Energy restriction and potential energy restriction mimetics

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Abstract

Energy restriction (ER; also known as caloric restriction) is the only nutritional intervention that has repeatedly been shown to increase lifespan in model organisms and may delay ageing in humans. In the present review we discuss current scientific literature on ER and its molecular, metabolic and hormonal effects. Moreover, criteria for the classification of substances that might induce positive ER-like changes without having to reduce energy intake are summarised. Additionally, the putative ER mimetics (ERM) 2-deoxy-d-glucose, metformin, rapamycin, resveratrol, spermidine and lipoic acid and their suggested molecular targets are discussed. While there are reports on these ERM candidates that describe lifespan extension in model organisms, data on longevity-inducing effects in higher organisms such as mice remain controversial or are missing. Furthermore, some of these candidates produce detrimental side effects such as immunosuppression or lactic acidosis, or have not been tested for safety in long-term studies. Up to now, there are no known ERM that could be recommended without limitations for use in humans.

Key words: Energy restriction: Energy restriction mimetics: Healthy ageing: Longevity: Lifespan

Introduction

Overweight and obesity are emerging problems all over the world – not only developed countries but also recently industrialised and developing countries are struggling under the burden of this epidemic(1). In Germany, one in five adults suffers from severe obesity, indicated by a BMI of 30 kg/m² or higher(2) and even 60 % of German adults are overweight (i.e. with a BMI of 25 kg/m² or higher)(3). Apart from limitations in everyday life such as impaired physical performance or dyspnoea caused by obesity, the incidence of associated disorders severely increases the more body weight is gained(4–6). In the past, chronic diseases like type 2 diabetes mellitus (T2DM), hypertension, CVD and cancer were mostly observed in the elderly. Nowadays, many young individuals are also constrained by these pathologies because of excess weight(7). It is estimated that the worldwide mortality from chronic diseases will increase up to 66 % in 2030(11). For this reason, food and health agencies such as the USDA (United States Department of Health and Human Services and United States Department of Agriculture) recommend choosing foods high in vitamins and minerals but low (to moderate) in energy density in order to decrease the risk of such diet-related diseases. Indeed, by changing Western eating patterns, enhancing physical activity levels and avoiding tobacco use, the risk of CVD mortality from chronic diseases will increase up to 66 % in 2030(1).

It has been hypothesised that ageing and its associated diseases may be the result of increasing amounts of altered nuclear and mitochondrial DNA, structurally aberrant proteins, and oxidised lipids that lead to structural and functional impairment of single cells and whole organisms(8–9). This may impede dealing with and recovering from endogenous and environmental stress, thereby favouring the development of age-related chronic diseases(10–11).

In the following, current literature on energy restriction (ER; also known as caloric restriction), the most promising method to counteract obesity and ageing, and on its underlying mechanisms such as anti-inflammatory and antioxidant effects is reviewed. Additionally, substances that have been discussed as potentially mimicking ER are also discussed.

Energy restriction and lifespan in model organisms

ER, a reduction in energy intake of 20 % (mild ER) to 50 % (severe ER)(12–13) without a reduction in essential nutrients or malnutrition(14), was shown to reduce obesity and prevent premature onset of chronic ageing-associated diseases(15). Up to now ER is the only intervention which reliably increases lifespan in various model organisms(10).

In an early study in 1935, rats on a hypoenergetic diet showed increased lifespan(16). Since then several studies have

Abbreviations: 2DG, 2-deoxy-d-glucose; AMPK, AMP-activated protein kinase; ER, energy restriction; ERM, energy restriction mimetic; FOXO, forkhead box O; GH, growth hormone; IGF-1, insulin-like growth factor 1; mTOR, mammalian target of rapamycin; mTORC1, mammalian target of rapamycin complex 1; NIA, United States National Institute on Aging; Nrf2, nuclear factor (erythroid-derived 2) like 2; p-AMPK, phosphorylated AMPK; PGC1α, PPAR γ coactivator 1-α; ROS, reactive oxygen species; RSV, resveratrol; SIRT, sirtuin; SOD, superoxide dismutase; SPD, spermidine; T2DM, type 2 diabetes mellitus; UCP, uncoupling protein.

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verified these findings in model organisms extending from yeast\(^{17}\) over invertebrate species like \textit{Caenorhabditis elegans}\(^{18}\) and \textit{Drosophila melanogaster} \(^{19}\) to rodents and primates\(^{20–22}\). Conversely, there are reports that ER may also shorten lifespan in mammals\(^{23}\) and a meta-analysis by Swindell\(^{24}\) concluded that the ER-induced lifespan increase in rodents depended on the genotype. Furthermore, it is questionable whether \textit{ad libitum}-fed animals are the adequate controls for ER studies because \textit{ad libitum} intake can lead to overweight and consequently shorten lifespan\(^{25}\). In contrast to an earlier study in rhesus monkeys at the Wisconsin National Primate Research Center\(^{22}\), Mattison \textit{et al.}\(^{26}\) found, on the one hand, that ER did not affect the animals’ lifespan. On the other hand, the ER-fed monkeys showed a later onset of age-related diseases than the \textit{ad libitum}-fed controls and therefore an increased healthspan.

In the Biosphere 2 study in the early 1990s a small number of human subjects unintentionally had limited access to food for about 2 years. Being sealed into a materially closed ecosystem these human subjects were farming and processing their own food, resulting in an energy-limited but nutrient-dense diet. This ER of up to 30\% led to phenotypes similar to those known from ER studies in model organisms\(^{27–29}\). As with rodents and primates, the human subjects displayed decreased body weight and temperature, lowered fasting glucose and insulin levels, and reduced BMR and blood pressure compared with before the study\(^{27,29}\). A 25\% reduction in energy intake for 6 months in the statistically high-powered CALERIE (Comprehensive Assessment of the Long-term Effects of Reducing Intake of Energy) study by the United States National Institute on Aging (NIA) showed results consistent with these findings. Although there are few data on ER in humans, it is known that the inhabitants of Okinawa Island traditionally consumed a modified Japanese diet containing about 20\% less energy than the Western high-fat diet. Interestingly, the number of centenarians in this population is 4- to 5-fold higher than in Western populations and the Okinawan life expectancy is the highest in the world\(^{30}\). However, in the Okinawan population aged under 65 years, there seems to be a trend towards a lower life expectancy compared with the older generation. This is most probably due to a change from traditional dietary patterns to energy-dense Western-type diets that was initiated in the 1960s\(^{31}\).

**Physical appearance during energy restriction**

In addition to a reduction in body weight and fat content\(^{22,32}\), body temperature also decreases under ER\(^{33,34}\). In model organisms on a lifelong ER, a decline in growth and delayed sexual maturity occurred\(^{34}\) possibly resulting in reduced reproduction rates. In these hypenergetic-fed animals, a shift from development and reproduction towards maintenance can be observed. Interestingly, changes in activity levels upon ER feeding seem to be species dependent. While rodents undergoing ER showed higher activity levels than \textit{ad libitum}-fed controls\(^{35}\), primates displayed mostly unchanged activity patterns compared with the controls\(^{32,34}\).

Fig. 1 summarises some of the suggested targets that energy restriction might address.

