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NUTRITION, SIRTUINS AND AGING

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ABSTRACT: Beyond our inherited genetic make-up environmental factors are central for health and disease and finally determine our life span. Amongst the environmental factors nutrition plays a prominent role in affecting a variety of degenerative processes that are linked to aging. The exponential increase of non-insulin-dependent diabetes mellitus in industrialized nations as a consequence of a long-lasting caloric supernutrition is an expression of this environmental challenge that also affects aging processes. The most consistent effects along the environmental factors that slow down aging – from simple organisms to rodents and primates – have been observed for caloric restriction. In the yeast Saccharomyces cerevisiae, the fruit fly Drosophila melanogaster and the nematode Caenorhabditis elegans, sirtuins (silencing information regulators) have been identified to mediate as "molecular sensors" the effects of caloric restriction on aging processes. Sirtuins are NAD$^+$-dependent deacetylases that are activated when e.g. cell energy status is low and the NAD$^+$ over NADH ratio is high. As a consequence transcription rates of a variety of genes including that of the apoptosis inducing p53 gene are reduced. Moreover, in C. elegans, sirtuins were shown to interact with proteins of the insulin/IGF-1 signaling cascade of which several members are known to extend life span of the nematodes when mutated. Downstream targets of this pathway include genes that encode antioxidative enzymes such as superoxide dismutase (SOD) whose transcription is activated when receptor activation by insulin/IGF is low or when sirtuins are active and the ability of cells to resist oxidative damage appears to determine their life span. Amongst dietary factors that activate sirtuins are certain polyphenols such as quercetin and resveratrol. Whereas their ability to affect life span has been demonstrated in simple organisms, their efficacy in mammals awaits proof of principle.

KEY WORDS: Aging, Apoptosis, Calorie Restriction, Insulin/IGF-1 Signaling, Sirtuins.

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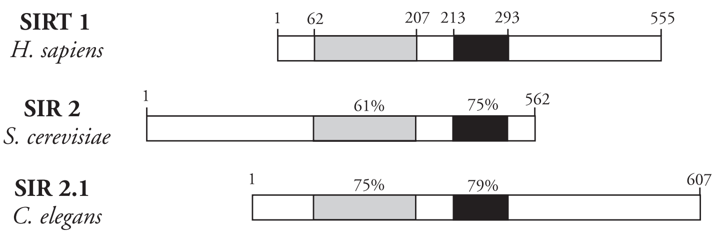
CALORIC INTAKE AND AGING

Within the last century the mean life expectancy of humans in the industrialized world has increased dramatically and this process seems to continue. It is suggested that by 2050 about 5% of the population in developed countries will be older than 85 years as compared to 1% in 1994 (Tuljapurkar *et al.*, 2000). This development is due to advances in disease prevention and treatment, and improvements in nutrition and infant mortality (Bernarducci and Owens, 1996). At the same time, however, there is a tremendous increase in "prosperity diseases" such as non-insulin dependent diabetes mellitus (NIDDM) that can be largely attributed to the increased secretion of adipokines due to the increased mass of adipose tissue in obese subjects (Schinner *et al.*, 2005). Since development of coronary heart disease and cancer, which are the two predominant causes for deaths in industrialized countries (Hoyert *et al.*, 1999), are clearly associated with obesity (Bray, 2004), it has to be concluded that obesity and its co-morbidities, that cluster to form the "metabolic syndrome", decrease the life-span which is counteracted by modern pharmacological interventions (Moon and Kashyap, 2004). This is further substantiated by the fact that people of a few countries known for their longevity have experienced lower life expectancies following the change in nutritional conditions towards high caloric diets (Walker and Walker, 1993). Since aging is generally associated with a reduction in insulin-sensitivity and a diminished glycemic control both of which are closely related to the accumulation of fat especially in the abdominal cavity (Barzilai and Gupta, 1999; Gupta *et al.*, 2000), it has to be suggested that the detrimental consequences of such physiological aging phenomena can be dramatically enforced when high loads of fuel are provided.

