to cause induction of mitochondrial metabolism and ROS production [240–242]. Moreover, health-promoting effects were demonstrated to be reduced if subjects exposed to physical activity were cotreated with antioxidant supplements [186,243].

Conclusions

Taken together, the data summarized and discussed in this review support the conclusion that CR, glucose restriction, and moderate physical activity share, at least in part, common mechanistic features that may influence the aging process, i.e., enhanced mitochondrial activity and subsequently increased ROS formation that ultimately induce an adaptive response (increased defense mechanisms and improved stress resistance), which culminates in metabolic health and extended longevity.

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