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**Fig. 1.** Schematic overview of the suggested targets that energy restriction might address.
Blood parameters during energy restriction

As expected from epidemiological studies, in rodent models reduced body weight in the ER groups was accompanied by a decreased risk of developing age-related diseases such as T2DM(50). In initially overweight as well as in normal-weight subjects, fasting plasma glucose and insulin were reduced(37,38) whereas adiponectin levels(59) and insulin sensitivity increased(40) upon energy restriction. Interestingly, consuming energy-restricted diets even reversed existing diabetic changes like insulin resistance(41). ER regimens were also seen to attenuate hypertension(42) and hyperlipidaemia(43). The latter observation might also be due to changes in nutrient utilisation. Since, in order to compensate for reduced glucose intake upon ER, gluconeogenesis from lipids and amino acids is induced(44). In contrast, as shown in rats, glycolysis is reduced under ER(45).

Moreover, improved gluoregulation reduces glycation reactions, in which blood glucose molecules react with amino acids, proteins or nucleic acids in Maillard reactions to form advanced glycation endproducts (AGE)(46). These can bind to their receptors (receptors for AGE; RAGE) in various tissues, thereby inducing renal, vascular or neurological changes that are observed in ageing-related pathologies(40,47). Thus, reducing blood glucose levels via ER might contribute to lowering the risk of developing age-dependent diseases such as CVD(48), neurodegenerative diseases(49) and cancer(50,51).

While insulin secretion is decreased, plasma concentrations of gluocorticoids usually increase when food intake is limited(52). As to be expected from reduced growing rates and the slowing down of maturation, levels of anabolic hormones like leptin and insulin, testosterone, oestradiol(53) and follicle-stimulating hormone(54) decreased upon ER in several species. However, changes in levels of steroid hormones seem to be dependent on the species, as no changes were seen in primates. In contrast, plasma concentrations of the catabolic adipokine nectin(39) and of the steroid hormone-binding protein(54) tend to increase by ER. Probably, increased steroid hormone-binding protein levels contribute to the reduced availability of insulin resistance(41). ER regimens were also seen to attenuate hypertension(42) and hyperlipidaemia(43). The latter observation might also be due to changes in nutrient utilisation. Since, in order to compensate for reduced glucose intake upon ER, gluconeogenesis from lipids and amino acids is induced(44). In contrast, as shown in rats, glycolysis is reduced under ER(45).

Cellular processes affected by energy restriction

Energy restriction and oxidative status

Recently, an increasing number of researchers have become interested in the hormesis effect on healthy ageing(66). This phenomenon describes how a low dose of a stimulus (for example, ROS) induces a positive effect and a high dose of the same stimulus induces a negative effect(67). As far as ER is concerned, limiting energy supply can be seen as a factor causing mild stress in an organism which activates mechanisms of endogenous stress response, thereby improving the overall protection against stress(68,69). Consistently, enhanced expression of heat shock proteins and antioxidant enzymes under ER support this hypothesis(70). Since accumulated stress is observed in age-related disease, ER might enhance healthspan and lifespan via hormesis(68).

Although oxidative damage accumulates during ageing(71) and is characteristic of age-related diseases such as CVD(72), cancer(73) and neurodegenerative pathologies(74,75), it is not known whether increased ROS levels are the cause or a consequence of ageing.

ER is assumed to alleviate the age-associated increase in oxidative stress(76), probably via improving endogenous stress response mechanisms(40) including several redox-sensitive transcription factors. Indeed, in mice undergoing ER antioxidant enzymes under the transcriptional control of the redox-sensitive transcription factor nuclear factor (erythroid-derived 2) like 2 (Nrf2) such as NQO1 (NAD(P)H dehydrogenase, quinone 1) and cancer(50,51).

Nrf2 protects the organism from the harmful effects of oxidative stress(76). Nrf2 transactivates the expression of genes encoding enzymes that may prevent oxidative damage to cellular structures or remove damaged molecules(79). Apart from NQO1, Nrf2 also induces the transcription of phase II enzymes like glutathione-S-transferases in mice(79). Moreover, Nrf2 knock-out mice showed decreased expressions of the antioxidant enzymes catalese, haem oxygenase 1 (HO1) and superoxide dismutase (SOD1) in mice(79). By stimulating proteasomal degradation, Nrf2 can further reduce the burden of oxidatively damaged macromolecules(83). Interestingly, the expression and transcriptional activity of Nrf2 were shown to decline in tissues of ageing rodents(82,83) and ER was shown to enhance the activity of Nrf2. In mice under ER several Nrf2 target genes were up-regulated, which protected these mice from tumorigenesis. However, this anti-cancer effect was diminished in Nrf2 knock-out mice(84), suggesting a contribution of Nrf2 to the health-promoting effects induced by ER(85). Additionally, oxidative damage to macromolecules seems to be reduced upon ER(76), but these beneficial effects often occur only after several months of ER(66).

However, in lifespan studies, mutants expressing higher levels of antioxidant enzymes did not always live longer than the controls. While a SOD1 knock-out decreased lifespan in mice(87), higher activity of SOD1 (CuZnSOD) did not promote longevity in mice(88). Although higher levels of oxidative damaged DNA could be detected in animals with reduced SOD2 (MnSOD) activity, a SOD2 knock-out trial did not reveal different lifespans in transgenic v. control mice(89). Similarly, supplementing mice with...
antioxidants like glutathione\(^{90}\), vitamin C\(^{91}\), lipoic acid or coenzyme Q10\(^{92}\) did not increase lifespan compared with the untreated controls.

However, in the case of catalase, overexpression of this antioxidant enzyme extended lifespan in mice\(^{93}\).

**Energy restriction and inflammation**

While Nrf2, which is thought to be protective against age-related diseases\(^{95}\) was shown to decrease with age\(^{94,95}\) the transcription factors NF-κB, activator protein-1 and hypoxia inducible factor-1 were shown to be up-regulated age-dependently and in the presence of ROS\(^{10}\). When activated, they contribute to the development of inflammation and associated disorders, for example, arthritis\(^{94}\), cancer, atherosclerosis or neurodegenerative diseases\(^{95,96}\).

NF-κB leads to the expression of pro-inflammatory genes encoding cytokines, chemokines and inflammatory cell adhesion which in turn worsen the ROS overload and further promote the activity of NF-κB\(^{97}\). ER consistently decreased the transcriptional activity of NF-κB and hypoxia inducible factor-1\(^{98-100}\). However, the precise mechanism of how ROS increases the activity of these transcription factors is largely unknown\(^{100,101}\). Most likely the reduced load of ROS contributes to a well-balanced cellular redox state, thereby potentially preventing the development of disadvantageous age-associated pathologies\(^{100}\).

Inflammation increases with age\(^{102}\) and body weight\(^{103}\) and is often related to tissue injury, organ dysfunction, fibrosis, several chronic diseases and ageing in general\(^{104}\).

ER increased the anti-inflammatory hormones adiponectin, ghrelin and corticosteroids\(^{660}\) and PPAR transcription factors as well as NF-κB inhibitor α\(^{105,106}\) in the plasma of human subjects and rodents. In contrast, NF-κB targets such as inflammation-promoting PG, thromboxanes and other cytokines (TNFα, IL-6, inducible NO synthase, vascular cell adhesion molecule and intercellular adhesion molecule) were decreased\(^{32,99,105,106}\). In line with these findings, a diet rich in fat and sugar may increase systemic inflammation\(^{77}\).

