



Review Article

Extending life span by increasing oxidative stress

Michael Ristow ^{a, b, *}, Sebastian Schmeisser ^a^a Department of Human Nutrition, Institute of Nutrition, University of Jena, D-07743 Jena, Germany^b Department of Clinical Nutrition, German Institute of Human Nutrition Potsdam-Rehbrücke, D-14558 Nuthetal, Germany

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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

* Corresponding author at: Department of Human Nutrition, Institute of Nutrition, University of Jena, D-07743 Jena, Germany.

E-mail address: mr@mrlistow.org (M. Ristow).

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 extends 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 atherogenic dyslipidemia [69]. However, more research, especially long-term studies, is needed to evaluate the putative effect of low-carbohydrate 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, intracellular 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 longevity 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 invertebrates. 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 corresponding 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 neurodegeneration [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 restriction 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 restriction 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]. Furthermore, 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 mitochondrial 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 anserina* [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-

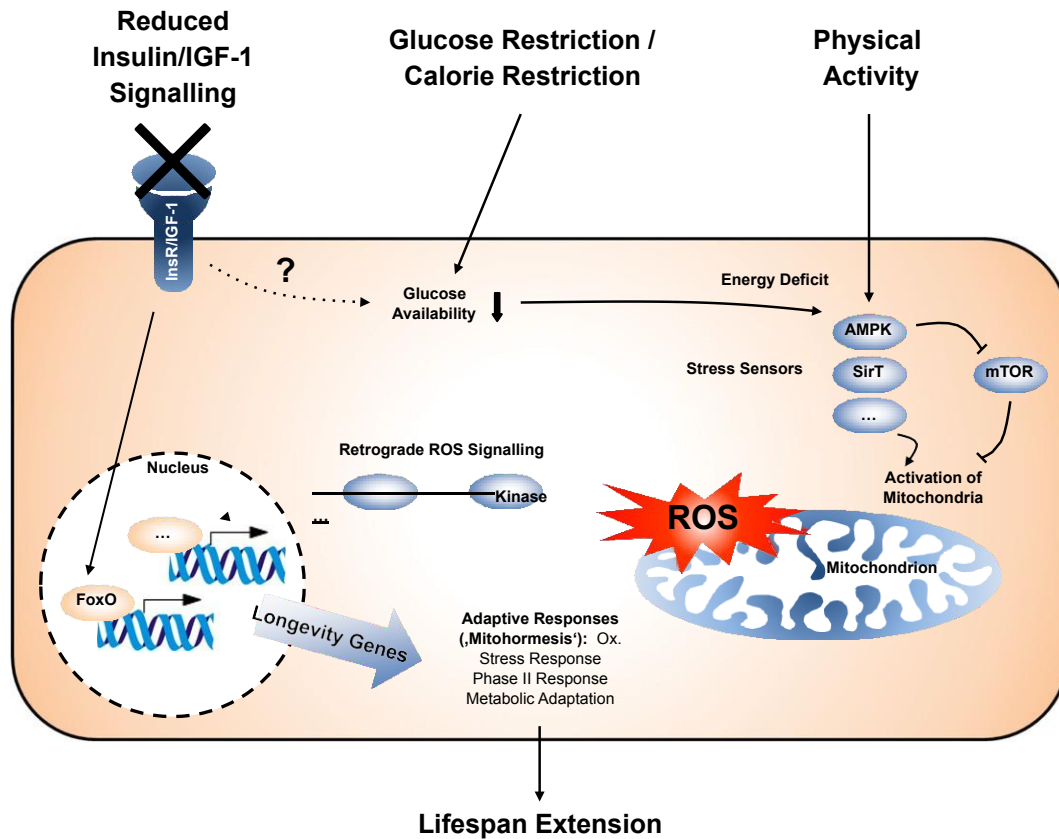


Fig. 1. Life-span-extending interventions generate mitochondrial ROS signals to activate longevity-promoting genes. For calorie and especially glucose restriction, but also for physical exercise, evidence exists that these interventions extend life span in various model organisms, but also increase mitochondrial 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].

Conclusion

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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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References

- McCay, C. M.; Crowell, M. F.; Maynard, L. A. The effect of retarded growth upon the length of the life span and upon ultimate body size. *J. Nutr.* 10:63–79; 1935.
- Fontana, L.; Partridge, L.; Longo, V. D. Extending healthy life span—from yeast to humans. *Science* 328:321–326; 2010.
- Fontana, L.; Meyer, T. E.; Klein, S.; Holloszy, J. O. Long-term calorie restriction is highly effective in reducing the risk for atherosclerosis in humans. *Proc. Natl. Acad. Sci. U. S. A.* 101:6659–6663; 2004.
- Heilbronn, L. K.; de Jonge, L.; Frisard, M. I.; DeLany, J. P.; Larson-Meyer, D. E.; Rood, J.; Nguyen, T.; Martin, C. K.; Volaufova, J.; Most, M. M.; Greenway, F. L.; Smith, S. R.; Deutsch, W. A.; Williamson, D. A.; Ravussin, E. Effect of 6-month calorie restriction on biomarkers of longevity, metabolic adaptation, and oxidative stress in overweight individuals: a randomized controlled trial. *JAMA* 295: 1539–1548; 2006.
- Fontana, L.; Klein, S. Aging, adiposity, and calorie restriction. *JAMA* 297:986–994; 2007.
- Colman, R. J.; Anderson, R. M.; Johnson, S. C.; Kastman, E. K.; Kosmatka, K. J.; Beasley, T. M.; Allison, D. B.; Cruzen, C.; Simmons, H. A.; Kemnitz, J. W.; Weindruch, R. Caloric restriction delays disease onset and mortality in rhesus monkeys. *Science* 325: 201–204; 2009.
- Ingram, D. K.; Roth, G. S.; Lane, M. A.; Ottinger, M. A.; Zou, S.; de Cabo, R.; Mattison, J. A. The potential for dietary restriction to increase longevity in humans: extrapolation from monkey studies. *Biogerontology* 7:143–148; 2006.
- Weindruch, R. Will dietary restriction work in primates? *Biogerontology* 7: 169–171; 2006.
- Rubner, M. III. Das Wachstumsproblem und die Lebensdauer des Menschen und einiger Säugetiere vom energetischen Standpunkt aus betrachtet. In: Rubner, M. (Ed.), Das Problem der Lebensdauer und seine Beziehungen zum Wachstum und der Ernährung. Oldenbourg, Munich/Berlin, pp. 127–208; 1908.
- Pearl, R. The Rate of Living: being an account of some experimental studies on the biology of life duration. Knopf, New York; 1928.