In all mammalian species studied so far, caloric restriction (CR) with a reduction of caloric intake from 25% to 60% of that of control animals fed ad libitum, extends the life span, provided that all essential nutrients are present in sufficient amounts in the diet (Koubova and Guarente, 2003). Besides conserving insulin-sensitivity, CR blunts sexual maturation and fecundity, which allows long-term survival through energy sparing (Holliday, 1989). It appears that a reduced white adipose tissue mass (WAT) serves as the prime signal for these effects since mice engineered to have less WAT live longer, although they do not eat less (Bluher *et al.*, 2003).

Fig. 1. Sirtuins from humans, *S. cerevisiae* and *C. elegans*. The numbers denote the amino acids in the polypeptide sequence. The binding sites for NAD$^+$ (grey), the substrate (black) and the percentage of homology to SIRT1 are indicated.



MOLECULAR LINKS OF CR AND AGING

In the yeast *Saccharomyces cerevisiae* it has been shown that the gene *sir2* is required for CR to act in slowing down aging (Anderson *et al.*, 2003). Sir2-like proteins, so-called sirtuins (silencing information regulators), are a family of NAD$^+$-dependent deacetylases conserved from simple organisms to humans (Smith *et al.*, 2000) (Fig. 1). In humans, seven sirtuins (SIRT1-7) have been identified (Frye, 2000) with SIRT1 being the most extensively studied. SIRT1, SIRT2, SIRT3 and SIRT5 convert NAD$^+$ and the acetylated substrate into deacetylated products, nicotinamide and *O*-acetyl-ADP-ribose. No such NAD$^+$-dependent deacetylase activity has been reported for SIRT4, SIRT6 and SIRT7 (Grubisha *et al.*, 2005). SIRT-1 is localized to the nucleus where it functions to silence chromatin by deacetylating histones in targeted regions of the genome (Michishita *et al.*, 2005). SIRT6 and SIRT7 also possess a nuclear localization but have different subnuclear distribution (Michishita *et al.*, 2005). SIRT-2 is located in the cytoplasm, whereas SIRT3, SIRT4 and SIRT5 are mitochondrial proteins (Michishita *et al.*, 2005). SIRT3 and SIRT5 may function as a link between metabolic and aging processes in humans given that mitochondria are organelles centrally involved in both aging and energy metabolism (Merry, 2004) and both proteins are affected by the NAD$^+$/NADH-ratio (Blander and Guarente, 2004). The easiest explanation for an increase in the NAD$^+$/NADH-ratio might be the lowered flux through glycolysis and tricarboxylic acid (TCA) cycle when glucose levels in the cell decrease. In yeast, however, it has been shown that cells respond to reduced glucose in the media by shunting more of the carbon to the TCA cycle to enhance the efficiency of ATP-generation by respiration instead of exploiting glycolysis when glucose offering is not restricted (Lin *et al.*, 2002). Although NAD$^+$- and NADH-levels have not been determined in *S. cerevisiae* under CR, it was suggested that increased respiration in restricted yeast is associated with an increased NAD$^+$/NADH-ratio in order to account for the activation of Sir2 measured (Koubova and Guarente, 2003). That the NAD$^+$/NADH-ratio can be regulated in a very complex way was shown in the livers of fasted mice where NAD$^+$-levels were increased by fasting and returned to control levels by refeeding without significant changes of NADH-levels (Rodgers *et al.