**Energy restriction and mitochondrial metabolism**

The cellular energy suppliers mitochondria are very sensitive to oxidative damage and are a considerable ROS source themselves\(^{109}\). Most importantly, these double-membraned organelles contain a subset of different proteins that generate ATP as an energy source for the organism. However, in the oxidation steps of the mitochondrial electron chain, superoxide radicals are formed from leaking electrons and molecular oxygen\(^{110,111}\). Leakage of electrons and therefore basal ROS production increases once the mitochondria become old\(^{109,112,113}\) and decreased efficiency in mitochondrial ATP production during ageing leads to less energy and more ROS\(^{114}\), which in turn further damages mitochondria. Similar to other antioxidant defence mechanisms, the activity of the mitochondrial antioxidant defence enzyme SOD2 (MnSOD) also declines with increasing age\(^{115}\), thereby favouring the vicious cycle of less efficient enzymes in the mitochondrial respiratory chain. That leads to an increase in oxidative stress resulting in further mitochondrial destruction.

Thus, it seems useful to promote mitochondrial turnover by enhancing biogenesis of new mitochondria and degradation of old and damaged ones. Biogenesis of mitochondria is a complex multistep interplay of various transcripts and proteins with nuclear, cytosolic and mitochondrial origin\(^{116}\). It may be activated in response to different stimuli like nutrient supply, endocrine signals, growth factors and changes in temperature\(^{117}\). PPAR γ coactivator 1α (PGC1α) is thought to be the key modulator of mitochondrial biogenesis and function, as it regulates the activity of other important factors in mitochondrial biogenesis like nuclear respiratory factors 1 and 2 and oestrogen-related receptors\(^{118}\). In turn, nuclear respiratory factors 1 and 2 may induce mitochondrial transcription factor A, which is essential in controlling the expression of mitochondrial genes\(^{119}\). Oestrogen-related receptors target genes are thought to be involved in nutrient-degrading processes, oxidative phosphorylation and mitochondrial dynamics\(^{119}\).

ER was shown to induce mitochondrial biogenesis by enhancing the expression of PGC1α and nuclear respiratory factor 1 as well as their downstream target mitochondrial transcription factor A\(^{120}\). Moreover, PGC1α seems to be involved in mitochondrial function as it enhanced the activity of respiratory chain enzyme complexes III and IV in a cell culture model\(^{121}\). In line with these findings, AMP-activated protein kinase (AMPK), which is known to be up-regulated upon ER, was shown to affect mitochondrial development and metabolism positively\(^{122}\).

Importantly, ER-induced unselective and selective autophagic degradation may remove old and damaged mitochondria\(^{123}\), thereby probably reducing oxidative stress. Indeed, ER enhanced mitochondrial turnover in mouse liver, measured as reduced mitochondrial half-life\(^{124}\).

**Energy restriction and autophagic processes**

Autophagy is an intracellular, lysosomal degradation process that aims at recycling cell organelles, proteins and macromolecules. In all types of autophagy (macroautophagy, microautophagy and chaperone-mediated autophagy) the freight is sequestered and transported to lysosomes, where it is degraded by different hydrolases\(^{125}\). While in macro- and microautophagy heterogeneous cytoplasmic content is taken up into vesicles, chaperone-mediated autophagy is able to target, transport and degrade specific proteins\(^{123,125,126}\). However, macroautophagy may also selectively incorporate cargo (such as mitochondria) into autophagosomes, which later fuse with lysosomes to form autolysosomes\(^{127}\). In the (auto)lysosomes, acidic hydrolases shed organelles and molecules into their components. After transport back to the cytoplasm, these components can be reused for biosynthetic or energy-generating processes\(^{128}\). Fig. 2 shows the major events of macroautophagy.

For the best-studied type of autophagy, macroautophagy (in the following referred to as autophagy), it was shown that autophagy-related proteins (ATG) initiate and perform this lysosomal degradation pathway\(^{129}\). These ATG are mostly activated under catabolic conditions. Many of them have been
shown to be activated by sirtuins (SIRT) and/or inhibited by mammalian target of rapamycin (mTOR) complex 1 (mTORC1), which are induced or repressed by ER, respectively. Consistently, ATG's such as the mammalian ATG8 homologue LC3 were induced upon ER treatment.

The reason why autophagy is induced upon ER is most probably because ER causes a shift from development and reproduction towards maintenance. Under these circumstances autophagy is necessary for recycling of cellular material required for rebuilding essential cell structures and for generating energy. Interestingly, in lifespan experiments the pro-longevity effect of ER depended on autophagy induction. In line with the finding that autophagy induction contributed to the pro-longevity effect of ER, autophagy was shown to decrease during the ageing process. By accelerating the turnover of proteins, membranes and other organelles, autophagy also contributes to maintaining efficiency of peroxisomes, ER and importantly mitochondria. Thus, active autophagy could possibly counteract the age-related increase in ROS generation/oxidative stress. This potentially reduces the risk for neurodegenerative diseases like Alzheimer’s, Huntington’s or Parkinson’s in which the accumulation of redundant proteins, followed by functional impairments and the death of post-mitotic neurons are observed. As autophagy removes aggregation-prone redundant or damaged molecules and apoptotic bodies, increased autophagy might attenuate the progress of neurodegenerative diseases. Indeed, autophagy was found to be dysregulated in patients suffering from Alzheimer’s disease, Parkinson’s disease and Huntington’s disease. Therefore, targeting autophagy induction, for example, by ER, seems to be a valuable strategy for delaying the onset of these diseases and increasing healthspan and lifespan.

### Molecular targets of energy restriction

**AMP-activated protein kinase**

In the case of limited energy supply, ATP levels decline and the AMP:ATP ratio increases which in turn activates, i.e. phosphorylates, the nutrient sensor AMPK. Thus, high levels of phosphorylated AMPK (p-AMPK) are indicative of low energy supply. Additionally, AMPK appears to be regulated by hormones like leptin and adiponectin, ghrelin and thyroxine.

AMPK phosphorylation leads to activation of various downstream targets such as SIRT1, PGC1α and some forkhead box O (FOXO). This in turn leads to deacetylation of transcription factors and other proteins, biogenesis of mitochondria and stress defence mechanisms. Additionally, AMPK was shown to inhibit the central kinase mTOR, which in its activated complex mTORC1 promotes cell growth and proliferation.

Indeed, AMPK activation has been found to promote longevity in model organisms. Whereas decreased AMPK activity reduced the lifespan of D. melanogaster, an increase in lifespan could be observed in yeast and C. elegans with activated AMPK.

However, the results concerning the effect of ER on AMPK activity are inconsistent. While some studies found increased p-AMPK in the heart, muscle and liver of lifelong ER-fed mice, others did not observe any changes in p-AMPK levels.

Interesting insights come from studies that supplemented model organisms with substances that mimic ER. It could be shown that AMPK is necessary for the life-prolonging effect of such ER mimetics. Additionally, the deacetylase SIRT1 was also shown to be necessary for ER-mimetic-induced lifespan extension.