- 11 Harman, D. Aging: a theory based on free radical and radiation chemistry. *J. Gerontol.* 11:298–300; 1956.
- 12 Harman, D. Origin and evolution of the free radical theory of aging: a brief personal history, 1954–2009. *Biogerontology* 10:773–781; 2009.
- 13 Houthoofd, K.; Braeckman, B. P.; Lenaerts, I.; Brys, K.; De Vreese, A.; Van Eygen, S.; Vanfleteren, J. R. Axenic growth up-regulates mass-specific metabolic rate, stress resistance, and extends life span in *Caenorhabditis elegans*. *Exp. Gerontol.* 37:1371–1378; 2002.
- 14 Hulbert, A. J.; Clancy, D. J.; Mair, W.; Braeckman, B. P.; Gems, D.; Partridge, L. Metabolic rate is not reduced by dietary-restriction or by lowered insulin/IGF-1 signalling and is not correlated with individual lifespan in *Drosophila melanogaster*. *Exp. Gerontol.* 39:1137–1143; 2004.
- 15 Koizumi, A.; Weindruch, R.; Walford, R. L. Influences of dietary restriction and age on liver enzyme activities and lipid peroxidation in mice. *J. Nutr.* 117: 361–367; 1987.
- 16 Semsei, I.; Rao, G.; Richardson, A. Changes in the expression of superoxide dismutase and catalase as a function of age and dietary restriction. *Biochem. Biophys. Res. Commun.* 164:620–625; 1989.
- 17 Rao, G.; Xia, E.; Nadakavukaren, M. J.; Richardson, A. Effect of dietary restriction on the age-dependent changes in the expression of antioxidant enzymes in rat liver. *J. Nutr.* 120:602–609; 1990.
- 18 Pieri, C.; Falasca, M.; Marcheselli, F.; Moroni, F.; Recchioni, R.; Marmocchi, F.; Lupidi, G. Food restriction in female Wistar rats. V. Lipid peroxidation and antioxidant enzymes in the liver. *Arch. Gerontol. Geriatr.* 14:93–99; 1992.
- 19 Youngman, L. D.; Park, J. Y.; Ames, B. N. Protein oxidation associated with aging is reduced by dietary restriction of protein or calories. *Proc. Natl Acad. Sci. U. S. A.* 89:9112–9116; 1992.
- 20 Xia, E.; Rao, G.; Van Remmen, H.; Heydari, A. R.; Richardson, A. Activities of antioxidant enzymes in various tissues of male Fischer 344 rats are altered by food restriction. *J. Nutr.* 125:195–201; 1995.
- 21 Masoro, E. J. Hormesis and the antiaging action of dietary restriction. *Exp. Gerontol.* 33:61–66; 1998.
- 22 Barros, M. H.; Bandy, B.; Tahara, E. B.; Kowaltowski, A. J. Higher respiratory activity decreases mitochondrial reactive oxygen release and increases life span in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 279:49883–49888; 2004.
- 23 Schulz, T. J.; Zarse, K.; Voigt, A.; Urban, N.; Birringer, M.; Ristow, M. Glucose restriction extends *Caenorhabditis elegans* life span by inducing mitochondrial respiration and increasing oxidative stress. *Cell Metab.* 6:280–293; 2007.
- 24 Sharma, P. K.; Agrawal, V.; Roy, N. Mitochondria-mediated hormetic response in life span extension of calorie-restricted *Saccharomyces cerevisiae*. *Age* 33: 143–154; 2010.
- 25 Zuin, A.; Carmona, M.; Morales-Ivorra, I.; Gabrielli, N.; Vivancos, A. P.; Ayte, J.; Hidalgo, E. Lifespan extension by calorie restriction relies on the Sty1 MAP kinase stress pathway. *EMBO J.* 29:981–991; 2010.
- 26 Rattan, S. I.; Demirovic, D. Hormesis as a mechanism for the anti-aging effects of calorie restriction. In: Everitt, A.V., Rattan, S.I.S., Couteur, D.G., Cabo, R.D. (Eds.), *Calorie Restriction, Aging and Longevity*. Springer Netherlands, Dordrecht, pp. 233–245; 2010.
- 27 Mesquita, A.; Weinberger, M.; Silva, A.; Sampaio-Marques, B.; Almeida, B.; Leao, C.; Costa, V.; Rodrigues, F.; Burhans, W. C.; Ludovico, P. Caloric restriction or catalase inactivation extends yeast chronological lifespan by inducing H₂O₂ and superoxide dismutase activity. *Proc. Natl Acad. Sci. U. S. A.* 107:15123–15128; 2010.
- 28 Iwasaki, K.; Gleiser, C. A.; Masoro, E. J.; McMahan, C. A.; Seo, E. J.; Yu, B. P. Influence of the restriction of individual dietary components on longevity and age-related disease of Fischer rats: the fat component and the mineral component. *J. Gerontol.* 43:B13–B21; 1988.
- 29 Stoltzner, G. Effects of life-long dietary protein restriction on mortality, growth, organ weights, blood counts, liver aldolase and kidney catalase in Balb/C mice. *Growth* 41:337–348; 1977.
- 30 Leto, S.; Kokkonen, G. C.; Barrows Jr., C. H. Dietary protein, life-span, and biochemical variables in female mice. *J. Gerontol.* 31:144–148; 1976.
- 31 Fernandes, G.; Yunis, E. J.; Good, R. A. Influence of diet on survival of mice. *Proc. Natl Acad. Sci. U. S. A.* 73:1279–1283; 1976.
- 32 Min, K. J.; Tatar, M. Restriction of amino acids extends lifespan in *Drosophila melanogaster*. *Mech. Ageing Dev.* 127:643–646; 2006.
- 33 Grandison, R. C.; Piper, M. D.; Partridge, L. Amino-acid imbalance explains extension of lifespan by dietary restriction in *Drosophila*. *Nature* 462: 1061–1064; 2009.
- 34 Zimmerman, J. A.; Malloy, V.; Krajcik, R.; Orentreich, N. Nutritional control of aging. *Exp. Gerontol.* 38:47–52; 2003.
- 35 Miller, R. A.; Buehner, G.; Chang, Y.; Harper, J. M.; Sigler, R.; Smith-Wheelock, M. Methionine-deficient diet extends mouse lifespan, slows immune and lens aging, alters glucose, T4, IGF-I and insulin levels, and increases hepatocyte MIF levels and stress resistance. *Ageing Cell* 4:119–125; 2005.
- 36 Perrone, C. E.; Mattocks, D. A.; Jarvis-Morar, M.; Plummer, J. D.; Orentreich, N. Methionine restriction effects on mitochondrial biogenesis and aerobic capacity in white adipose tissue, liver, and skeletal muscle of F344 rats. *Metabolism* 59: 1000–1011; 2010.
- 37 De, A. K.; Chipalkatti, S.; Aiyar, A. S. Some biochemical parameters of ageing in relation to dietary protein. *Mech. Ageing Dev.* 21:37–48; 1983.
- 38 Meissner, B.; Boll, M.; Daniel, H.; Baumeister, R. Deletion of the intestinal peptide transporter affects insulin and TOR signaling in *Caenorhabditis elegans*. *J. Biol. Chem.* 279:36739–36745; 2004.
- 39 Mair, W.; Piper, M. D.; Partridge, L. Calories do not explain extension of life span by dietary restriction in *Drosophila*. *PLoS Biol.* 3:e223; 2005.
- 40 Lin, S. J.; Kaeblerlein, M.; Andalis, A. A.; Sturtz, L. A.; Defossez, P. A.; Culotta, V. C.; Fink, G. R.; Guarente, L. Calorie restriction extends *Saccharomyces cerevisiae* lifespan by increasing respiration. *Nature* 418:344–348; 2002.
- 41 Lin, S. J.; Defossez, P. A.; Guarente, L. Requirement of NAD and SIR2 for life-span extension by calorie restriction in *Saccharomyces cerevisiae*. *Science* 289: 2126–2128; 2000.
- 42 Kaeblerlein, M.; Kirkland, K. T.; Fields, S.; Kennedy, B. K. Sir2-independent life span extension by calorie restriction in yeast. *PLoS Biol.* 2:E296; 2004.
- 43 Agarwal, S.; Sharma, S.; Agrawal, V.; Roy, N. Caloric restriction augments ROS defense in *S. cerevisiae* by a Sir2p independent mechanism. *Free Radic. Res.* 39: 55–62; 2005.
- 44 Guarente, L.; Picard, F. Calorie restriction—the SIR2 connection. *Cell* 120: 473–482; 2005.
- 45 Smith Jr., D. L.; McClure, J. M.; Matecic, M.; Smith, J. S. Calorie restriction extends the chronological lifespan of *Saccharomyces cerevisiae* independently of the sirtuins. *Ageing Cell* 6:649–662; 2007.
- 46 Roux, A. E.; Leroux, A.; Alaamery, M. A.; Hoffman, C. S.; Chartrand, P.; Ferbeyre, G.; Rokeach, L. A. Pro-aging effects of glucose signaling through a G protein-coupled glucose receptor in fission yeast. *PLoS Genet.* 5:e1000408; 2009.
- 47 Wick, A. N.; Drury, D. R.; Nakada, H. I.; Wolfe, J. B. Localization of the primary metabolic block produced by 2-deoxyglucose. *J. Biol. Chem.* 224:963–969; 1957.
- 48 Sols, A.; Crane, R. K. Substrate specificity of brain hexokinase. *J. Biol. Chem.* 210: 581–595; 1954.
- 49 Garriga-Canut, M.; Schoenike, B.; Qazi, R.; Bergendahl, K.; Daley, T. J.; Pfender, R. M.; Morrison, J. F.; Ockuly, J.; Stafstrom, C.; Sutula, T.; Roopra, A. 2-Deoxy-D-glucose reduces epilepsy progression by NRSF-CtBP-dependent metabolic regulation of chromatin structure. *Nat. Neurosci.* 9:1382–1387; 2006.
- 50 Lane, M. A.; Ingram, D. K.; Roth, G. S. 2-Deoxy-D-glucose feeding in rats mimics physiologic effects of calorie restriction. *J. Anti Aging Med.* 1:327–336; 1998.
- 51 Ingram, D. K.; Anson, R. M.; de Cabo, R.; Mamczarz, J.; Zhu, M.; Mattison, J.; Lane, M. A.; Roth, G. S. Development of calorie restriction mimetics as a longevity strategy. *Ann. N. Y. Acad. Sci.* 1019:412–423; 2004.
- 52 Duan, W.; Mattison, M. P. Dietary restriction and 2-deoxyglucose administration improve behavioral outcome and reduce degeneration of dopaminergic neurons in models of Parkinson's disease. *J. Neurosci.* 57:195–206; 1999.
- 53 Sinclair, D. A. Toward a unified theory of caloric restriction and longevity regulation. *Mech. Ageing Dev.* 126:987–1002; 2005.
- 54 Zhu, Z.; Jiang, W.; McGinley, J. N.; Thompson, H. J. 2-Deoxyglucose as an energy restriction mimetic agent: effects on mammary carcinogenesis and on mammary tumor cell growth in vitro. *Cancer Res.* 65:7023–7030; 2005.
- 55 Ingram, D. K.; Zhu, M.; Mamczarz, J.; Zou, S.; Lane, M. A.; Roth, G. S.; deCabo, R. Calorie restriction mimetics: an emerging research field. *Ageing Cell* 5:97–108; 2006.
- 56 Minor, R. K.; Smith Jr., D. L.; Sossong, A. M.; Kaushik, S.; Poosala, S.; Spangler, E. L.; Roth, G. S.; Lane, M.; Allison, D. B.; de Cabo, R.; Ingram, D. K.; Mattison, J. A. Chronic ingestion of 2-deoxy-D-glucose induces cardiac vacuolization and increases mortality in rats. *Toxicol. Appl. Pharmacol.* 243:332–339; 2010.
- 57 Hardie, D. G.; Hawley, S. A.; Scott, J. W. AMP-activated protein kinase: development of the energy sensor concept. *J. Physiol.* 574:7–15; 2006.
- 58 Apfeld, J.; O'Connor, G.; McDonagh, T.; DiStefano, P. S.; Curtis, R. The AMP-activated protein kinase aak-2 links energy levels and insulin-like signals to lifespan in *C. elegans*. *Genes Dev.* 18:3004–3009; 2004.
- 59 Greer, E. L.; Dowlatshahi, D.; Banko, M. R.; Villen, J.; Hoang, K.; Blanchard, D.; Gygi, S. P.; Brunet, A. An AMPK-FOXO pathway mediates longevity induced by a novel method of dietary restriction in *C. elegans*. *Curr. Biol.* 17:1646–1656; 2007.
- 60 Pan, D. A.; Hardie, D. G. A homologue of AMP-activated protein kinase in *Drosophila melanogaster* is sensitive to AMP and is activated by ATP depletion. *Biochem. J.* 367:179–186; 2002.
- 61 Onken, B.; Driscoll, M. Metformin induces a dietary restriction-like state and the oxidative stress response to extend *C. elegans* healthspan via AMPK, LKB1, and SKN-1. *PLoS One* 5:e8758; 2010.
- 62 Kotani, K.; Peroni, O. D.; Minokoshi, Y.; Boss, O.; Kahn, B. B. GLUT4 glucose transporter deficiency increases hepatic lipid production and peripheral lipid utilization. *J. Clin. Invest.* 114:1666–1675; 2004.
- 63 McCarter, R.; Mejia, W.; Ikeno, Y.; Monnier, V.; Kewitt, K.; Gibbs, M.; McMahan, A.; Strong, R. Plasma glucose and the action of calorie restriction on aging. *J. Gerontol. A Biol. Sci. Med. Sci.* 62:1059–1070; 2007.
- 64 Lee, S. J.; Murphy, C. T.; Kenyon, C. Glucose shortens the life span of *C. elegans* by downregulating DAF-16/FOXO activity and aquaporin gene expression. *Cell Metab.* 10:379–391; 2009.
- 65 Schlotterer, A.; Kukudov, G.; Bozorgmehr, F.; Hutter, H.; Du, X.; Oikonomou, D.; Ibrahim, Y.; Pfisterer, F.; Rabbani, N.; Thornalley, P.; Sayed, A.; Fleming, T.; Humpert, P.; Schwenger, V.; Zeier, M.; Hamann, A.; Stern, D.; Brownlee, M.; Bierhaus, A.; Nawroth, P.; Morcos, M. C. *C. elegans* as model for the study of high glucose mediated lifespan reduction. *Diabetes* 58:2450–2456; 2009.
- 66 Nordmann, A. J.; Nordmann, A.; Briel, M.; Keller, U.; Yancy Jr., W. S.; Brehm, B. J.; Bucher, H. C. Effects of low-carbohydrate vs low-fat diets on weight loss and cardiovascular risk factors: a meta-analysis of randomized controlled trials. *Arch. Intern. Med.* 166:285–293; 2006.
- 67 Hession, M.; Rolland, C.; Kulkarni, U.; Wise, A.; Broom, J. Systematic review of randomized controlled trials of low-carbohydrate vs low-fat/low-calorie diets in the management of obesity and its comorbidities. *Obes. Rev.* 10:36–50; 2009.
- 68 Volek, J. S.; Phinney, S. D.; Forsythe, C. E.; Quann, E. E.; Wood, R. J.; Puglisi, M. J.; Kraemer, W. J.; Bibus, D. M.; Fernandez, M. L.; Feinman, R. D. Carbohydrate restriction has a more favorable impact on the metabolic syndrome than a low fat diet. *Lipids* 44:297–309; 2009.