*, 2005). Besides changes in energy state associated with altered NAD$^+$-levels, alterations of nicotinamide concentrations are likely to contribute to the physiological regulation of sirtuins. Nicotinamide, a product of the deacetylation reaction, is a potent inhibitor of Sir2 like proteins with an IC$_{50}$-value of around 120 µM, whereas other NAD$^+$-analogues show IC$_{50}$-values that are not consistent with a physiological role (Schmidt *et al.*, 2004). It has been shown that increased dosage of key enzymes for mammalian NAD$^+$-biosynthesis increase total cellular NAD$^+$-levels and enhance the transcriptional regulation activity of a mouse Sir2 orthologue (Revollo *et al.*, 2004). Such a strategy was shown also to protect injured axons in a SIRT1-dependent fashion in a Wallerian degeneration model (Araki *et al.*, 2004). These studies suggest that mammalian Sir2 orthologues are sensitive to metabolic pathways that regulate the levels of NAD$^+$. Moreover, independent on the mechanisms that control the activity of sirtuins the evolutionary conservation of the regulated activity of sirtuins suggests that they represent a set of effector proteins in a signal transduction pathway important for survival that monitors cellular energy and redox states. This holds true also for the nematode *Caenorhabditis elegans* in which *sir-2.1* deletion significantly suppressed the enhanced longevity of calorie-restricted mutants such as *unc-13* and *eat-2* (Wang and Tissenbaum, 2005). Moreover, a *sir-2.1* deletion strain is short lived and stress sensitive (Wang and Tissenbaum, 2005) whereas increased dosage of *sir-2.1* extends the adult life span of worms by up to 50% (Tissenbaum and Guarente, 2001). The signaling pathways through which sirtuins transfer the metabolic alterations into effects on life span are currently under investigation. One of the best characterized pathways playing a prominent role for aging is the insulin/IGF-1 signaling pathway (Fig. 2). Originally identified in *C. elegans* (Friedman and Johnson, 1988) as closely linked to aging processes, studies in *S. cerevisiae*, *D. melanogaster* and in mice demonstrate its central role in controlling aging and this holds most likely true also for humans (Barbieri *et al.*, 2003). In *C. elegans*, the effect of insulin/IGF-1 signaling is completely dependent on DAF-16, a (Lin *et al.*, 1997; Ogg *et al.*, 1997; Kenyon, 2005). DAF-16 is translocated into the nucleus when upstream signals of the insulin/IGF-1 pathway, that includes the DAF-2 insulin receptor-like protein, the AGE-1 PI3-kinase, the DAF-18 PTEN lipid phosphatase, and the serine/threonine kinases PDK-1, AKT-1, AKT-2 and SGK-1 (Fig. 2), are absent (Kenyon, 2005; Hertweck *et al.*, 2004). In contrast when the insulin/IGF-1 cascade is activated, nuclear translocation of DAF-16 is prevented by phosphorylation (Fig. 2) (Wolkow *et al.*, 2000; Hertweck *et al.*, 2004). When central members of the insulin-/IGF-1 pathway, such as DAF-2 or AGE-1 lose their function due to mutations, life span in *C. elegans* is increased two-fold as compared to wild-type animals (Kenyon *et al.*, 1993; Araki et al., 2004). Animals with weak alleles of the *age-1* and *daf-2* genes can bypass dauer formation as a non-feeding, stress-resistant larval state that allows dispersal under adverse conditions, and turn into long-living adults in a *daf-16* dependent manner (Kenyon *et al.*, 1993; Barbieri *et al.*, 2003).