**Sirtuins**

Various studies have shown that SIRT1 is induced by ER. SIRT1 is part of the sirtuin family – a group of energy-sensing protein deacetylases. In an energy-dependent manner, SIRT1 regulates the activity of many proteins, for example, transcription factors, by changing their state of acetylation. Using NAD+ as a cofactor (the concentration of which rises upon limited nutrient supply), SIRT1 catalyses the transfer of a lysine-bound acetyl group from a protein substrate to NAD+. Therefore, forming nicotinamide, O-acetyl-ADP-ribose and the deacetylated substrate.
Via deacetylation of histones, SIRT1 might modulate the expression of several genes involved in energy metabolism. Due to their lysine and arginine residues, histones are charged positively and they form tight complexes with the negatively charged DNA, thereby impeding the RNA polymerase access to the DNA. The acetylation of lysine and arginine residues in the histone weakens its interaction with the DNA, which renders the DNA sequences more susceptible for binding of the polymerase, leading to enhanced gene transcription. Thus, deacetylating histones might down-regulate gene expression.

Additional substrates of SIRT1 include proteins that are involved in mitochondrial biogenesis and turnover. By deacetylating PGC1α and thereby enhancing its activity, SIRT1 might induce mitochondrial biogenesis. Moreover, SIRT1 seems to enhance the activity of ATG5, and 7 and 8, which promotes autophagy induction. Similar to ER, increased expression of SIRT1 (or its homologues) in yeast, C. elegans, D. melanogaster and mice enhanced lifespan. Mice overexpressing SIRT1 revealed an improved glucose homeostasis, including increased insulin sensitivity and were protected from developing T2DM. High-fat diet-fed mice with increased SRT1 expression seemed to be resistant to the metabolic syndrome. Moreover, SIRT1 was shown to prevent metabolic syndrome-induced tumour development. Via interacting with and inhibiting the binding sites of PPARγ (a transcription factor that regulates lipid metabolism), SIRT1 reduces the expression of its target genes in white adipocytes. Thus, SIRT1 may also inhibit lipid metabolism, storage and enhance fat mobilisation in white adipose tissue.

Conversely, some studies found that ER increased lifespan even in the absence of SIRT1 and other SIRTs. It could be possible that the signalling proteins of the interdependent network SIRT1, AMPK and PGC1α can in part compensate for each other.

PPARγ coactivator 1α

The key target of Sirt1 and AMPK, the co-activator of the transcription factor PPARγ, PGC1α is also central for mediating the ER-induced lifespan-enhancing effect. When activated, PGC1α might even prevent age-related undesirable changes in skeletal and heart muscle and in adipose tissue. Apart from its role in mitochondrial biogenesis and function in all cell types, in skeletal muscle PGC1α is thought to affect the type of muscle fibres. Furthermore, PGC1α stimulates adaptive thermogenesis in brown adipose tissue. PGC1α activity appears to prevent loss of mitochondrial function in muscles, protects from distributional and functional changes of adipose tissue and affects the energy metabolism by favouring lipids instead of carbohydrates as a main energy source.

While some ER studies found that nuclear PGC1α levels (and subsequent increase in mitochondrial biogenesis) increased under limited nutrient supply, others did not observe any differences in nuclear PGC1α protein concentrations in rodent tissues such as heart, liver, white adipose tissue and brain. However, in mice the SIRT1-mediated activation of PGC1α also led to increased gluconeogenesis, enhanced glucose mobilisation and reduced levels of glycolysis.

Forkhead box O

FOXO transcription factors were also shown to be activated by SIRT1. FOXO are involved in stress defence and energy metabolism and induce the transcription of genes that promote autophagy and cell death.

Apart from deacetylation, which activates FOXO, they can be regulated negatively by phosphorylation. After being phosphorylated, FOXO are shuttled from the nucleus into the cytoplasm, which reduces the expression of FOXO target genes.

Insulin-like growth factor 1

The IGF-1 signalling pathway is also negatively affected under ER. The reduced IGF-1 signalling observed under ER is usually accompanied by lowered growth hormone (GH) concentrations, since GH promotes IGF expression. An ER-induced reduction in plasma levels of free IGF-1 was shown to be pro-apoptotic and anti-proliferative and is therefore potentially chemopreventive.

Indeed, GH and IGF-1 receptor deficiencies were shown to increase lifespan, whereas GH overexpression led to premature ageing and shortened lifespan in mice. Dwarf mice that display reduced IGF-1/GH signalling lived considerably longer compared with normal-sized wild-type mice, mainly because they had a reduced incidence of neoplasms. Furthermore, it was shown that low IGF-1 levels were related to survival in long-lived humans.

Additionally to decreased levels of growth factors and hormones, the reduction of insulin secretion upon ER feeding could also account for its chemopreventive effect. Hyperinsulinaemia is strongly related to high levels of oxidative stress and reduced rates of autophagy, leading to increased generation of damaged macromolecules and decreased degradation of damaged proteins.

Another consequence of increased IGF-1 signalling is the induction of phosphoinositide-dependent protein kinase, which in turn activates protein kinase B by phosphorylation, thereby activating mTOR.

Mammalian target of rapamycin

mTOR is a serine/threonine protein kinase that is involved in several regulatory processes. It is suggested that it influences cell growth, including proliferation, transcription and protein synthesis, as well as cell survival. Due to its central role in proliferation and cellular growth processes, mTOR has long been studied as a molecular target for cancer therapy. Inhibiting mTOR activity genetically or pharmacologically increased lifespan in yeast and D. melanogaster. Consistent with decreased IGF-1 and GH signalling, which are upstream regulators of mTOR, mTOR signalling is also reduced in dwarf and ER-fed mice. Inhibition of mTOR via rapamycin (a mTOR inhibitor) can extend lifespan in some species.
leads to autophagy induction\(^{(213,214)}\) possibly via dephosphorylation of ATG13 and the mammalian Atg1 homologues Unc-51 like autophagy activating kinases 1 and 2, which are essential for mTOR-dependent autophagy\(^{(130,151)}\).

**Limitations and adverse effects of energy restriction**

Evaluating ER as a potential pro-longevity treatment, it is necessary to keep in mind some of its adverse effects (see Table 1). First of all, it seems almost infeasible to follow a lifelong ER regimen that requires a reduction of energy intake of up to 40\%\(^{(215)}\) without being malnourished at any time. Another critical issue in ER experiments might be decreased bone mineral density as observed in primates and rodents\(^{(32)}\) as well as in a long-term ER study in human subjects\(^{(216)}\). Deficiencies in skin wound healing – seen in restrictively fed rats\(^{(217)}\) – and decline in concentrations of gonadal steroids\(^{(34)}\) could impair health-related quality of life. Furthermore, symptoms such as starving, cold and reduced libido persist during long-term ER and the social aspect of eating food should not be neglected\(^{(36)}\). Importantly, ER-fed old mice seem to be more susceptible to infections\(^{(218)}\), which could even decrease lifespan.

An alternative to ER is the ‘alternate day fasting’ regimen. In this modified ER concept, dietary intake is partially or completely limited every other day, whereas food consumption remains unrestricted on the remaining days\(^{(219)}\). Although it could be expected that a higher food intake in \textit{ad libitum} phases compensates or even overcompensates for energy shortage during the restriction days, several health-promoting effects were seen in alternate day fasting compared with control groups with unlimited food access\(^{(220–225)}\). However, this nutritional concept also seems hard to follow in modern society and possible side effects need to be studied in more detail.