- 69 Forsythe, C. E.; Phinney, S. D.; Fernandez, M. L.; Quann, E. E.; Wood, R. J.; Bibus, D. M.; Kraemer, W. J.; Feinman, R. D.; Volek, J. S. Comparison of low fat and low carbohydrate diets on circulating fatty acid composition and markers of inflammation. *Lipids* 43:65–77; 2008.
- 70 Quarrie, J. K.; Riabowol, K. T. Murine models of life span extension. *Sci. Aging Knowledge Environ.* 2004:re5; 2004.
- 71 Brown-Borg, H. M.; Borg, K. E.; Meliska, C. J.; Bartke, A. Dwarf mice and the ageing process. *Nature* 384:33; 1996.
- 72 Pendergrass, W. R.; Li, Y.; Jiang, D.; Wolf, N. S. Decrease in cellular replicative potential in "giant" mice transfected with the bovine growth hormone gene correlates to shortened life span. *J. Cell. Physiol.* 156:96–103; 1993.
- 73 Steger, R. W.; Bartke, A.; Cecim, M. Premature ageing in transgenic mice expressing different growth hormone genes. *J. Reprod. Fertil. Suppl.* 46:61–75; 1993.
- 74 Holzenberger, M.; Dupont, J.; Ducos, B.; Leneuve, P.; Geloan, A.; Even, P. C.; Cervera, P.; Le Bouc, Y. IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. *Nature* 421:182–187; 2003.
- 75 Kappeler, L.; De Magalhães Filho, C. M.; Dupont, J.; Leneuve, P.; Cervera, P.; Perin, L.; Loudes, C.; Blaise, A.; Klein, R.; Epelbaum, J.; Le Bouc, Y.; Holzenberger, M. Brain IGF-1 receptors control mammalian growth and lifespan through a neuroendocrine mechanism. *PLoS One* 6:e254; 2008.
- 76 Bitto, A.; Lerner, C.; Torres, C.; Roell, M.; Malaguti, M.; Perez, V.; Lorenzini, A.; Hrelia, S.; Ikeno, Y.; Matzko, M. E.; McCarter, R.; Sell, C. Long-term Igf-1 exposure decreases autophagy and cell viability. *PLoS One* 5:e12592; 2010.
- 77 Kahn, C. R. Insulin action, diabetogenes, and the cause of type II diabetes. *Diabetes* 43:1066–1084; 1994.
- 78 Biddinger, S. B.; Kahn, C. R. From mice to men: insights into the insulin resistance syndromes. *Annu. Rev. Physiol.* 68:123–158; 2006.
- 79 Brüning, J. C.; Michael, M. D.; Winnay, J. N.; Hayashi, T.; Hörsch, D.; Accili, D.; Goodyear, L. J.; Kahn, C. R. A muscle-specific insulin receptor knockout exhibits features of the metabolic syndrome of NIDDM without altering glucose tolerance. *Mol. Cell* 2:559–569; 1998.
- 80 Blüher, M.; Kahn, B. B.; Kahn, C. R. Extended longevity in mice lacking the insulin receptor in adipose tissue. *Science* 299:572–574; 2003.
- 81 Selman, C.; Lingard, S.; Choudhury, A. I.; Batterham, R. L.; Claret, M.; Clements, M.; Ramadan, F.; Okkenhaug, K.; Schuster, E.; Blanc, E.; Piper, M. D.; Al-Qassab, H.; Speakman, J. R.; Carmignac, D.; Robinson, I. C.; Thornton, J. M.; Gems, D.; Partridge, L.; Withers, D. J. Evidence for lifespan extension and delayed age-related biomarkers in insulin receptor substrate 1 null mice. *FASEB J.* 22:807–818; 2008.
- 82 Taguchi, A.; Wartschow, L. M.; White, M. F. Brain IRS2 signaling coordinates life span and nutrient homeostasis. *Science* 317:369–372; 2007.
- 83 Friedman, D. B.; Johnson, T. E. A mutation in the age-1 gene in *Caenorhabditis elegans* lengthens life and reduces hermaphrodite fertility. *Genetics* 118:75–86; 1988.
- 84 Kenyon, C.; Chang, J.; Gensch, E.; Rudner, A.; Tabtiang, R. A *C. elegans* mutant that lives twice as long as wild type. *Nature* 366:461–464; 1993.
- 85 Kimura, K. D.; Tissenbaum, H. A.; Liu, Y.; Ruvkun, G. daf-2, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. *Science* 277:942–946; 1997.
- 86 Clancy, D. J.; Gems, D.; Harshman, L. G.; Oldham, S.; Stocker, H.; Hafen, E.; Leevers, S. J.; Partridge, L. Extension of life-span by loss of CHICO, a *Drosophila* insulin receptor substrate protein. *Science* 292:104–106; 2001.
- 87 Tatar, M.; Kopelman, A.; Epstein, D.; Tu, M. P.; Yin, C. M.; Garofalo, R. S. A mutant *Drosophila* insulin receptor homolog that extends life-span and impairs neuroendocrine function. *Science* 292:107–110; 2001.
- 88 Brys, K.; Castelein, N.; Matthijssens, F.; Vanfleteren, J. R.; Braeckman, B. P. Disruption of insulin signalling preserves bioenergetic competence of mitochondria in ageing *Caenorhabditis elegans*. *BMC Biol.* 8:91; 2010.
- 89 van Heemst, D.; Beekman, M.; Mooijaart, S. P.; Heijmans, B. T.; Brandt, B. W.; Zwaan, B. J.; Slagboom, P. E.; Westendorp, R. G. Reduced insulin/IGF-1 signalling and human longevity. *Aging Cell* 4:79–85; 2005.
- 90 Pawlikowska, L.; Hu, D.; Huntsman, S.; Sung, A.; Chu, C.; Chen, J.; Joyner, A.; Schork, N. J.; Hsueh, W. C.; Reiner, A. P.; Psaty, B. M.; Atzmon, G.; Barzilai, N.; Cummings, S. R.; Browner, W. S.; Kwok, P. Y.; Ziv, E. Association of common genetic variation in the insulin/IGF1 signaling pathway with human longevity. *Aging Cell* 8:460–472; 2009.
- 91 Lakowski, B.; Hekimi, S. The genetics of caloric restriction in *Caenorhabditis elegans*. *Proc. Natl Acad. Sci. U. S. A.* 95:13091–13096; 1998.
- 92 Bartke, A.; Masternak, M. M.; Al-Regaiey, K. A.; Bonkowski, M. S. Effects of dietary restriction on the expression of insulin-signaling-related genes in long-lived mutant mice. *Interdiscip. Top. Gerontol.* 35:69–82; 2007.
- 93 Houthoofd, K.; Braeckman, B. P.; Johnson, T. E.; Vanfleteren, J. R. Life extension via dietary restriction is independent of the Ins/IGF-1 signalling pathway in *Caenorhabditis elegans*. *Exp. Gerontol.* 38:947–954; 2003.
- 94 Min, K. J.; Yamamoto, R.; Buch, S.; Pankratz, M.; Tatar, M. *Drosophila* lifespan control by dietary restriction independent of insulin-like signaling. *Aging Cell* 7:199–206; 2008.
- 95 Bonkowski, M. S.; Dominici, F. P.; Arum, O.; Rocha, J. S.; Al Regaiey, K. A.; Westbrook, R.; Spong, A.; Panici, J.; Masternak, M. M.; Koppchick, J. J.; Bartke, A. Disruption of growth hormone receptor prevents calorie restriction from improving insulin action and longevity. *PLoS One* 4:e4567; 2009.
- 96 Brown-Borg, H. M.; Rakoczy, S. G.; Romanick, M. A.; Kennedy, M. A. Effects of growth hormone and insulin-like growth factor-1 on hepatocyte antioxidant enzymes. *Exp. Biol. Med. (Maywood)* 227:94–104; 2002.
- 97 Clancy, D. J.; Gems, D.; Hafen, E.; Leevers, S. J.; Partridge, L. Dietary restriction in long-lived dwarf flies. *Science* 296:319; 2002.