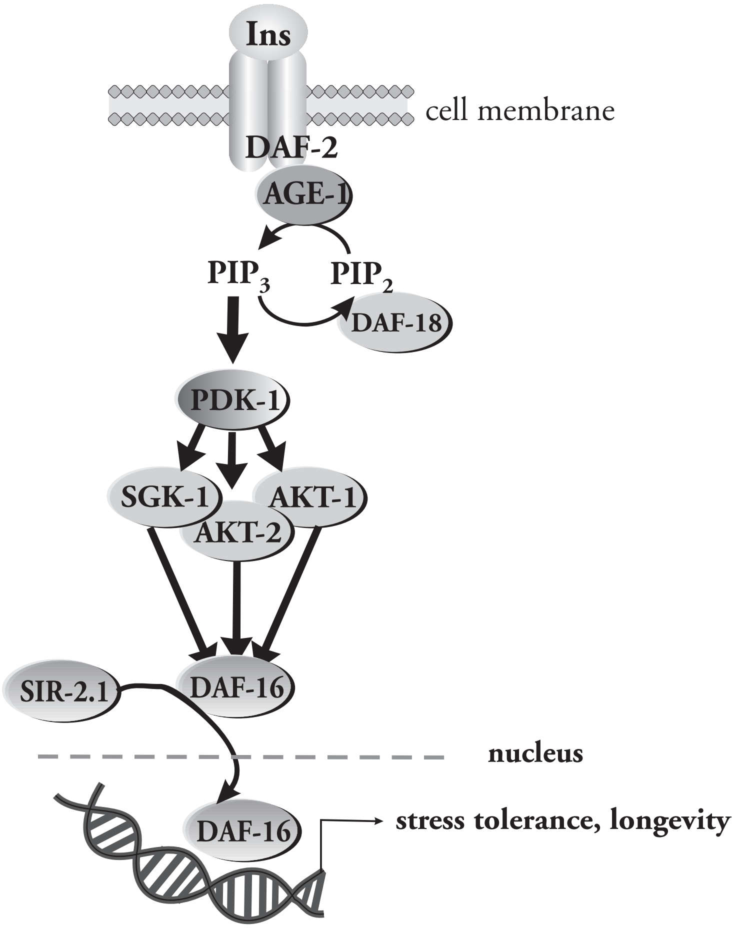


Fig. 2. Insulin/IGF-1 (Ins) signaling pathway in *C. elegans*. The insulin receptor DAF-2 affects stress response and life span via the forkhead transcription factor DAF-16. DAF-16 when phosphorylated through the serine/threonine kinases AKT-1, AKT-2 and SGK-1 remains in the cytosol whereas in the absence of Ins-signals DAF-16 translocates in the nucleus and genes that code for proteins involved in stress response and longevity are transcribed. Sir-2.1 by deacetylation of DAF-16 promotes translocation into the nucleus.

Recent studies show that sirtuins do interfere with the insulin/IGF-1 signaling pathway. In mammalian cells, SIRT1 and the FOXO transcription factor FOXO3 form a complex in response to oxidative stress, which leads to deacetylation of FOXO3 (Brunet *et al.*, 2004). As a consequence, FOXO3's ability to induce cell cycle arrest and resistance to oxidative stress is induced but its ability to induce cell death is inhibited (Brunet *et al.*, 2004). Thus, one way by which members of the Sir2 family of proteins may increase organismal longevity is by directing FOXO-dependent responses away from apoptosis and towards increased stress resistance (Brunet *et al.*, 2004). Downstream transcriptional targets of the nuclear FOXOs, including DAF-16, are e.g. heat-shock proteins, such as Hsp-16, involved in the synthesis of GSH (Escobedo *et al.*, 2004) and the reduction of GSSG to GSH (Baek *et al.*, 2000) and enzymes that detoxify reactive oxygen species (ROS), such as superoxide dismutase (SOD) or catalase (Yanase *et al.*, 2002). Consequently, as a general response to increased stress - either caused by ROS or CR - an increased stress defense program is initiated. In agreement with this, all long-lived mutants of *C. elegans* show an increased ability to respond to different stresses, including heat, UV, and ROS, irrespective of whether the genes providing the longevity phenotype are involved in the insulin/IGF-1 signaling pathway or not (Johnson *et al.*, 2002). Evidences from *C. elegans isp-1* mutants, that carry a missense mutation in the "Rieske" iron sulfur protein of complex III of the electron transport chain which results in low oxygen consumption, decreased sensitivity to ROS, and increased life span, suggest that especially mitochondrial ROS could affect the aging process by their detrimental effects on mitochondrial proteins and/or DNA (Feng *et al.*, 2001). A key role for ROS in aging can be further derived from results obtained in *daf-2/isp-1* double mutants, because they did not show additive effects on adult life span versus the *daf-2* or *isp-1* single mutants, that both displayed an increased ROS-resistance versus the wild-type worms, but based on mutations that do not interfere in a sense of common signaling (Feng *et al.*, 2001). Thus, the resistance to ROS associated with the high activity of detoxifying enzymes in the *daf-2* mutants, and the resistance to ROS associated with a low respiratory rate of the *isp-1* mutants, suggests that longevity in these mutants is due to a high ROS-detoxification capacity and a low ROS-production rate, respectively. In rodents, CR is also recognized to slow the rate of accrual of age-related oxidative stress (Merry, 2004). Although the oxidation products of proteins, lipids and DNA accumulate as a characteristic of aging processes, activation of redox sensitive transcription factors may have an even greater impact on cell function than the accumulation of these non-specific oxidative markers (Merry, 2004).

