



REVIEW

Interventions to Slow Aging in Humans: Are We Ready?

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Summary

The workshop entitled ‘Interventions to Slow Aging in Humans: Are We Ready?’ was held in Erice, Italy, on October 8–13, 2013, to bring together leading experts in the biology and genetics of aging and obtain a consensus related to the discovery and development of safe interventions to slow aging and increase healthy lifespan in humans. There was consensus that there is sufficient evidence that aging interventions will delay and prevent disease onset for many chronic conditions of adult and old age. Essential pathways have been identified, and behavioral, dietary, and pharmacologic approaches have emerged. Although many gene targets and drugs were discussed and there was not complete consensus about all interventions, the participants selected a subset of the most promising strategies that could be tested in humans for their

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effects on healthspan. These were: (i) dietary interventions mimicking chronic dietary restriction (periodic fasting mimicking diets, protein restriction, etc.); (ii) drugs that inhibit the growth hormone/IGF-I axis; (iii) drugs that inhibit the mTOR–S6K pathway; or (iv) drugs that activate AMPK or specific sirtuins. These choices were based in part on consistent evidence for the pro-longevity effects and ability of these interventions to prevent or delay multiple age-related diseases and improve healthspan in simple model organisms and rodents and their potential to be safe and effective in extending human healthspan. The authors of this manuscript were speakers and discussants invited to the workshop. The following summary highlights the major points addressed and the conclusions of the meeting.

Key words: aging; anti-aging; centenarians; longevity regulation; dietary restriction; lifespan studies; longevity gene.

Introduction

Human aging and age-associated diseases are emerging as among the greatest challenges and financial burdens faced by developed and developing countries (Christensen *et al.*, 2009) (http://esa.un.org/wpp/documentation/pdf/WPP2010_Volume-I_Comprehensive-Tables.pdf). Although average life expectancy has increased dramatically in the last 100 years, this has not been accompanied by an equivalent increase in healthy life expectancy, which has been termed healthspan (Hung *et al.*, 2011). Research related to longevity extension has traditionally been viewed with skepticism and with concerns that it could lead to an increase in the size of the elderly population and the prevalence of diseases associated with aging. However, studies in a wide range of organisms have demonstrated that major lifespan extension is accompanied by reduced or delayed morbidity in most cases (Fontana *et al.*, 2010). Data from experimental studies in invertebrates and rodents have consistently shown that both chronic dietary restriction (DR) and mutations in nutrient and growth signaling pathways can extend longevity by 30–50%. These interventions can also lower the prevalence of age-related loss of function and multiple diseases, including tumors, cardiovascular disease, and neurodegeneration (Fontana *et al.*, 2010). DR protects against diabetes, cancer, cardiovascular disease, sarcopenia, and neurodegeneration of certain brain regions in rhesus monkeys and also extends lifespan (Mattison *et al.*, 2012; Colman *et al.*, 2014). In humans, long-term DR causes several metabolic and molecular changes that protect against age-related pathologies, including changes in markers for type 2 diabetes, hypertension, cardiovascular disease, cancer, and dementia (Cava & Fontana, 2013). Moreover, chronic DR ameliorates the expected age-associated alterations in myocardial stiffness, autonomic function, and skeletal muscle gene expression changes (Mattison *et al.*, 2012; Cava & Fontana, 2013; Colman *et al.*, 2014). However, the duration and severity of the DR regimen that is required for optimal benefits is not feasible for most people and is likely to be associated with undesirable side effects. Therefore, the focus of this consensus meeting in Erice was on less drastic dietary interventions and drugs that target nutrient-response pathways and mimic the effects of DR, but that are practical, realistic, and safe.

There was a general consensus among this panel of experts on the following points: (i) aging can be slowed by many interventions; (ii) slowing aging typically delays or prevents a range of chronic diseases of old age; (iii) dietary, nutraceutical, and pharmacologic interventions that

modulate relevant intracellular signaling pathways and can be considered for human intervention have been identified. Additional potential targets will continue to emerge as research progresses; and (iv) it is now necessary to cautiously proceed to test these interventions in humans. Based on a vote taken on the last day of the workshop, the strategies believed to be most promising by the panel of invited experts and authors of this manuscript are as follows:

- 1 Pharmacological inhibition of the GH/IGF-1 axis
- 2 Protein restriction and Fasting Mimicking Diets
- 3 Pharmacological inhibition of the TOR–S6K pathway
- 4 Pharmacological regulation of certain sirtuin proteins and the use of spermidine and other epigenetic modulators
- 5 Pharmacological inhibition of inflammation
- 6 Chronic metformin use

These choices were based in part on: (i) consistent evidence for their pro-longevity effects in simple model organisms and rodents; (ii) evidence for their ability to prevent or delay multiple age-related diseases and conditions; and/or (iii) clinical evidence for their safety in small mammals and/or nonhuman primates. Below, we present the salient points related to each of the promising strategies. Other ideas discussed at the meeting are described elsewhere (Gems, 2014).