- 98 Al-Regaiey, K. A.; Masternak, M. M.; Bonkowski, M.; Sun, L.; Bartke, A. Long-lived growth hormone receptor knockout mice: interaction of reduced insulin-like growth factor I/insulin signaling and caloric restriction. *Endocrinology* 146:851–860; 2005.
- 99 Bonkowski, M. S.; Rocha, J. S.; Masternak, M. M.; Al Regaiey, K. A.; Bartke, A. Targeted disruption of growth hormone receptor interferes with the beneficial actions of caloric restriction. *Proc. Natl Acad. Sci. U. S. A.* 103:7901–7905; 2006.
- 100 Narasimhan, S. D.; Yen, K.; Tissenbaum, H. A. Converging pathways in lifespan regulation. *Curr. Biol.* 19:R657–R666; 2009.
- 101 Yen, K.; Mobbs, C. V. Evidence for only two independent pathways for decreasing senescence in *Caenorhabditis elegans*. *Age (Dordrecht)* 32:39–49; 2010.
- 102 Yechoor, V. K.; Patti, M. E.; Ueki, K.; Laustsen, P. G.; Saccone, R.; Raunyar, R.; Kahn, C. R. Distinct pathways of insulin-regulated versus diabetes-regulated gene expression: an in vivo analysis in MIRKO mice. *Proc. Natl Acad. Sci. U. S. A.* 101:16525–16530; 2004.
- 103 Brooks, N. L.; Trent, C. M.; Raetzsch, C. F.; Flurkey, K.; Boysen, G.; Perfetti, M. T.; Jeong, Y. C.; Klebanov, S.; Patel, K. B.; Khodush, V. R.; Kupper, L. L.; Carling, D.; Swenberg, J. A.; Harrison, D. E.; Combs, T. P. Low utilization of circulating glucose after food withdrawal in Snell dwarf mice. *J. Biol. Chem.* 282:35069–35077; 2007.
- 104 Katic, M.; Kennedy, A. R.; Leykin, I.; Norris, A.; McGettrick, A.; Gesta, S.; Russell, S. J.; Blüher, M.; Maratos-Flier, E.; Kahn, C. R. Mitochondrial gene expression and increased oxidative metabolism: role in increased lifespan of fat-specific insulin receptor knock-out mice. *Aging Cell* 6:827–839; 2007.
- 105 Russell, S. J.; Kahn, C. R. Endocrine regulation of ageing. *Nat. Rev. Mol. Cell Biol.* 8:681–691; 2007.
- 106 Westbrook, R.; Bonkowski, M. S.; Strader, A. D.; Bartke, A. Alterations in oxygen consumption, respiratory quotient, and heat production in long-lived GHRKO and Ames dwarf mice, and short-lived bGH transgenic mice. *J. Gerontol. A Biol. Med. Sci.* 64:443–451; 2009.
- 107 Wiederkehr, A.; Wollheim, C. B. Implication of mitochondria in insulin secretion and action. *Endocrinology* 147:2643–2649; 2006.
- 108 Ristow, M. Oxidative metabolism in cancer growth. *Curr. Opin. Clin. Nutr. Metab.* 9:339–345; 2006.
- 109 Fukui, H.; Moraes, C. T. The mitochondrial impairment, oxidative stress and neurodegeneration connection: reality or just an attractive hypothesis? *Trends Neurosci.* 31:251–256; 2008.
- 110 Tatsuta, T.; Langer, T. Quality control of mitochondria: protection against neurodegeneration and ageing. *EMBO J.* 27:306–314; 2008.
- 111 Trifunovic, A.; Larsson, N. G. Mitochondrial dysfunction as a cause of ageing. *J. Intern. Med.* 263:167–178; 2008.
- 112 Bratic, I.; Trifunovic, A. Mitochondrial energy metabolism and ageing. *Biochim. Biophys. Acta* 1797:961–967; 2010.
- 113 Masoro, E. J.; Yu, B. P.; Bertrand, H. A. Action of food restriction in delaying the aging process. *Proc. Natl Acad. Sci. U. S. A.* 79:4239–4241; 1982.
- 114 Nisoli, E.; Tonello, C.; Cardile, A.; Cozzi, V.; Bracciale, R.; Tedesco, L.; Falcone, S.; Valerio, A.; Cantoni, O.; Clementi, E.; Moncada, S.; Carruba, M. O. Calorie restriction promotes mitochondrial biogenesis by inducing the expression of eNOS. *Science* 310:314–317; 2005.
- 115 Lopez-Lluch, G.; Hunt, N.; Jones, B.; Zhu, M.; Jamieson, H.; Hilmer, S.; Cascajo, M. V.; Allard, J.; Ingram, D. K.; Navas, P.; de Cabo, R. Calorie restriction induces mitochondrial biogenesis and bioenergetic efficiency. *Proc. Natl Acad. Sci. U. S. A.* 103:1768–1773; 2006.
- 116 Selman, C.; Phillips, T.; Staib, J. L.; Duncan, J. S.; Leeuwenburgh, C.; Speakman, J. R. Energy expenditure of calorically restricted rats is higher than predicted from their altered body composition. *Mech. Ageing Dev.* 126:783–793; 2005.
- 117 Alvers, A. L.; Fishwick, L. K.; Wood, M. S.; Hu, D.; Chung, H. S.; Dunn Jr, W. A.; Aris, J. P. Autophagy and amino acid homeostasis are required for chronological longevity in *Saccharomyces cerevisiae*. *Aging Cell* 8:353–369; 2009.
- 118 D'Antona, G.; Ragni, M.; Cardile, A.; Tedesco, L.; Dossena, M.; Bruttini, F.; Caliaro, F.; Corsetti, G.; Bottinelli, R.; Carruba, M. O.; Valerio, A.; Nisoli, E. Branched-chain amino acid supplementation promotes survival and supports cardiac and skeletal muscle mitochondrial biogenesis in middle-aged mice. *Cell Metab.* 12:362–372; 2010.
- 119 Ames, B. N. Increasing longevity by tuning up metabolism: to maximize human health and lifespan, scientists must abandon outdated models of micronutrients. *EMBO Rep.* 6:S20–S24 (Special Issue); 2005.
- 120 Warburton, D. E.; Nicol, C. W.; Bredin, S. S. Health benefits of physical activity: the evidence. *Can. Med. Assoc. J.* 174:801–809; 2006.
- 121 Lanza, I. R.; Short, D. K.; Short, K. R.; Raghavakamal, S.; Basu, R.; Joyner, M. J.; McConnell, J. P.; Nair, K. S. Endurance exercise as a countermeasure for aging. *Diabetes* 57:2933–2942; 2008.
- 122 Zarse, K.; Bossecker, A.; Müller-Kuhr, L.; Siems, K.; Hernandez, M. A.; Berendsohn, W. G.; Birringer, M.; Ristow, M. The phytochemical glucarubinone promotes mitochondrial metabolism, reduces body fat, and extends lifespan of *Caenorhabditis elegans*. *Horm. Metab. Res.* 43:241–243; 2011.
- 123 Schulz, T. J.; Westermann, D.; Isken, F.; Voigt, A.; Laube, B.; Thierbach, R.; Kuhlow, D.; Zarse, K.; Schomburg, L.; Pfeiffer, A. F. H.; Tschöpe, C.; Ristow, M. Activation of mitochondrial energy metabolism protects against cardiac failure. *Aging (Albany)* 2:843–853; 2010.
- 124 Bonawitz, N. D.; Rodeheffer, M. S.; Shadel, G. S. Defective mitochondrial gene expression results in reactive oxygen species-mediated inhibition of respiration and reduction of yeast life span. *Mol. Cell. Biol.* 26:4818–4829; 2006.
- 125 Zarse, K.; Schulz, T. J.; Birringer, M.; Ristow, M. Impaired respiration is positively correlated with decreased life span in *Caenorhabditis elegans* models of Friedreich ataxia. *FASEB J.* 21:1271–1275; 2007.