As mentioned above, sirtuins as activated by CR could initiate an antioxidative response by interfering with the insulin/IGF-1 signaling cascade (Fig. 2). In support of this it was demonstrated that longevity induced by increased SIR-2.1 activity in *C. elegans* is dependent on DAF-16 (Tissenbaum and Guarente, 2001). It was also demonstrated that *daf-2/sir-2.1* double mutants show life spans similar to *daf-2* mutants, indicating that SIR-2.1 functions either upstream of DAF-2 or in a parallel pathway to the DAF-2 insulin/IGF-1 signaling chain that finally converges at the level of DAF-16 (Fig. 2) (Wang and Tissenbaum). However, since long living eat-2 mutants do not require the activity of DAF-16 (Lakowski and Hekimi, 1998) whereas they need a functional SIR-2.1 for longevity (Tissenbaum and Guarente, 2001) it must be concluded that life span regulation by *sir-2.1* and *daf-16* occurs not via simple linear pathways.

CELLULAR LINKS BETWEEN CALORIC INTAKE AND AGING

Fat cells through a number of mechanisms seem to interfere with aging processes and WAT now emerges as being pivotal in controlling the life span of many organisms. As a matter of fact, sirtuins could be the central factor linking a reduction in WAT mass to life span extension since it was shown that human SIRT1 for example represses peroxisome proliferator-activated receptor γ (PPAR-γ) transactivation and thereby inhibits lipid accumulation in adipocytes (Picard *et al.*, 2004). In the most simplistic way it appears that reproductive function and insulin resistance, both of which are associated with accelerated aging, are also closely related to body fat mass and are both affected by hormones produced by adipocytes (Mora and Pessin, 2002). Moreover, free fatty acids released constantly into the circulation, especially from visceral WAT by its relative high number of β-adrenoceptors, induce insulin resistance when not oxidized immediately as is the case in the elderly (Toth and Tchernof, 2000; Möller and Kaufmann, 2005. Interestingly, human adipocytes show an increased expression of SIRT1 when they are incubated in serum from calorie-restricted rats, whereas both insulin and IGF-1 are to suppress SIRT1 up-regulation when added to the serum (Cohen *et al.*, 2004). In WAT, SIRT1 by inhibition of PPAR-γ not only acts as a repressor of genes involved in fat storage but also of genes that control adipocyte differentiation (Picard et al., 2004).