**Energy restriction mimetics**

Because of its limitations, alternatives to ER that could prevent or retard the onset of ageing-related pathologies to a similar extent as ER are being sought. Since it takes very long to generate data on the influence of dietary regimens, supplements or pharmacological substances on human lifespan and age-related diseases there is only limited information on general ageing patterns in specific organs and tissues. Using broad-range microarrays, gene expression profiles associated with the ageing process have been identified. By comparing these patterns of aged individuals to subjects undergoing ER and those supplemented with potential ERM, the anti-ageing potential of candidate substances can easily be assessed\(^{(92)}\). Results of ongoing studies identifying potential ERM are published regularly by the NIA Interventions Testing Program. A comprehensive review on ER effects with a focus on epigenetic changes was recently published by Chung \textit{et al.}\(^{(224)}\). Moreover, Ingram \textit{et al.}\(^{(225)}\) have established criteria that should be met before a substance is considered an ERM. First, candidate substances should not significantly influence long-term dietary intake in order to prevent positive results merely because of reducing energy consumption. Second, the candidate substance should induce metabolic, hormonal and physiological changes that are comparable with the effects of ER. And third, it should induce an ER-like stress response. It is hypothesised that through meeting these three criteria, dietary supplementation with the candidate substance may have a positive influence on healthspan and lifespan\(^{(226)}\). According to Selman, the elongation of healthspan by putative ERM candidates should be more emphasised\(^{(227)}\).

The criterion proposed by Lane \textit{et al.}\(^{(228)}\) resembles these points. They state that without altering food intake the potential ERM should target energy metabolism since this seems to be the underlying mechanism of the beneficial ER effects.

**Putative energy restriction mimetic candidate substances**

ERM target various molecules and processes that have been described in the Cellular processes affected by energy restriction section. A putative ERM that targets glucoregulation and inhibits glycolysis is the glucose analogue 2-deoxy-D-glucose (2DG)\(^{(36)}\). Recently, glucosamine has been discussed as mimicking a low-carbohydrate diet and thus potentially acting as an ERM\(^{(229)}\).

Further ERM candidates are the biguanides that have been used in the treatment of T2DM since they lower insulin/IGF-1 signalling and activate AMPK\(^{(36)}\). While formerly phenformin and metformin were used alongside metformin, nowadays only metformin is approved for T2DM treatment. Thus, despite studies showing a prolonged lifespan in phenformin- and butformin-supplemented rats\(^{(230–231)}\), metformin currently is the best studied biguanide in terms of mimicking ER. Oxaloacetate seems to activate AMPK-related pathways, thereby increasing lifespan in a nematode model. However, supplementation trials in mice did not reveal any pro-longevity effect of oxaloacetate\(^{(32)}\).

**Table 1. ‘Side effects’ of energy restriction**

<table>
<thead>
<tr>
<th>Negative effect seen upon energy restriction</th>
<th>Model organism in which side effect was seen</th>
<th>References</th>
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<tbody>
<tr>
<td>Immunosuppression (increased sensitivity to viral/bacterial infections)</td>
<td>\textit{Mus musculus}</td>
<td>((218))</td>
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<tr>
<td>Loss of bone mineral density</td>
<td>Rodents, primates and man</td>
<td>((32))</td>
</tr>
<tr>
<td>Impaired wound healing</td>
<td>\textit{Rattus norvegicus}</td>
<td>((217))</td>
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<tr>
<td>Delayed sexual maturity and reduced levels of sexual hormones</td>
<td>\textit{Macaca mulatta}</td>
<td>((34,54))</td>
</tr>
<tr>
<td>Decreased body temperature</td>
<td>\textit{Mus musculus}</td>
<td>((33))</td>
</tr>
<tr>
<td>Social exclusion and missing feasibility of energy restriction</td>
<td>\textit{R. norvegicus}</td>
<td>((36))</td>
</tr>
</tbody>
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Another substance class suggested as possessing pro-longevity effects are anti-lipolytic compounds such as the nicotinic acid derivative acipimox or 3,5-dimethylpyrazole. Additionally to their lowering effect on plasma lipids, they are thought to be glucose- and insulin-decreasing as well as autophagy-inducing agents. These observations all point to potential ER-like effects. Unfortunately, studies looking at lifespan upon/after treatment with anti-lipolytic drugs are rare.

Further ERM candidates can be found among SIRT stimulators, particularly stilbenes. Systemic SIRT1 activators are known for their metabolism-stimulating and body fat-reducing effects. The best-studied member of this group is resveratrol (RSV), which has been shown to increase lifespan in some species. Nicotinamide might also be considered a putative ERM since it was shown to activate SIRT. Interestingly, nicotinamide and RSV induce autophagy which might contribute to their ER-mimicking potential. Autophagy-inducing agents might also promote ER-like phenotypes independently of SIRT regulation. Spermidine (SPD) was shown to induce autophagy and thereby increase lifespan in some species without affecting SIRT. Another target molecule for ERM is the proliferation and cell growth-promoting kinase mTOR. Indeed, supplementation experiments with the pharmacological mTOR inhibitor rapamycin produced promising results.

Furthermore, agents that affect mitochondrial biogenesis or the circadian rhythm may mediate ER-mimicking effects. Antioxidants such as lipoic acid that reduce ROS production by improving mitochondrial performance or increasing the production of endogenous antioxidants are also interesting putative ERM. Because of its influence on the circadian rhythm, melatonin might be helpful in managing body weight and preventing chronic diseases and premature deaths.

Sometimes anorectics like amphetamines are discussed as ERM candidates. But due to their considerable reduction of food and consequently energy intake, anorectics cannot be considered ERM according to the criteria mentioned above.

Postulated mechanisms and potential limitations of the best-studied and most promising energy restriction mimetics

The following section will focus on postulated mechanisms and potential limitations of the best-studied and most promising ERM as found in recent literature (for a summary, see Table 2).

2-Deoxy-D-glucose

2DG (Fig. 3) was one of the first substances discussed as a possible ERM. As a glucose analogue it differs from glucose only in that it lacks one hydroxyl group at C2. After intestinal absorption and entering the circulation, 2DG is taken up into cells using the same transporters as glucose. 2DG is phosphorylated immediately by a hexokinase and accumulates intracellularly since it cannot be metabolised by phosphohexose isomerase. Thereby, it competitively inhibits glucose utilisation, reduces the amounts of available energy and might consequently mimic the effects of ER.