- Thierbach, R.; Schulz, T. J.; Isken, F.; Voigt, A.; Mietzner, B.; Drewes, G.; von Kleist-Retzow, J. C.; Wiesner, R. J.; Magnuson, M. A.; Puccio, H.; Pfeiffer, A. F.; Steinberg, P.; Ristow, M. Targeted disruption of hepatic frataxin expression causes impaired mitochondrial function, decreased life span, and tumor growth in mice. *Hum. Mol. Genet.* 14:3857–3864; 2005.
- Powers III, R. W.; Kaerberlein, M.; Caldwell, S. D.; Kennedy, B. K.; Fields, S. Extension of chronological life span in yeast by decreased TOR pathway signaling. *Genes Dev.* 20:174–184; 2006.
- Bonawitz, N. D.; Chatenay-Lapointe, M.; Pan, Y.; Shadel, G. S. Reduced TOR signaling extends chronological life span via increased respiration and upregulation of mitochondrial gene expression. *Cell Metab.* 5:265–277; 2007.
- Zid, B. M.; Rogers, A. N.; Katewa, S. D.; Vargas, M. A.; Kolipinski, M. C.; Lu, T. A.; Benzer, S.; Kapahi, P. 4E-BP extends lifespan upon dietary restriction by enhancing mitochondrial activity in *Drosophila*. *Cell* 139:149–160; 2009.
- Gwinn, D. M.; Shackelford, D. B.; Egan, D. F.; Mihaylova, M. M.; Mery, A.; Vasquez, D. S.; Turk, B. E.; Shaw, R. J. AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol. Cell* 30:214–226; 2008.
- Harman, D. The biologic clock: the mitochondria? *J. Am. Geriatr. Soc.* 20:145–147; 1972.
- Lapointe, J.; Hekimi, S. When a theory of aging ages badly. *Cell. Mol. Life Sci.* 67: 1–8; 2010.
- Harrington, L. A.; Harley, C. B. Effect of vitamin E on lifespan and reproduction in *Caenorhabditis elegans*. *Mech. Ageing Dev.* 43:71–78; 1988.
- Phillips, J. P.; Campbell, S. D.; Michaud, D.; Charbonneau, M.; Hilliker, A. J. Null mutation of copper/zinc superoxide dismutase in *Drosophila* confers hypersensitivity to paraquat and reduced longevity. *Proc. Natl. Acad. Sci. U. S. A.* 86: 2761–2765; 1989.
- Orr, W. C.; Sohal, R. S. Extension of life-span by overexpression of superoxide dismutase and catalase in *Drosophila melanogaster*. *Science* 263:1128–1130; 1994.
- Parke, T. L.; Elia, A. J.; Dickinson, D.; Hilliker, A. J.; Phillips, J. P.; Boulianne, G. L. Extension of *Drosophila* lifespan by overexpression of human SOD1 in motoneurons. *Nat. Genet.* 19:171–174; 1998.
- Melov, S.; Ravenscroft, J.; Malik, S.; Gill, M. S.; Walker, D. W.; Clayton, P. E.; Wallace, D. C.; Malfroy, B.; Doctrow, S. R.; Lithgow, G. J. Extension of life-span with superoxide dismutase/catalase mimetics. *Science* 289:1567–1569; 2000.
- Moskovitz, J.; Bar-Noy, S.; Williams, W. M.; Requena, J.; Berlett, B. S.; Stadtman, E. R. Methionine sulfoxide reductase (MsrA) is a regulator of antioxidant defense and lifespan in mammals. *Proc. Natl. Acad. Sci. U. S. A.* 98:12920–12925; 2001.
- Bakae, V. V.; Lyudmila, M. B. Effect of ascorbic acid on longevity in the nematode *Caenorhabditis elegans*. *Biogerontology* 3 (Suppl. 1):12–16; 2002.
- Ruan, H.; Tang, X. D.; Chen, M. L.; Joiner, M. L.; Sun, G.; Brot, N.; Weissbach, H.; Heinemann, S. H.; Iversen, L.; Wu, C. F.; Hoshi, T. High-quality life extension by the enzyme peptide methionine sulfoxide reductase. *Proc. Natl. Acad. Sci. U. S. A.* 99:2748–2753; 2002.
- Ishii, N.; Senoo-Matsuda, N.; Miyake, K.; Yasuda, K.; Ishii, T.; Hartman, P. S.; Furukawa, S. Coenzyme Q10 can prolong *C. elegans* lifespan by lowering oxidative stress. *Mech. Ageing Dev.* 125:41–46; 2004.
- Huang, T. T.; Naemuddin, M.; Elchuri, S.; Yamaguchi, M.; Kozy, H. M.; Carlson, E. J.; Epstein, C. J. Genetic modifiers of the phenotype of mice deficient in mitochondrial superoxide dismutase. *Hum. Mol. Genet.* 15:1187–1194; 2006.
- Zou, S.; Sinclair, J.; Wilson, M. A.; Carey, J. R.; Liedo, P.; Oropeza, A.; Kalra, A.; de Cabo, R.; Ingram, D. K.; Longo, D. L.; Wolkow, C. A. Comparative approaches to facilitate the discovery of longevity interventions: effects of tocopherols on lifespan of three invertebrate species. *Mech. Ageing Dev.* 128:222–226; 2007.
- Kim, J.; Takahashi, M.; Shimizu, T.; Shirasawa, T.; Kajita, M.; Kanayama, A.; Miyamoto, Y. Effects of a potent antioxidant, platinum nanoparticle, on the lifespan of *Caenorhabditis elegans*. *Mech. Ageing Dev.* 129:322–331; 2008.
- Quick, K. L.; Ali, S. S.; Arch, R.; Xiong, C.; Wozniak, D.; Dugan, L. L. A carboxyfullerene SOD mimetic improves cognition and extends the lifespan of mice. *Neurobiol. Aging* 29:117–128; 2008.
- Dai, D. F.; Santana, L. F.; Vermulst, M.; Tomazela, D. M.; Emond, M. J.; MacCoss, M. J.; Gollahan, K.; Martin, G. M.; Loeb, L. A.; Ladiges, W. C.; Rabinovitch, P. S. Overexpression of catalase targeted to mitochondria attenuates murine cardiac aging. *Circulation* 119:2789–2797; 2009.
- Shibamura, A.; Ikeda, T.; Nishikawa, Y. A method for oral administration of hydrophilic substances to *Caenorhabditis elegans*: effects of oral supplementation with antioxidants on the nematode lifespan. *Mech. Ageing Dev.* 130: 652–655; 2009.
- Greenberg, E. R.; Baron, J. A.; Tosteson, T. D.; Freeman Jr, D. H.; Beck, G. J.; Bond, J. H.; Colacchio, T. A.; Collier, J. A.; Frankl, H. D.; Haile, R. W., et al. A clinical trial of antioxidant vitamins to prevent colorectal adenoma. Polyp Prevention Study Group. *N. Engl. J. Med.* 331:141–147; 1994.
- Liu, S.; Ajani, U.; Chae, C.; Hennekens, C.; Buring, J. E.; Manson, J. E. Long-term beta-carotene supplementation and risk of type 2 diabetes mellitus: a randomized controlled trial. *JAMA* 282:1073–1075; 1999.
- Rautalahti, M. T.; Virtamo, J. R.; Taylor, P. R.; Heinonen, O. P.; Albanes, D.; Haukka, J. K.; Edwards, B. K.; Karkkainen, P. A.; Stolzenberg-Solomon, R. Z.; Huttunen, J. The effects of supplementation with alpha-tocopherol and beta-carotene on the incidence and mortality of carcinoma of the pancreas in a randomized, controlled trial. *Cancer* 86:37–42; 1999.
- Virtamo, J.; Edwards, B. K.; Virtanen, M.; Taylor, P. R.; Malila, N.; Albanes, D.; Huttunen, J. K.; Hartman, A. M.; Hietanen, P.; Maenpaa, H.; Koss, L.; Nordling, S.; Heinonen, O. P. Effects of supplemental alpha-tocopherol and beta-carotene on urinary tract cancer: incidence and mortality in a controlled trial (Finland). *Cancer Causes Control* 11:933–939; 2000.