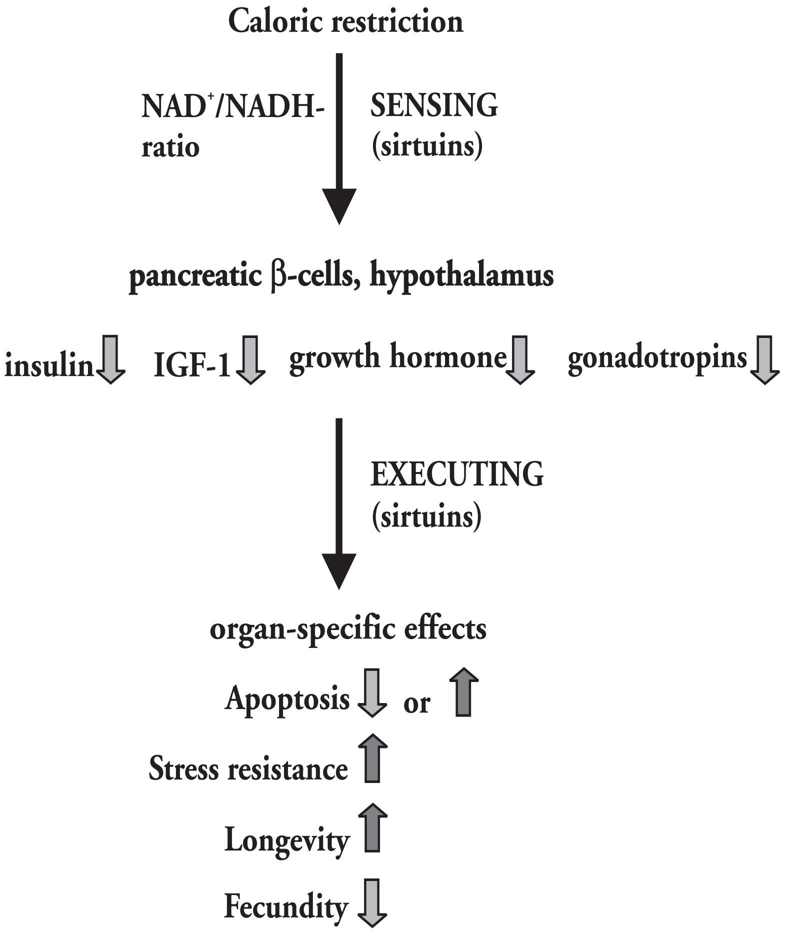
Apoptosis is another cellular process that can be influenced by sirtuins and that affects aging. In human embryonic kidney cells, SIRT1 was shown to deacetylate the DNA-repair factor Ku70, leading to the sequestration of pro-apoptotic bax away from mitochondria and thereby prevent stress-induced apoptotic cell death (Cohen *et al.*, 2004). The inhibition of apoptosis by sirtuins also includes the deacetylation of other proteins with crucial importance for apoptosis, for example of the pro-apoptotic tumor suppressor protein p53 resulting in negative regulation of p53-mediated transcriptional activation (Smith, 2002). Therefore, besides effects in WAT, sirtuins seem to have a high impact on aging by promoting the long-term survival especially of irreplaceable cells. Apoptosis is of crucial importance for the determination of mammalian life span since cells with a targeted disruption in the *p66shc* gene, one of the down-stream targets of p53, display an impaired p53-mediated apoptotic stress response but *p66shc*$^{-/-}$ mice showed a 30% prolonged life span as compared to wild-type animals (Migliaccio *et al.*, 1999). Conversely, a hyperactive allele of p53 confers enhanced tumor surveillance in transgenic mice but animals at the same time develop early organ degeneration and signs of premature aging (Tyner *et al.*, 2002). These findings substantiate a dual function of apoptosis for the organisms health state. It provides critical tumor surveillance during the reproductive life period but contributes to organ dysfunction and aging later on in life. SIRT1 could play the role of a double-edged sword in this context as HIC1 (Hypermethylated in cancer 1), an epigenetically regulated transcriptional repressor of SIRT1 is inactivated by hypermethylation not only in cancer but also during aging and results in an up-regulated SIRT1 expression and inactivation of p53 in normal and transformed mammalian cells (Chen *et al.*, 2005). Nevertheless, whereas CR reduces apoptosis especially in irreplaceable cells, suggesting that those are critical life span determinators, in organs with a high cell turn-over, as for example the gastrointestinal tract, apoptosis is increased by CR. Increased apoptosis in these organs appears not to affect life span but seems to prevent carcinogenesis in experimental models (Holt et al., 1998; Hursting and Kari, 1999).