Biomarkers to assess the efficacy of interventions

The successful development and implementation of interventions to promote healthspan will require the use of aging biomarkers. Biomarkers are defined here as biological characteristics that can be objectively measured and evaluated as indicators of age-related normal and pathogenic processes. In practice, such biomarkers should aid in diagnosing individual aging phenotypes, predicting the progression of these phenotypes, selecting possible interventions, and evaluating the effects and outcomes of such interventions. Biomarkers are key for the development, testing, and implementation of interventions in aging because of the great difficulty in performing clinical trials. Indeed, biomarkers of disease are often an aid in clinical trials. For example, to stratify patients so that only those likely to respond to a particular therapy receive it. Interventions in aging require biomarkers for all the same reasons, and also as surrogates for determining the efficacy and safety of interventions that often require very long time scales. There are three categories of such biomarkers that should be distinguished:

Biomarkers of critical pro-aging mechanisms

These biomarkers are needed to define the parameters that should be targeted in interventions. A disease can be defined as a disorder or abnormality of structure or function. Some have argued that aging cannot be abnormal because everyone suffers from it, which has led to the reluctance to accept aging as the equivalent of a disease. This and the extended period of time that is required to evaluate the effect of interventions on aging essentially constrain clinical trials to testing the efficacy of experimental interventions. Hence, biomarkers are needed to predict the effect of interventions over the long term.

Despite the great progress in our understanding of how lifespan can be modulated in experimental animals, human aging itself remains less well understood. Biomarkers need to be defined for those processes deemed critical for healthy human aging. To develop such biomarkers, integrated animal–human studies are needed with an important role for systems biology to understand better the molecular basis of the critical pro-aging processes targeted for intervention and the mechanistic rationale of potential interventions. Data on

epigenetic changes and other 'omics' level alterations with potential significance for critical pro-aging molecular pathways are important to collect.

Companion biomarkers

For each of the experimental interventions deemed promising enough for limited human testing, biomarkers need to be developed that are able to: (i) identify individuals who would benefit from the treatment; and (ii) help understand and assess the molecular target(s) of the agent being studied. The high failure rate of new therapies tested in clinical development accentuates the critical importance of discovering biomarker readouts that can predict efficacy.

Safety biomarkers

The development of 30% of new drug candidates is halted due to unforeseen toxicity and other adverse side effects in clinical studies. This is especially relevant for interventions in aging, which cannot be tested easily in clinical trials and need to be applied over extended periods of time. Accordingly, greater emphasis must be placed on the development and use of safety biomarkers in studies of interventions in aging. In this respect, it is possible to profit from safety biomarkers that have been developed and accepted over the years, for example, to detect toxicity in the kidney and liver.

Companion biomarkers and safety biomarkers are not specific to aging or age-related disease. The challenge to the field is to develop a consensus panel of biomarkers of aging that can be used in clinical trials, that is, biomarkers in category 1 above. The following is a list of potential biomarkers or methods to assess biological age discussed at the workshop. It does not represent a comprehensive listing of all possible biomarkers, but a list of potential markers and methods to assess biological age and the risk of developing age-related diseases suggested by the participants and discussed at the meeting.

- 1 Standard frailty measurements (e.g., walking speed at older ages, hand grip, VO_{2max})
- 2 Insulin, insulin resistance, fasting blood glucose + GTT, HbA1c, adiponectin, DEXA (abdominal adiposity)
- 3 LDL, HDL, blood pressure, pulse wave velocity, intima media thickness, left ventricular diastolic function/pressure
- 4 Inflammatory markers (e.g., CRP, IL-6, TNF- α)
- 5 Cognitive performance (cognitive tests, fMRIs)
- 6 Lymphocyte number, lymphoid/myeloid ratio
- 7 IGF-I, T3
- 8 Epigenetic profile (e.g., DNA methylation)
- 9 Renal clearance (age 60–90)