- Heart Protection Study Collaborative Group MRC/BHF Heart Protection Study of antioxidant vitamin supplementation in 20,536 high-risk individuals: a randomised placebo-controlled trial. *Lancet* 360:23–33; 2002.
- Sacco, M.; Pellegrini, F.; Roncaglioni, M. C.; Avanzini, F.; Tognoni, G.; Nicolucci, A. Primary prevention of cardiovascular events with low-dose aspirin and vitamin E in type 2 diabetic patients: results of the Primary Prevention Project (PPP) trial. *Diabetes Care* 26:3264–3272; 2003.
- Zureik, M.; Galan, P.; Bertrais, S.; Mennen, L.; Czernichow, S.; Blacher, J.; Ducimetiere, P.; Hercberg, S. Effects of long-term daily low-dose supplementation with antioxidant vitamins and minerals on structure and function of large arteries. *Arterioscler. Thromb. Vasc. Biol.* 24:1485–1491; 2004.
- Czernichow, S.; Bertrais, S.; Blacher, J.; Galan, P.; Briancon, S.; Favier, A.; Safar, M.; Hercberg, S. Effect of supplementation with antioxidants upon long-term risk of hypertension in the SU.VI.MAX study: association with plasma antioxidant levels. *J. Hypertens.* 23:2013–2018; 2005.
- Czernichow, S.; Couthous, A.; Bertrais, S.; Vergnaud, A. C.; Dauchet, L.; Galan, P.; Hercberg, S. Antioxidant supplementation does not affect fasting plasma glucose in the Supplementation with Antioxidant Vitamins and Minerals (SU.VI.MAX) study in France: association with dietary intake and plasma concentrations. *Am. J. Clin. Nutr.* 84:395–399; 2006.
- Cook, N. R.; Albert, C. M.; Gaziano, J. M.; Zaharris, E.; MacFadyen, J.; Danielson, E.; Buring, J. E.; Manson, J. E. A randomized factorial trial of vitamins C and E and beta carotene in the secondary prevention of cardiovascular events in women: results from the Women's Antioxidant Cardiovascular Study. *Arch. Intern. Med.* 167:1610–1618; 2007.
- Kataja-Tuomola, M.; Sundell, J. R.; Mannisto, S.; Virtanen, M. J.; Kontto, J.; Albanes, D.; Virtamo, J. Effect of alpha-tocopherol and beta-carotene supplementation on the incidence of type 2 diabetes. *Diabetologia* 51:47–53; 2008.
- Sesso, H. D.; Buring, J. E.; Christen, W. G.; Kurth, T.; Belanger, C.; MacFadyen, J.; Bubes, V.; Manson, J. E.; Glynn, R. J.; Gaziano, J. M. Vitamins E and C in the prevention of cardiovascular disease in men: the Physicians' Health Study II randomized controlled trial. *JAMA* 300:2123–2133; 2008.
- Katsiki, N.; Manes, C. Is there a role for supplemented antioxidants in the prevention of atherosclerosis? *Clin. Nutr.* 28:3–9; 2009.
- Lin, J.; Cook, N. R.; Albert, C.; Zaharris, E.; Gaziano, J. M.; Van Denburgh, M.; Buring, J. E.; Manson, J. E. Vitamins C and E and beta-carotene supplementation and cancer risk: a randomized controlled trial. *J. Natl. Cancer Inst.* 101:14–23; 2009.
- Song, Y.; Cook, N. R.; Albert, C. M.; Van Denburgh, M.; Manson, J. E. Effects of vitamins C and E and beta-carotene on the risk of type 2 diabetes in women at high risk of cardiovascular disease: a randomized controlled trial. *Am. J. Clin. Nutr.* 90:429–437; 2009.
- Bjelakovic, G.; Nikolova, D.; Simonetti, R. G.; Gluud, C. Antioxidant supplements for prevention of gastrointestinal cancers: a systematic review and meta-analysis. *Lancet* 364:1219–1228; 2004.
- Bairati, I.; Meyer, F.; Gelinis, M.; Fortin, A.; Nabid, A.; Brochet, F.; Mercier, J. P.; Tetu, B.; Harel, F.; Masse, B.; Vigneault, E.; Vass, S.; del Vecchio, P.; Roy, J. A randomized trial of antioxidant vitamins to prevent second primary cancers in head and neck cancer patients. *J. Natl. Cancer Inst.* 97:481–488; 2005.
- Hercberg, S.; Ezzedine, K.; Guinot, C.; Preziosi, P.; Galan, P.; Bertrais, S.; Estaquio, C.; Briancon, S.; Favier, A.; Latreille, J.; Malvy, D. Antioxidant supplementation increases the risk of skin cancers in women but not in men. *J. Nutr.* 137: 2098–2105; 2007.
- Bardia, A.; Tleyjeh, I. M.; Cerhan, J. R.; Sood, A. K.; Limburg, P. J.; Erwin, P. J.; Montori, V. M. Efficacy of antioxidant supplementation in reducing primary cancer incidence and mortality: systematic review and meta-analysis. *Mayo Clin. Proc.* 83:23–34; 2008.
- Lawenda, B. D.; Kelly, K. M.; Ladas, E. J.; Sagar, S. M.; Vickers, A.; Blumberg, J. B. Should supplemental antioxidant administration be avoided during chemotherapy and radiation therapy? *J. Natl. Cancer Inst.* 100:773–783; 2008.
- Myung, S. K.; Kim, Y.; Ju, W.; Choi, H. J.; Bae, W. K. Effects of antioxidant supplements on cancer prevention: meta-analysis of randomized controlled trials. *Ann. Oncol.* 21:166–179; 2010.
- Albanes, D.; Heinonen, O. P.; Taylor, P. R.; Virtamo, J.; Edwards, B. K.; Rautalahti, M.; Hartman, A. M.; Palmgren, J.; Freedman, L. S.; Haapakoski, J.; Barrett, M. J.; Pietinen, P.; Malila, N.; Tala, E.; Liippo, K.; Salomaa, E. R.; Tangrea, J. A.; Teppo, L.; Askin, F. B.; Taskinen, E.; Erozan, Y.; Greenwald, P.; Huttunen, J. K. Alpha-Tocopherol and beta-carotene supplements and lung cancer incidence in the alpha-Tocopherol, beta-Carotene Cancer Prevention Study: effects of base-line characteristics and study compliance. *J. Natl. Cancer Inst.* 88:1560–1570; 1996.
- Omenn, G. S.; Goodman, G. E.; Thornquist, M. D.; Balmes, J.; Cullen, M. R.; Glass, A.; Keogh, J. P.; Meyskens, F. L.; Valanis, B.; Williams, J. H.; Barnhart, S.; Hammar, S. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. *N. Engl. J. Med.* 334:1150–1155; 1996.
- Vivekananthan, D. P.; Penn, M. S.; Sapp, S. K.; Hsu, A.; Topol, E. J. Use of antioxidant vitamins for the prevention of cardiovascular disease: meta-analysis of randomized trials. *Lancet* 361:2017–2023; 2003.
- Lonn, E.; Bosch, J.; Yusuf, S.; Sheridan, P.; Pogue, J.; Arnold, J. M.; Ross, C.; Arnold, A.; Sleight, P.; Probstfield, J.; Dagenais, G. R. Effects of long-term vitamin E supplementation on cardiovascular events and cancer: a randomized controlled trial. *JAMA* 293:1338–1347; 2005.
- Bjelakovic, G.; Nikolova, D.; Gluud, L. L.; Simonetti, R. G.; Gluud, C. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. *JAMA* 297:842–857; 2007.
- Ward, N. C.; Wu, J. H.; Clarke, M. W.; Puddey, I. B.; Burke, V.; Croft, K. D.; Hodgson, J. M. The effect of vitamin E on blood pressure in individuals with type 2