Amongst the irreplaceable cells that may be considered as prime life span regulators, neuronal cells are in focus. Interestingly, the brain responds to CR with conservation of organ mass, which could be essential for the organism to reach the maximal life span (Weindruch and Sohal, 1997). Neuronal cells are, moreover, especially vulnerable to oxidative stress which is associated with advanced aging as supported by experimental findings in a variety of human neurodegenerative diseases. For example in some cases of amyotrophic lateral sclerosis (ALS), defects of SOD are found in all cells, but motoneurons are most affected (Rosen *et al.*, 1994). Most interestingly, in *Drosophila* carrying Cu/Zn SOD null mutations, transgenic expression of human SOD specifically in motoneurons could rescue the lifespan of the short-lived SOD null mutant and extends lifespan of wild-type animals by up to 40% (Parkes *et al.*, 1998). Overexpression of SOD only during the development period or in muscles did not affect life span of *Drosophila* suggesting that increased SOD-activity only in mature motoneurons is able to mediate life span extension (Parkes *et al.*, 1999). In *C. elegans* neurons also were proven to have a massive effect on life span. Specifically mutations in neuronal genes, which are associated with decreased sensory perception, can extend the mean life span by up to 50% without affecting feeding rate, development or fertility (Apfeld and Kenyon, 1999). This demonstrates that the effects by which neurons affect life span are rather direct. The intensity of the insulin/IGF-1 signaling pathway seems to mediate these effects of neurons on the life span in *C. elegans* as *daf-2* mutations only in neurons arrest the worms in their development at the dauer larval stage, a phenotype typically found in animals with reduced insulin/IGF-1 signaling (Apfeld and Kenyon, 1999). Moreover, the dauer arrest phenotype in *C. elegans* carrying *daf-2* mutations in all cells, could be rescued by neuronal expression of a functional *daf-2* but not when expressed in muscle or intestine (Wolkow *et al.*, 2000). Other studies in *C. elegans* show that removal of gustatory and olfactory neurons results in longer life span (Alcedo and Kenyon, 2004). A deletion mutant for thioredoxin, an antioxidative protein with restricted expression in ASI and ASJ sensory neurons and in intestine, shows also a reduced life span (Jee *et al.*, 2005). Taken together, neurons are obviously critical regulators of life span. However, it is yet not understood of whether these effects are dependent on the survival of neurons and the conservation of neuronal cell mass, which may be achieved by a low insulin/IGF-1 signaling and/or high sirtuin activity and in consequence by a lowered apoptosis rate, or whether neurons exert beneficial effects on other cell types which are reduced when neuronal activity is impaired. Results from *unc-64* and *unc-31* mutant *C. elegans* lines demonstrate altered Ca^{2+}-regulated secretion -

most probably of an insulin-like ligand of the DAF-2 receptor - in combination with an increased adult life span and constitutive dauer formation. This suggests that the neuroendocrine signals protect additional cells from age-related damage (Ailion *et al.*, 1999). That the neuroendocrine system plays a pivotal role for controlling aging in higher animals and probably in humans as well comes from studies in rodents. Calorically restricted mice and rats, that live longer than when fed ad libitum, show lower levels of growth hormone, which in turn reduces the levels of circulating IGF-1 and also of thyroid stimulating hormone and gonadotropins (Mobbs *et al.*, 2001). Conversely, levels of glucocorticoids, catecholamines and glucagon are increased by CR (Mobbs *et al.*, 2001). That those neuroendocrine changes can be directly linked to slowed aging was demonstrated in growth hormone receptor deficient mice that showed significantly lower levels of IGF-1 and an extended life span versus wild-type mice (Coschigano *et al.*, 2000).

To define the input signals for the neuroendocrine responses that contribute to aging processes is even more complicated as assessing the output signals. However, glucose appears to represent a central and important metabolite. Specific hypothalamic neurons do sense - like the β-cells in the pancreas - glucose by the metabolic conversion of NAD$^+$ to NADH and respond to changes in the NAD$^+$/NADH-ratio with altered neurotransmission (Yang *et al.*, 1999). It appears possible that the sirtuins, as NAD$^+$-dependent deacetylases, are also important regulators in neuronal cells that, when the NAD$^+$/NADH-ratio is high, inhibit apoptosis and/or affect neurosecretion. Sirtuins thus could sense the energy status and when glucose levels are lowered by CR not only a reduced secretion of insulin from the pancreatic ß-cells but also of growth hormone and gonadotropins from hypothalamic neurons occurs (Fig. 3). In turn, lowered insulin and IGF-1 levels reduce the activity of the insulin/IGF-1 signaling pathway and the reduced gonadotropin levels result in reduced reproductive capabilities; features characteristically observed under CR (Fig. 3). That sirtuins in neurons play an important role for the healthiness of the whole organism is suggested by studies showing that *C. elegans* that overexpress a pathogenic version of huntingtin in touch-receptor neurons are protected from pathology by an overexpression of *sir-2.1* (Parker *et al.*, 2005). Moreover, in mouse neurons overexpressing a mutant huntingtin, apoptotic cell death was suppressed by an activator of sirtuins and the effects could be blocked specifically using sirtuin inhibitors (Parker *et al.*, 2005). The importance of sirtuins for neuroprotection is finally stressed by the fact that NAD$^+$-levels decrease in degenerating axons and that preventing this axonal NAD$^+$-decline efficiently protects from degeneration (Wang *et al.*, 2005).