Dietary interventions mimicking calorie restriction

Intermittent and prolonged fasting

Fasting is the most extreme of the DR interventions because it requires the complete elimination of nutrients. The best-characterized form of fasting evaluated in both rodent and human studies is intermittent or alternate-day fasting (IF or ADF), which involves the feeding on every other day for long periods of time (Trepanowski *et al.*, 2011). Prolonged fasting (PF), in which water but not food is consumed for two or more consecutive days, is also becoming a well-investigated intervention, particularly in rodent models and lower eukaryotes (Longo & Mattson, 2014). The mechanisms of action of fasting are best understood in the

yeast *Saccharomyces cerevisiae*, in which a switch from glucose-containing medium to water causes the downregulation of the Tor–S6K and Ras–adenylate cyclase–PKA pathways, and the consequent activation of the stress resistance transcription factors Msn2/4 and Gis1, which regulate many protective and metabolic genes (Wei *et al.*, 2008). These changes, similar to those observed in starved worms and mice, promote resistance to both multiple toxins and longevity extension. In mice, fasting reduces circulating IGF-I and causes downregulation of PI3K–AKT, Tor–S6K, and adenylate cyclase–PKA signaling, which, analogously to yeast, results in the activation of multiple transcription factors including members of the FOXO family of forkhead transcription factors (Cheng *et al.*, 2014).

Different types of PF or IF have been shown to extend the lifespan of bacteria, worms, and rodents (Longo & Mattson, 2014). In mouse models, IF helps to prevent or delay the progression of myocardial infarction, diabetes, stroke, Alzheimer's disease, and Parkinson's disease (Longo & Mattson, 2014; Mattson, 2014). Similarly, PF protects mice against the adverse effects of chemotherapy and ischemia/reperfusion-mediated toxicity as well as cancer progression and promotes stem cell-dependent regeneration and immune system rejuvenation in old animals (Mauro *et al.*, 2014, Safdie *et al.*, 2009, Longo & Mattson 2014 Cheng *et al.*, 2014; Levine *et al.*, 2014). Although the effect of chronic cycles of PF on healthspan is not known, these studies point to PF, which could be carried out in humans as infrequently as once a month or less, as a potent inducer of protective systems and a potential alternative to chronic CR and IF.

A number of PF and IF clinical studies in humans are yielding very promising results in support of the possibility that they are sufficiently safe and effective to be considered for long-term clinical trials focused on healthspan (Longo & Mattson, 2014). Among the initial but solid evidence for efficacy is that PF followed by a vegetarian diet reduces both inflammation and pain in patients with rheumatoid arthritis (Michalsen *et al.*, 2005). Preliminary studies suggest that PF may also decrease adverse effects of chemotherapy in humans (Safdie *et al.*, 2009), a finding now being tested in multiple larger randomized clinical trials. Part of the protective effects of fasting against aging and disease may be mediated by the reduction in IGF-1, glucose, and insulin (see following sections).

The health effects of IF in humans have been investigated more extensively than those of PF. For example, 3 weeks of alternate-day fasting reduces body weight, body fat, and plasma insulin concentrations in both men and women (Heilbronn *et al.*, 2005), while a diet of 500–600 calories on 2 of 7 days per week induces loss of abdominal fat, improves insulin sensitivity, and reduces blood pressure (Harvie *et al.*, 2010).

PF and IF have few adverse effects, but could be dangerous for subjects of very low BMI, those who are frail and old, and patients with diabetes receiving insulin or insulin-like drugs. Thus, the lack of medical supervision in subjects undergoing IF or PF could result in severe adverse effects. For example, the insulin-sensitizing effects of PF can cause severe hypoglycemia and even death in patients with diabetes treated with insulin. Although major adverse effects caused by IF or PF are rare and usually reversible, these examples should underscore the potency of these interventions and their potential to cause remarkably global and beneficial effects, but also cause detrimental effects if the underlying mechanism of action are not well understood and they are not properly tested clinically and implemented. These issues point to the need for the identification and preclinical/clinical testing of fasting mimicking diets that match or even surpass the effects of fasting while minimizing the burden and adverse effects associated with water-only regimens.

Protein restriction or selective amino acid restriction

The benefits attributed to reduced calorie intake could be due, in part, to the concomitant restriction of proteins or individual essential amino acids. Restriction of calories in the form of protein contributes to the benefits of DR on animal longevity (Gallinetti *et al.*, 2013; Mirzaei *et al.*, 2014). Restriction of individual essential amino acids, including methionine and tryptophan, can also extend longevity (Spindler, 2009). Notably it remains unclear whether the underlying mechanisms responsible for increased longevity overlap with those of other DR regimens. It is also not known whether there is some specificity to the effects of restriction of different essential amino acids.

Amino acid levels are sensed by at least two evolutionarily conserved mechanisms: one involving GCN2 (general control nonderepressible 2) and the other involving mTOR. In mammals, mTOR is activated by amino acids, particularly leucine, while GCN2 is activated by the absence of many individual amino acids. Mechanisms of protection downstream of GCN2 activation and mTOR repression subsequent to protein/amino acid starvation remain largely unknown. However, the transcription factor ATF4 is stabilized upon GCN2 activation and could be a key mediator in a variety of extended longevity models, including hypopituitary dwarf models and methionine restriction (Li *et al.*, 2014).