<table>
<thead>
<tr>
<th>ERM candidate</th>
<th>Molecular mechanism underlying the pro-longevity effect</th>
<th>Model organism, in which lifespan increase was shown</th>
<th>Limitations or adverse effects</th>
<th>References</th>
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<tr>
<td>2-Deoxy-D-glucose</td>
<td>Inhibition of glucose utilisation</td>
<td>Caenorhabditis elegans</td>
<td>Cardiotoxicity. No lifespan increase in healthy mammals (13,40,142,245)</td>
<td>(238)</td>
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<tr>
<td>Metformin</td>
<td>AMPK activation</td>
<td>C. elegans, Mus musculus, Drosophila</td>
<td>Lactic acidosis (231,252,253,256)</td>
<td>(231)</td>
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<tr>
<td>Rapamycin</td>
<td>mTOR inhibition</td>
<td>Saccharomyces cerevisiae, C. elegans, D. melanogaster, M. musculus</td>
<td>Immunosuppression (206,266–268,336)</td>
<td>(206)</td>
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<tr>
<td>Resveratrol</td>
<td>SIRT1 activation, AMPK activation, antioxidant</td>
<td>S. cerevisiae, C. elegans, D. melanogaster, Nothobranchius furzeri</td>
<td>No lifespan increase in healthy mammals on standard chow (170,274,279–281,283)</td>
<td>(170)</td>
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<tr>
<td>Spermidine</td>
<td>Autophagy induction</td>
<td>S. cerevisiae, C. elegans, D. melanogaster, M. musculus, human immune cells</td>
<td>High polyamine levels may promote tumour progression, long-term studies in mammals missing</td>
<td>(8,133,291,313)</td>
</tr>
<tr>
<td>Lipoic acid</td>
<td>Antioxidant</td>
<td>C. elegans, D. melanogaster, M. musculus, D. melanogaster</td>
<td>No lifespan increase in animals fed low-energy diet</td>
<td>(19,22,314–316)</td>
</tr>
</tbody>
</table>

AMPK, AMP-activated protein kinase; mTOR, mammalian target of rapamycin; SIRT1, sirtuin 1.
Indeed, in rodents, adding 2DG to the diet led to ER-similar phenotypes(252) including decreased body weight, blood glucose(240), insulin(241), body temperature(242) and heart rate(241). Moreover, 2DG was shown to induce protection against oxidative stress(243).

Additionally, application of 2DG elevated protein levels of SIRT1 and p-AMPK in MCF-7 cancer cells(142). Since the inhibition of glycolytic processes by 2DG is followed by a limitation of cellular energy supply, ATP levels are reduced and subsequently the AMP:ATP ratio is increased leading to AMPK activation which increases the NAD⁺:NADH ratio and activates SIRT1(244).

In fact, Schulz et al.(245) reported enhanced mean and maximum lifespan of C. elegans upon 2DG supplementation. However, these results could not be verified in mammals. Long-term studies in rodents revealed cardiotoxic effects of 2DG treatment at concentrations which were necessary for ER-like effects(13). In a long-term feeding trial in rats, 2DG was shown to reduce lifespan dose-dependently(13). However, at lower concentrations which did not seem toxic, 2DG exerted health-promoting effects in a 5-week supplementation study in tumour-prone rats where it inhibited cancer growth(142).

In conclusion, 2DG-induced ER-like phenotypes in laboratory animals are possibly outweighed by its cardiomyopathic effects observed in some studies. Toxicity thresholds should therefore be considered when planning long-term or even lifelong 2DG supplementation studies. Thus, further studies on dose-dependent efficiency and toxicity are needed.

Metformin

The biguanide metformin (Fig. 4) has been used for treating diabetes for a long time(246). Due to its potential of inducing ER-like effects, metformin has been considered an ERM candidate substance(251). Metformin was shown to suppress gluconeogenesis and promote insulin sensitivity and glycolysis in diabetic subjects(246,247). Moreover, intestinal glucose absorption and plasma glucose and lipid concentrations were decreased(248).

In different tumour-prone rodent models and in diabetic patients incidence and progression of cancer were decreased by metformin(50,251,249,250). Additionally, risk factors for the development of CVD were reduced upon metformin treatment(251). Most probably, the delay in the onset of chronic diseases such as cancer and hypertension is the main reason for the longevity-promoting effect seen in C. elegans and mice(231,252).

Interestingly, metformin caused a reduction in body weight in several studies without significantly decreasing energy intake(6,231). Since a reduction in body weight is associated with an increased AMPK activation, it seems reasonable to assume a contribution of AMPK to the beneficial effects of metformin. Indeed, the levels of p-AMPK were found to be elevated upon metformin supplementation(6,253). Potential longevity-promoting effects of AMPK and its downstream targets have been described earlier in this article.

Metformin probably addresses further targets that also contribute to the observed increase in lifespan(248). In rodent models metformin was seen to mimic the hepatic and muscular transcriptional ER response(6,254,255). These gene expression data indicate improved mitochondrial function, glucose and lipid metabolism, reduced apoptotic rates and inflammation, as well as enhanced stress response. In particular, mRNA expression and nuclear protein levels of the central antioxidant transcription factor Nrf2 were found to be up-regulated. Additionally, mRNA concentrations of its downstream targets SOD, NQO1 and NQO2 were elevated compared with the control animals. In contrast, expression of pro-inflammatory genes like NF-κB seemed decreased after metformin treatment(6).

Despite these promising effects regarding the delay of the ageing process, some impairments of chronic metformin application have to be taken into account. Metformin is suggested as causing lactic acidosis when applied at higher doses for a longer period(256). Indeed, microarray analyses revealed increased mRNA concentrations of cytosolic lactate dehydrogenase (a key enzyme in lactic acid generation) in the liver and muscle of metformin-supplemented mice(60). However, metformin was applied at doses that were higher than the doses used in human subjects and the toxicity of metformin seems to depend on the dose. Current meta-analyses and reviews on metformin-treated T2DM patients concluded that the increased incidence of lactic acidosis more probably resulted from an underlying systemic dysfunction than from the metformin treatment(257,258). Moreover, metformin supplementation may be contraindicated in individuals suffering from renal diseases(259) since metformin induced renal failure in rodents when applied at high concentrations. Despite metformin being a promising ERM candidate, supplementation studies in healthy mammals have not always observed a clear lifespan-enhancing effect(226). For example, a study in healthy rats supplemented with metformin failed to show a pro-longevity effect(231), whereas a study in C57BL/6 mice showed longevity-promoting effects after metformin treatment(60).

Rapamycin

Rapamycin (Fig. 5), a complex macrolide antibiotic, has been used because of its immunosuppressive effects(260) after organ
transplantations and because of its antiproliferative properties in cancer treatment\(^{261,262}\). It has long been known that rapamycin exerts its antiproliferative effects via inhibition of mTOR signalling\(^{263}\). Possibly, reduced mTOR activation decreases cancer risk and therefore prolongs survival in rapamycin-treated subjects. After discovering that deleterious TOR mutations in yeast promoted longevity, rapamycin was suggested as being an ERM in 2006\(^{266}\). Indeed, it has been shown to decelerate senescence in vitro (in non-cancerous cell lines) and to possess lifespan-enhancing properties in several model organisms such as *Saccharomyces cerevisiae*\(^{268,264}\), *C. elegans*, *D. melanogaster*\(^{265,266}\) and mice\(^{266–268}\)

How rapamycin interferes with mTOR is still under discussion. It has been hypothesised that rapamycin could bind to the heterodimer of the FK506 binding protein (FKBP)-type peptidyl-prolyl cis-trans-isomerase and the FKBP–rapamycin-associated protein. As such a heterotrimer, rapamycin might form complexes with mTOR, thereby inhibiting the formation of the complex mTORC1 and consequently cell proliferation and cell growth\(^{269}\). Of interest, via inhibition of mTORC1, rapamycin was also shown to activate autophagy by up-regulating ATG1\(^{266,270,271}\). Similar to findings from ER studies, prolonged lifespan induced by low doses of rapamycin was shown to depend on autophagy since rapamycin failed to enhance survival in atg1- and atg7-deficient yeast\(^{264}\). Several beneficial outcomes of rapamycin administration, which may contribute to its pro-longevity effect, were observed in clinical and pre-clinical studies; namely, rapamycin seemed to alleviate the ageing-related diseases T2DM, the metabolic syndrome, atherosclerosis, neurodegeneration and some kinds of cancer\(^{272,273}\). However, body weight does not appear to be affected by rapamycin administration in mice fed standard diets\(^{267}\).