- diabetes: a randomized, double-blind, placebo-controlled trial. *J. Hypertens.* 25: 227–234; 2007.
- [175] Lippman, S. M.; Klein, E. A.; Goodman, P. J.; Lucia, M. S.; Thompson, I. M.; Ford, L. G.; Parnes, H. L.; Minasian, L. M.; Gaziano, J. M.; Hartline, J. A.; Parsons, J. K.; Bearden III, J. D.; Crawford, E. D.; Goodman, G. E.; Claudio, J.; Winquist, E.; Cook, E. D.; Karp, D. D.; Walther, P.; Lieber, M. M.; Kristal, A. R.; Darke, A. K.; Arnold, K. B.; Ganz, P. A.; Santella, R. M.; Albanes, D.; Taylor, P. R.; Probstfield, J. L.; Jagpal, T. J.; Crowley, J. J.; Meyskens Jr, F. L.; Baker, L. H.; Coltman Jr, C. A. Effect of selenium and vitamin E on risk of prostate cancer and other cancers: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). *JAMA* 301:39–51; 2009.
- 176 Barja, G. Oxygen radicals, a failure or a success of evolution? *Free Radic. Res. Commun.* 18:63–70; 1993.
- 177 Rhee, S. G.; Chang, T. S.; Bae, Y. S.; Lee, S. R.; Kang, S. W. Cellular regulation by hydrogen peroxide. *J. Am. Soc. Nephrol.* 14:S211–S215; 2003.
- 178 Kaelin Jr, W. G. ROS: really involved in oxygen sensing. *Cell Metab.* 1:357–358; 2005.
- 179 Connor, K. M.; Subbaram, S.; Regan, K. J.; Nelson, K. K.; Mazurkiewicz, J. E.; Bartholomew, P. J.; Aplin, A. E.; Tai, Y. T.; Aguirre-Ghiso, J.; Flores, S. C.; Melendez, J. A. Mitochondrial H₂O₂ regulates the angiogenic phenotype via PTEN oxidation. *J. Biol. Chem.* 280:16916–16924; 2005.
- 180 Guzy, R. D.; Hoyos, B.; Robin, E.; Chen, H.; Liu, L.; Mansfield, K. D.; Simon, M. C.; Hammerling, U.; Schumacker, P. T. Mitochondrial complex III is required for hypoxia-induced ROS production and cellular oxygen sensing. *Cell Metab.* 1: 401–408; 2005.
- 181 Guzy, R. D.; Schumacker, P. T. Oxygen sensing by mitochondria at complex III: the paradox of increased reactive oxygen species during hypoxia. *Exp. Physiol.* 91:807–819; 2006.
- 182 Chandel, N. S.; Budinger, G. R. The cellular basis for diverse responses to oxygen. *Free Radic. Biol. Med.* 42:165–174; 2007.
- 183 Veal, E. A.; Day, A. M.; Morgan, B. A. Hydrogen peroxide sensing and signaling. *Mol. Cell* 26:1–14; 2007.
- 184 Owusu-Ansah, E.; Yavari, A.; Mandal, S.; Banerjee, U. Distinct mitochondrial retrograde signals control the G1–S cell cycle checkpoint. *Nat. Genet.* 40: 356–361; 2008.
- 185 Finley, L. W.; Haigis, M. C. The coordination of nuclear and mitochondrial communication during aging and calorie restriction. *Ageing Res. Rev.* 8:173–188; 2009.
- 186 Ristow, M.; Zarse, K.; Oberbach, A.; Klötting, N.; Birringer, M.; Kiehnopf, M.; Stumvoll, M.; Kahn, C. R.; Blüher, M. Antioxidants prevent health-promoting effects of physical exercise in humans. *Proc. Natl Acad. Sci. U. S. A.* 106: 8665–8670; 2009.
- 187 Loh, K.; Deng, H.; Fukushima, A.; Cai, X.; Boivin, B.; Galic, S.; Bruce, C.; Shields, B. J.; Skiba, B.; Ooms, L. M.; Stepto, N.; Wu, B.; Mitchell, C. A.; Tonks, N. K.; Watt, M. J.; Febbraio, M. A.; Crack, P. J.; Andrikopoulos, S.; Tiganis, T. Reactive oxygen species enhance insulin sensitivity. *Cell Metab.* 10:260–272; 2009.
- 188 Lee, S. J.; Hwang, A. B.; Kenyon, C. Inhibition of respiration extends *C. elegans* life span via reactive oxygen species that increase HIF-1 activity. *Curr. Biol.* 20: 2131–2136; 2010.
- 189 Yang, W.; Hekimi, S. A Mitochondrial superoxide signal triggers increased longevity in *Caenorhabditis elegans*. *PLoS Biol.* 8:e1000556; 2010.
- 190 Woo, D. K.; Shadel, G. S. Mitochondrial stress signals revise an old aging theory. *Cell* 144:11–12; 2011.
- 191 Boveris, A.; Chance, B. The mitochondrial generation of hydrogen peroxide: general properties and effect of hyperbaric oxygen. *Biochem. J.* 134:707–716; 1973.
- 192 Cypser, J. R.; Johnson, T. E. Multiple stressors in *Caenorhabditis elegans* induce stress hormesis and extended longevity. *J. Gerontol. A Biol. Sci. Med. Sci.* 57: B109–B114; 2002.
- 193 Yanase, S.; Hartman, P. S.; Ito, A.; Ishii, N. Oxidative stress pretreatment increases the X-radiation resistance of the nematode *Caenorhabditis elegans*. *Mutat. Res.* 426:31–39; 1999.
- 194 Turrens, J. F. Mitochondrial formation of reactive oxygen species. *J. Physiol.* 552: 335–344; 2003.
- 195 Conti, B.; Sanchez-Alavez, M.; Winsky-Sommerer, R.; Morale, M. C.; Lucero, J.; Brownell, S.; Fabre, V.; Huitron-Resendiz, S.; Henriksen, S.; Zorrilla, E. P.; de Lecea, L.; Bartfai, T. Transgenic mice with a reduced core body temperature have an increased life span. *Science* 314:825–828; 2006.
- 196 Hosono, R.; Mitsui, Y.; Sato, Y.; Aizawa, S.; Miwa, J. Life span of the wild and mutant nematode *Caenorhabditis elegans*: effects of sex, sterilization, and temperature. *Exp. Gerontol.* 17:163–172; 1982.
- 197 Ali, S. S.; Marcondes, M. C.; Bajova, H.; Dugan, L. L.; Conti, B. Metabolic depression and increased ROS production by isolated mitochondria at moderately lower temperatures. *J. Biol. Chem.* 285:32522–32528; 2010.
- 198 Masoro, E. J. Influence of caloric intake on aging and on the response to stressors. *J. Toxicol. Environ. Health B Crit. Rev.* 1:243–257; 1998.
- 199 Yu, B. P.; Chung, H. Y. Stress resistance by caloric restriction for longevity. *Ann. N. Y. Acad. Sci.* 928:39–47; 2001.
- 200 Masoro, E. J. The role of hormesis in life extension by dietary restriction. *Interdiscip. Top. Gerontol.* 35:1–17; 2007.
- 201 Southam, C. M.; Ehrlich, J. Effects of extract of western red-cedar heartwood on certain wood-decaying fungi in culture. *Phytopathology* 33:517–524; 1943.
- 202 Calabrese, E. J.; Baldwin, L. A. Defining hormesis. *Hum. Exp. Toxicol.* 21:91–97; 2002.
- 203 Cypser, J. R.; Tedesco, P.; Johnson, T. E. Hormesis and aging in *Caenorhabditis elegans*. *Exp. Gerontol.* 41:935–939; 2006.
- 204 Rattan, S. I. Hormesis in aging. *Ageing Res. Rev.* 7:63–78; 2008.
- 205 Mattson, M. P. Hormesis defined. *Ageing Res. Rev.* 7:1–7; 2008.
- 206 Tapia, P. C. Sublethal mitochondrial stress with an attendant stoichiometric augmentation of reactive oxygen species may precipitate many of the beneficial alterations in cellular physiology produced by caloric restriction, intermittent fasting, exercise and dietary phytonutrients: 'mitohormesis' for health and vitality. *Med. Hypotheses* 66:832–843; 2006.
- 207 Sreekumar, R.; Unnikrishnan, J.; Fu, A.; Nygren, J.; Short, K. R.; Schimke, J.; Barazzoni, R.; Nair, K. S. Effects of caloric restriction on mitochondrial function and gene transcripts in rat muscle. *Am. J. Physiol. Endocrinol. Metab.* 283: E38–E43; 2002.
- 208 Kharade, S. V.; Mittal, N.; Das, S. P.; Sinha, P.; Roy, N. Mrg19 depletion increases *S. cerevisiae* lifespan by augmenting ROS defence. *FEBS Lett.* 579:6809–6813; 2005.
- 209 Piper, P. W.; Harris, N. L.; MacLean, M. Preadaptation to efficient respiratory maintenance is essential both for maximal longevity and the retention of replicative potential in chronologically ageing yeast. *Mech. Ageing Dev.* 127: 733–740; 2006.
- 210 Huang, T. T.; Carlson, E. J.; Gillespie, A. M.; Shi, Y.; Epstein, C. J. Ubiquitous overexpression of CuZn superoxide dismutase does not extend life span in mice. *J. Gerontol. A Biol. Sci. Med. Sci.* 55:B5–B9; 2000.
- 211 Bayne, A. C.; Sohal, R. S. Effects of superoxide dismutase/catalase mimetics on life span and oxidative stress resistance in the housefly, *Musca domestica*. *Free Radic. Biol. Med.* 32:1229–1234; 2002.
- 212 Keaney, M.; Gems, D. No increase in lifespan in *Caenorhabditis elegans* upon treatment with the superoxide dismutase mimetic EUK-8. *Free Radic. Biol. Med.* 34:277–282; 2003.
- 213 Andziak, B.; O'Connor, T. P.; Qi, W.; DeWaal, E. M.; Pierce, A.; Chaudhuri, A. R.; Van Remmen, H.; Buffenstein, R. High oxidative damage levels in the longest-living rodent, the naked mole-rat. *Aging Cell* 5:463–471; 2006.
- 214 Selman, C.; McLaren, J. S.; Meyer, C.; Duncan, J. S.; Redman, P.; Collins, A. R.; Duthie, G. G.; Speakman, J. R. Life-long vitamin C supplementation in combination with cold exposure does not affect oxidative damage or lifespan in mice, but decreases expression of antioxidant protection genes. *Mech. Ageing Dev.* 127:897–904; 2006.
- 215 Ran, Q.; Liang, H.; Ikeno, Y.; Qi, W.; Prolla, T. A.; Roberts II, L. J.; Wolf, N.; Van Remmen, H.; Richardson, A. Reduction in glutathione peroxidase 4 increases life span through increased sensitivity to apoptosis. *J. Gerontol. A Biol. Sci. Med. Sci.* 62:932–942; 2007.
- 216 Doonan, R.; McElwee, J. J.; Matthijssens, F.; Walker, G. A.; Houthoofd, K.; Back, P.; Matscheki, A.; Vanfleteren, J. R.; Gems, D. 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; 2008.
- 217 Heidler, T.; Hartwig, K.; Daniel, H.; Wenzel, U. *Caenorhabditis elegans* lifespan extension caused by treatment with an orally active ROS-generator is dependent on DAF-16 and SIR-2.1. *Biogerontology* 11:183–195; 2010.
- 218 Jang, Y. C.; van Remmen, H. The mitochondrial theory of aging: insight from transgenic and knockout mouse models. *Exp. Gerontol.* 44:256–260; 2009.
- 219 Jang, Y. C.; Perez, V. I.; Song, W.; Lustgarten, M. S.; Salmon, A. B.; Mele, J.; Qi, W.; Liu, Y.; Liang, H.; Chaudhuri, A.; Ikeno, Y.; Epstein, C. J.; Van Remmen, H.; Richardson, A. Overexpression of Mn superoxide dismutase does not increase life span in mice. *J. Gerontol. A Biol. Sci. Med. Sci.* 64:1114–1125; 2009.
- 220 Lapointe, J.; Stepanyan, Z.; Bigras, E.; Hekimi, S. Reversal of the mitochondrial phenotype and slow development of oxidative biomarkers of aging in long-lived Mclk1^{-/-} mice. *J. Biol. Chem.* 284:20364–20374; 2009.
- 221 Van Raamsdonk, J. M.; Hekimi, S. Deletion of the mitochondrial superoxide dismutase sod-2 extends lifespan in *Caenorhabditis elegans*. *PLoS Genet.* 5: e1000361; 2009.
- 222 Yen, K.; Patel, H. B.; Lublin, A. L.; Mobbs, C. V. SOD isoforms play no role in lifespan in ad lib or dietary restricted conditions, but mutational inactivation of SOD-1 reduces life extension by cold. *Mech. Ageing Dev.* 130:173–178; 2009.
- 223 Zhang, Y.; Ikeno, Y.; Qi, W.; Chaudhuri, A.; Li, Y.; Bokov, A.; Thorpe, S. R.; Baynes, J. W.; Epstein, C.; Richardson, A.; Van Remmen, H. Mice deficient in both Mn superoxide dismutase and glutathione peroxidase-1 have increased oxidative damage and a greater incidence of pathology but no reduction in longevity. *J. Gerontol. A Biol. Sci. Med. Sci.* 64:1212–1220; 2009.
- 224 Pun, P. B.; Gruber, J.; Tang, S. Y.; Schaffer, S.; Ong, R. L.; Fong, S.; Ng, L. F.; Cheah, I.; Halliwell, B. Ageing in nematodes: do antioxidants extend lifespan in *Caenorhabditis elegans*? *Biogerontology* 11:17–30; 2010.
- 225 Sanz, A.; Fernandez-Ayala, D. J.; Stefanatos, R. K.; Jacobs, H. T. Mitochondrial ROS production correlates with, but does not directly regulate lifespan in *Drosophila*. *Aging (Albany)* 2:200–203; 2010.
- 226 Zintl, S.; Schwitala, D.; Luce, K.; Hamann, A.; Osiewacz, H. D. Increasing mitochondrial superoxide dismutase abundance leads to impairments in protein quality control and ROS scavenging systems and to lifespan shortening. *Exp. Gerontol.* 45:525–532; 2010.
- 227 Lam, Y. T.; Stocker, R.; Dawes, I. W. The lipophilic antioxidants α -tocopherol and coenzyme Q10 reduce the replicative lifespan of *Saccharomyces cerevisiae*. *Free Radic. Biol. Med.* 49:237–244; 2010.
- 228 Lithgow, G. J.; White, T. M.; Melov, S.; Johnson, T. E. Thermotolerance and extended life-span conferred by single-gene mutations and induced by thermal stress. *Proc. Natl Acad. Sci. U. S. A.* 92:7540–7544; 1995.
- 229 Vanfleteren, J. R.; De Vreese, A. The gerontogenes age-1 and daf-2 determine metabolic rate potential in aging *Caenorhabditis elegans*. *FASEB J.* 9:1355–1361; 1995.