Fig. 3. Model of how caloric restriction extends the life span of organisms. Sirtuins are at the center of effectors by sensing the NAD$^+$/NADH-ratio and responding to changes by adjusting hormonal levels and executing a slowing of aging in other organs than the nervous system as well. (Modified from Koubova J, Guarente L. Genes & Development 2003; 17: 313-321).



TO FOOL BIOLOGY

Considering that sirtuins are central regulators involved in aging processes, the quest for molecules that activate sirtuins (sirtuin activators, STACs) has began by trying to mimic CR. Screening of a library for molecules that alter SIRT1 activity yielded quercetin and piceatannol, both of which are typical red wine phenolics (Palmieri *et al.*, 1999; Howitz *et al.*, 2003). Further analysis revealed that the most potent STAC was resveratrol, another polyphenol from red grapes (Howitz *et al.*, 2003). At a concentration of 25 µM of both NAD$^+$ and SIRT1-substrate, resveratrol at a concentration of 11 µM doubled the rate of deacetylation by SIRT1 and caused its maximal stimulation at 100-200 µM (Howitz *et al.*, 2003). Whereas resveratrol increased the affinities of SIRT1 for both, its substrate and for NAD$^+$ it displayed no effects on the apparent V_{max} of the enzyme (Howitz *et al.*, 2003). This raises the question of whether activation of sirtuins by STACs is as effective also *in vivo* where sirtuins probably work under V_{max} conditions. *In vivo* evidence, however, comes from studies in *S. cerevisiae* and *C. elegans* which showed that resveratrol at concentrations of 10 µM in yeast and 100 µM in the nematode extended the life span in dependence on *sir2* and *sir-2.1*, respectively (Howitz *et al.*, 2003; Wood *et al.*, 2004). Another critical point regarding the activation of sirtuins in mammals is the concentration needed to act as STAC since plasma levels of 10 µM or even higher are difficult to achieve. However, studies in mice suggest that resveratrol concentrations in the range of 0.5 µM are active in protecting mouse neurons from cell death by activation of sirtuins (Parker *et al.*, 2005), possibly due to resveratrol metabolites that must be much more potent STACs than resveratrol itself then. Independent on whether activation of sirtuins by nutritional or pharmacological interventions can be achieved in humans, it must be questioned whether "fooling" biology by CR-mimetics resembles in all aspects the complex alterations of metabolism that occur under CR. Although the activation of the downstream targets of sirtuins such as FOXOs may result in an increased expression of antioxidative enzymes, it is not known yet whether an increased defense status induced by a CR-mimetic is effective without dietary intervention. On background of a western diet high in energy and yielding in the long run high plasma glucose concentrations a CR-mimetic may not be able to protect from the detrimental effects of sustained high blood glucose levels. In diabetes, in which an absolute or relative lack of insulin results in reduced insulin-dependent signaling the effects of glucose caused pathologies are still seen, especially in tissues with an unrestricted uptake of glucose that show also enhanced ROS production and increased accumulation of advanced glycation end products (AGEs), both of which are commonly associated with accelerated aging (Osawa and Kato, 2005).

CONCLUSIONS

So far, restricting caloric intake is the only nutritional manoeuvre proven to slow down aging and this has been shown in numerous species, from simple organisms to primates. Sirtuins are key regulators in the response of an organism to CR. They are NAD^+-dependent deacetylases that transmit CR into a reduced activity of the insulin/IGF-1 signaling or impaired apoptosis, leading to an overall increased defense status and enhanced neuronal cell survival. CR is also transmitted into altered neuroendocrine signaling that in turn affects aging processes in other tissues. In search for CR-mimetics, several polyphenols as found in fruits and vegetables but especially those found in high concentrations in red wine were identified as potent activators of sirtuins. Although these compounds may increase the defense status of an organism, it is not established as yet whether sirtuin activation in higher animals or humans can accomplish all effects of CR on aging when a sedentary lifestyle is maintained.

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