A limitation for rapamycin use as an ERM in humans results from its immunosuppressive action. While mice housed in specific pathogen-free cages are unlikely to acquire infections despite being supplemented with immunosuppressants, humans may not benefit from rapamycin due to severe bacterial or viral diseases\(^{220}\). Moreover, animals treated with high rapamycin doses suffered from disturbed lipid or glucose homeostasis, skin irritations, anaemia or impaired wound healing\(^{268}\). Currently, studies are being carried out by the NIA to evaluate the most effective dose for promoting longevity in rodents\(^{268}\).

RSV (Fig. 6), a naturally occurring stilbene with antioxidant capacity\(^{274,275}\), is mainly found in the skin of red grapes and therefore in red wine\(^{275,276}\). Considerable amounts can also be detected in the roots of the medical plant Japanese knotweed\(^{277}\) and lower amounts in other fruits\(^{278}\). In a NIA in vitro screening programme, RSV was identified as a SIRT1 activator in 2003. As SIRT1 seems to be a crucial player in ER-mediated lifespan extension, RSV was additionally tested for its lifespan-enhancing effects in *S. cerevisiae*. Due to the promising results of this trial\(^{279}\) several studies have been conducted to verify the lifespan-enhancing properties of RSV. Indeed, RSV has shown survival enhancing effects in yeast, *C. elegans*, some *D. melanogaster* experiments\(^{170}\), *Notobranchius furzeri*\(^{280}\) and high-fat diet-fed mice\(^{281}\). Additionally, Morselli et al. proposed that RSV-mediated SIRT1 activation was followed by the induction of autophagy, which appears to be important for the lifespan-enhancing effect of ER\(^{8}\).

However, in other fly studies\(^{278,282}\) and in mice fed a standard chow RSV did not prolong lifespan\(^{268,283,284}\). Nevertheless, in standard chow-fed mice supplemented with RSV several tissues revealed ER-like gene expression patterns, and anti-inflammatory, cardio- and osteoprotective effects as well as improved locomotor activity were observed\(^{263}\).

While it seems that SIRT1 might be responsible for the beneficial effects of RSV\(^{281}\) the exact mechanism of SIRT1 remains unclear. RSV may be binding to the regulatory\(^{285}\) N-terminal subunit of SIRT1. Subsequently, a conformational change could take place, which in turn could lead to an enhanced SIRT1 deacetylation activity\(^{279}\). Thus, the red wine polyphenol would seem to be an allosteric SIRT1 activator\(^{8,279}\).

Intriguingly, there are reports that the observed increase in SIRT1 deacetylase activity due to RSV could have been a result of a methodological error\(^{286,287}\). If the enzyme activity measurement is not performed with fluorochrome-conjugated substrates (as it usually is) but with natural substrates, RSV does not increase SIRT1 activity\(^{286}\).

However, Park et al. showed that RSV inhibits cAMP-degrading phosphodiesterases, which in turn leads to an activation of the AMPK pathway. They further argue that this increases NAD\(^+\) levels and that SIRT1 activation is a consequence of a RSV-mediated rise in NAD\(^+\) levels\(^{289}\). These findings could explain why Pacholec et al. could not detect SIRT1 activation when measuring the enzyme activity in vitro\(^{286}\).

Interestingly, experiments in knockout animals have shown that apart from SIRT1\(^{170}\) AMPK is also necessary for the pro-longevity effect of RSV\(^{154}\). The beneficial effects of RSV on insulin
sensitivity, motor function, mitochondrial performance \( ^{(159,201)} \) as well as decreased body weight \( ^{(154,159)} \) were not seen in AMPK-deficient animals \( ^{(154)} \). A recent publication has described a mechanism for AMPK activation by RSV \textit{in vitro} that does not depend on SIRT1 but on the tyrosyl transfer-RNA synthetase. RSV may fit into the active site of the tyrosyl transfer-RNA synthetase, thereby leading to nuclear translocation of this enzyme and consequently activation of the poly(ADP-ribose) polymerase 1 which in turn could activate downstream targets such as AMPK, FOXO and SIRT. Intriguingly, this happened at up to 100-fold lower doses than in previous studies \( ^{(290)} \).

In contrast to many other ERM candidate substances, hardly any adverse effects have been reported for RSV so far. Only a study applying very high RSV doses observed cases of premature death \( ^{(290)} \). However, it needs to be kept in mind that the efficiency of RSV in reducing mortality rates in healthy mammals and the appropriate dose of RSV for supplementation are still under discussion.

**Spermidine**

Spermidine (SPD; Fig. 7) is a naturally occurring polyamine that is essential for various cellular processes. Along with other endogenous polyanimes such as spermine and putrescine it was shown to modulate various cellular processes such as proliferation, differentiation and cell death \( ^{(291)} \).

Looking at particular polyamines, putrescine and cadaverine turn out to be rather negatively annotated molecules since they are enhanced in tissues in chronic diseases such as cancer \( ^{(202)} \), Parkinson’s \( ^{(293)} \) and pancreatitis \( ^{(294)} \). In contrast, SPD seems to be protective against age-related pathological changes \( ^{(133)} \). Spermine and SPD have already been described as exerting anti-inflammatory effects \( ^{(295,296)} \). Additionally, their levels were shown to be reduced in Alzheimer’s disease \( ^{(297)} \). Thus, the restoration of SPD and spermine levels might ameliorate Alzheimer’s pathology. Moreover, due to its anti-inflammatory properties, SPD has been suggested for the treatment of multiple sclerosis \( ^{(298)} \) and sepsis \( ^{(299)} \).

As the endogenous synthesis of SPD declines with age \( ^{(300,301)} \), supplementation with SPD could increase the plasma concentrations at a higher age \( ^{(291)} \). Food rich in SPD includes soya and other beans, green tea and mushroom \( ^{(302,303)} \). SPD rich diets are consumed traditionally in Asian \( ^{(304)} \) and Mediterranean \( ^{(305)} \) regions.

Recently, elevated SPD relative to total polyamine concentrations have been observed in the plasma of centenarians compared with younger counterparts \( ^{(300)} \). Consistently, SPD supplementation in aged mice \( ^{(291)} \), \textit{C. elegans}, \textit{D. melanogaster} and human immune cells \( ^{(133)} \) increased lifespan.

Some researchers assume SPD to exert its longevity-promoting effects via induction of autophagy \( ^{(8,153)} \). Autophagy is known to be reduced at a higher age \( ^{(134,297)} \), and seems to counteract senescence when induced \( ^{(298,299)} \).

\textit{In vitro} experiments revealed that SPD treatment inhibited the activity of histone acetyltransferases in yeast and mouse liver extracts \( ^{(8,133)} \). Gene expression depends on the acetylation state of histones. The negatively charged chromatin can be wrapped around the histones more tightly when more of the lysine and arginine residues in the histone molecules remain deacetylated, i.e. positively charged. Thus, histone acetylation and deacetylation may alter the accessibility of certain DNA sections to transcriptionally active enzymes \( ^{(309)} \). Therefore, a high number of deacetylated histones – as observed after SPD treatment \( ^{(133)} \) – leads to modified expression of several genes \( ^{(309)} \). Recently it has been reported that SPD-treated yeast expressed Atg7, 11 and 15, which are involved in autophagy induction, at enhanced levels \( ^{(8)} \). Indeed, SPD failed to increase lifespan in atg-deficient yeast \( ^{(133)} \).