- 230 Honda, Y.; Honda, S. The daf-2 gene network for longevity regulates oxidative stress resistance and Mn-superoxide dismutase gene expression in *Caenorhabditis elegans*. *FASEB J.* 13:1385–1393; 1999.
- 231 Murphy, C. T.; McCarroll, S. A.; Bargmann, C. I.; Fraser, A.; Kamath, R. S.; Ahringer, J.; Li, H.; Kenyon, C. Genes that act downstream of DAF-16 to influence the lifespan of *Caenorhabditis elegans*. *Nature* 424:277–283; 2003.
- 232 Houthoofd, K.; Fidalgo, M. A.; Hoogewijs, D.; Braeckman, B. P.; Lenaerts, I.; Brys, K.; Matthijssens, F.; De Vreese, A.; Van Eygen, S.; Munoz, M. J.; Vanfleteren, J. R. Metabolism, physiology and stress defense in three aging *Ins/IGF-1* mutants of the nematode *Caenorhabditis elegans*. *Aging Cell* 4:87–95; 2005.
- 233 Dong, M. Q.; Venable, J. D.; Au, N.; Xu, T.; Park, S. K.; Cociorva, D.; Johnson, J. R.; Dillin, A.; Yates III, J. R. Quantitative mass spectrometry identifies insulin signaling targets in *C. elegans*. *Science* 317:660–663; 2007.
- 234 Arumugam, T. V.; Gleichmann, M.; Tang, S. C.; Mattson, M. P. Hormesis/ preconditioning mechanisms, the nervous system and aging. *Ageing Res. Rev.* 5: 165–178; 2006.
- 235 El-Khoury, R.; Sainsard-Chanet, A. Deletion of the mitochondrial NADH kinase increases mitochondrial DNA stability and life span in the filamentous fungus *Podospora anserina*. *Exp. Gerontol.* 45:543–549; 2010.
- 236 Nazarewicz, R. R.; Ziolkowski, W.; Vaccaro, P. S.; Ghafourifar, P. Effect of short-term ketogenic diet on redox status of human blood. *Rejuvenation Res.* 10: 435–440; 2007.
- 237 Higuchi, M.; Cartier, L. J.; Chen, M.; Holloszy, J. O. Superoxide dismutase and catalase in skeletal muscle: adaptive response to exercise. *J. Gerontol.* 40: 281–286; 1985.
- 238 Lindsted, K. D.; Tonstad, S.; Kuzma, J. W. Self-report of physical activity and patterns of mortality in Seventh-Day Adventist men. *J. Clin. Epidemiol.* 44: 355–364; 1991.
- 239 Manini, T. M.; Everhart, J. E.; Patel, K. V.; Schoeller, D. A.; Colbert, L. H.; Visser, M.; Tylavsky, F.; Bauer, D. C.; Goodpaster, B. H.; Harris, T. B. Daily activity energy expenditure and mortality among older adults. *JAMA* 296:171–179; 2006.
- 240 Davies, K. J.; Quintanilha, A. T.; Brooks, G. A.; Packer, L. Free radicals and tissue damage produced by exercise. *Biochem. Biophys. Res. Commun.* 107:1198–1205; 1982.
- 241 Chevon, S.; Moran, D. S.; Heled, Y.; Shani, Y.; Regev, G.; Abbou, B.; Berenshtein, E.; Stadtman, E. R.; Epstein, Y. Plasma antioxidant status and cell injury after severe physical exercise. *Proc. Natl Acad. Sci. U. S. A.* 100:5119–5123; 2003.
- 242 Powers, S. K.; Jackson, M. J. Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiol. Rev.* 88: 1243–1276; 2008.
- 243 Gomez-Cabrera, M. C.; Domenech, E.; Romagnoli, M.; Arduini, A.; Borrás, C.; Pallardo, F. V.; Sastre, J.; Vina, J. Oral administration of vitamin C decreases muscle mitochondrial biogenesis and hampers training-induced adaptations in endurance performance. *Am. J. Clin. Nutr.* 87:142–149; 2008.