However, in \textit{D. melanogaster}, recent data suggest a contribution of non-autophagic mechanisms to the improved response to oxidative stress upon SPD supplementation although these additional underlying signalling pathways have not been fully elucidated \( ^{(510,311)} \).

SPD is still a very young topic in longevity research; therefore studies on potential negative side effects are rare. However, elevated concentrations of polyamines are thought to be related to an increased incidence of cancer. Possibly, oxidative damage, caused by oxidation of polyamines in general, contributes to this effect \( ^{(309)} \).

Potential limitations in the use of SPD as an ERM result from observations that increased polyamine concentrations caused enhanced tumour progression \( ^{(312-314)} \). In fact, putative anticancer agents inhibit polyamine metabolism \( ^{(303)} \). However, in these studies polyamines were applied to model organisms with existing tumours or cells that had undergone oncogenic transformation \( ^{(291)} \). Up to now, there is no direct evidence for cancer-promoting effects of enhanced SPD intake in healthy animals. So far, however, there are no long-term studies in healthy mammals.

**Lipoic acid**

The disulfide derivative of octanoic acid is well known for its antioxidant activity that is also exerted by its reduced form dihydrolipoic acid \( ^{(315)} \). Apart from its antioxidant effect, lipoic acid (Fig. 8) is an essential cofactor for certain enzyme complexes, for example, pyruvate dehydrogenase and α-ketoglutarate dehydrogenase \( ^{(316)} \).

Due to the beneficial effects of antioxidants in general, it seems worth examining the healthspan- and lifespan-extending properties of lipoic acid.

Lipoic acid exists as two stereoisomers, \textit{R}-α-lipoic acid and \textit{S}-α-lipoic acid. The biologically more active stereoisomer...
R-α-lipoic acid can be synthesised endogenously from fatty acids (octanoic acid) and a sulfur source (cysteine) or taken up with food(317). Exogenously supplied lipoic acid is mostly bound to lysine. As lipoyllysine it can be found in vegetables, for example, spinach, broccoli and tomatoes, as well as in animal tissues like liver, heart and kidney(12).

According to some popular ageing theories, free radicals are probably a major reason for the development of ageing-associated diseases(10,248). Thus, enhancing the supply of exogenous antioxidants might counteract the process of ageing and increase lifespan similar to ER. However, even though lipoic acid is a potent antioxidant, there are only a few studies showing increased lifespan in lipoic acid-supplemented invertebrates (*C. elegans, D. melanogaster*)(318–320). While experiments in rodents did not find longevity-promoting effects under physiological conditions (in healthy animals with unrestricted food supply)(12,92,321) immunosuppressed mice receiving a lipoic acid-supplemented diet revealed longer lifespans than controls(322). Another experiment showed that mice that were re-fed *ad libitum* after being on ER lived longer when the *ad libitum* feed was supplemented with lipoic acid compared with non-supplemented *ad libitum* feed-receiving mice(12).

Although evidence for lifespan-enhancing properties in rodents is missing, there are several publications pointing to the notion that lipoic acid counteracts age-related disorders. Lipoic acid was shown to reduce oxidative stress and damage in the heart muscle and brain in rats, thereby potentially ameliorating cardiac and neurodegenerative diseases(319,323–326).

Apart from directly scavenging ROS and recycling of other antioxidants like vitamin C or glutathione(315), lipoic acid might also decrease oxidative damage to macromolecules by decreasing the amount of ROS that is produced within the mitochondrial electron chain(327). This protective effect may be mediated by enhancing the expression of UCP, which use the electrochemical gradient at the inner mitochondrial membrane to generate heat instead of ATP(328). Fatty acids are potent inducers of mitochondrial UCP(329,330). It is assumed that lipoic acid might induce uncoupling due to its structural similarity to fatty acids(327).

The controversial outcome of studies on lipoic acid regarding lifespan could be in part explained by the low stability of lipoic acid. Encapsulating lipoic acid in a cyclodextrin cavity increased its solubility(331) and stability(331,332). Interestingly only this complexed form of lipoic acid increased energy expenditure in old mice. Most probably this was mediated by the induction of UCP(333). Importantly, a positive correlation between the metabolic rate, mitochondrial uncoupling and the lifespan of mice was reported(334). However, clear evidence for a positive relationship between lipoic acid intake and lifespan in healthy mammals is still lacking.

For a brief summary of the suggested molecular targets of the ERM candidates reviewed in this paper, see Fig. 9.

![Fig. 9. Schematic overview of the suggested molecular targets of the energy restriction mimetic candidate substances 2-deoxy-D-glucose (2DG), metformin (MET), rapamycin (RAP), resveratrol (RSV), spermidine (SPD) and lipoic acid (LA). 2DG inhibits the central process of glycolysis, thereby favouring the activities of AMP-activated protein kinase (AMPK) and sirtuin (SIRT). MET increases AMPK activity, indirectly leading to increased autophagy and mitochondrial turnover. RAP inhibits mammalian target of rapamycin (mTOR) signalling, thereby favouring autophagy and inhibiting proliferative processes. In addition to its antioxidant capacity, RSV is thought to increase SIRT and AMPK activity. SPD might up-regulate the antioxidant response, enhance autophagy and decrease proliferation. LA might improve mitochondrial function, increase energy expenditure and reduce oxidative stress. ROS, reactive oxygen species.](https://www.cambridge.org/core/journals/nutrition-research-reviews/article/schematic-overview-of-the-suggested-molecular-targets-of-the-energy-restriction-mimetic-candidate-substances-2-deoxy-d-glucose-2dg-metformin-met-rapamycin-rap-resveratrol-rsv-spermidine-spd-and-lipoic-acid-la/16843611/16843611)
Conclusion

Despite various benefits of ER, such as a reduced risk for age-related chronic diseases, the limitations of ER need to be considered. In order to prevent adverse side effects like immunosuppression or loss of bone mineral density, ER should only imitate the positive effects. However, until now no substances have been found that repeatedly mimic the positive effects seen in restrictively fed models without adverse effects for mammals. In order to possibly improve the health-promoting effects or reduce negative side effects of single substances, ERM candidates should be tested for putative synergistic interactions. Moreover, it needs to be ascertained if other components found in Mediterranean or Asian (MediterrAsian\textsuperscript{429}) diets might exert ER-like effects similar to RSV and SPD. There are only a few studies that have investigated ER effects in humans, since lifespan studies in human subjects are difficult to perform (at the very least because of the length of human life). However, by means of measuring longevity biomarkers that have already been established in rodent or primate ER lifespan studies, putative healthspan- and lifespan-promoting effects in humans may be extrapolated. But due to the lack of feasibility of ER and the limitations of ERM candidate substances, alternative ER patterns should also be considered in future studies since there is strong evidence that moderate restriction regimens may improve healthspan\textsuperscript{420–425}. Thus, more research is needed in order to find new dietary strategies that imitate positive ER effects without its downsides.

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