The major factor controlling the longevity benefits of protein restriction in multiple species is the ratio of dietary protein to other macronutrient calorie sources (carbohydrates, fats), which is likely to affect aging in part through the regulation of mTORC1 signaling (Solon-Biet *et al.*, 2014). Longevity extension by methionine restriction in yeast requires GCN2 (Wu *et al.*, 2013), but other mechanisms including autophagy (Ruckenstuhl *et al.*, 2014) and retrograde signaling from mitochondria (Johnson & Johnson, 2014) are implicated. In yeast, deficiencies in serine, threonine and valine, extend longevity by a mechanism involving the down-regulation of orthologs of mammalian PDK and Tor-S6K proteins (Mirisola, *et al.*, 2014). In flies, reduced dietary methionine also extends longevity although the mechanism responsible for this effect is poorly understood (Lee *et al.*, 2014). It has been shown that GH signaling is necessary to discriminate levels of dietary methionine in mice (Brown-Borg *et al.* 2014). In rodents, protein/amino acid restriction also offers health benefits in models of acute stress and chronic disease. Eliminating tryptophan from the diet for 1 week increases the resistance to the acute stress of ischemia/reperfusion injury in liver and kidney and this depends on GCN2 (Peng *et al.*, 2012). However, GCN2 is no longer necessary for this beneficial effect when animals are also deprived of total protein. Instead, mTORC1 downregulation is key to stress resistance in this case. This effect could be mediated in part through the negative action of mTORC1 on insulin sensitivity, such that its downregulation allows for increased pro-survival signaling after reperfusion (Harputlugil *et al.*, 2014).

Benefits of short-term protein deprivation also include protection from intimal hyperplasia (Mauro *et al.*, 2014). Similarly, cycles of protein deprivation lasting 1 week, followed by a week of *ad libitum* access to a complete diet, protect against Tau phosphorylation in a mouse model of Alzheimer's disease (Parrella *et al.*, 2013). Finally, protein restriction has beneficial effects on metabolism, activating a fasting-like response that requires both GCN2 and PPAR-alpha (peroxisome proliferator-activated receptor-alpha), and that causes an increase in the fasting hormone FGF21 (Laeger *et al.*, 2014).

To date, very few studies have been performed in humans on the potential beneficial effects of protein and/or amino acid restriction on

aging processes or age-associated chronic diseases (Cavuto & Fenech, 2012; Mirzaei *et al.*, 2014). In agreement with mouse studies (Solon-Biet *et al.*, 2014), the lowest protein intake was associated with reduced risk for cancer incidence and overall mortality, but only in the 65 and younger group of individuals (Levine *et al.*, 2014). Because of the inefficient utilization of dietary protein associated with differences in protein quality (i.e., essential amino acid composition and digestibility), the minimum recommended intake, which has been set at 0.66 g/kg/day for men and women 18 years or older, is likely to be higher in the elderly compared to that in younger adults (Levine *et al.*, 2014).

Pharmacological interventions mimicking calorie restriction

Inhibitors of the TOR pathway

The mTOR pathway has now been linked to lifespan and healthspan in several major model organisms and species. For instance, reduced mTOR signaling through genetic or pharmacological interventions leads to lifespan extension in yeast, worms, flies, and mice (Johnson *et al.*, 2013), and studies are currently being conducted in primates and humans. Thus, mTOR signaling is a major candidate for targeted interventions. The mTOR kinase exists in two complexes: mTORC1 and mTORC2 (Laplane & Sabatini, 2012). Most studies indicate that reduced mTORC1 signaling confers longevity benefits (Johnson *et al.*, 2013). However, individually reducing either mTORC1 or mTORC2 signaling extends worm lifespan (Vellai *et al.*, 2003; Soukas *et al.*, 2009). In addition to being responsive to insulin/IGF signaling, mTORC1 is activated by amino acids through the RAG GTPase complex and suppressed by stress signals or energy deficiency (Kim *et al.*, 2013). In short, mTORC1 activation leads to protein translation and cell growth, whereas its inhibition blocks growth and induces stress response pathways such as autophagy (Laplane & Sabatini, 2012).

Many of the interventions that extend lifespan in model organisms have the effect of reducing mTORC1 signaling (Johnson *et al.*, 2013; Kennedy & Pempacker, 2014). These include protein and calorie/dietary restriction and reduced insulin/IGF signaling, as well as activation of AMP kinase and possibly of sirtuins, raising the question of whether the benefits of these interventions are, at least in part, dependent on their effects on mTORC1. Another fundamental question involves the downstream pathways of mTORC1 that mediate the longevity effects. Two major substrates of mTORC1, S6 kinase and 4E-BP1, are both linked to longevity. Loss of S6 kinase promotes longevity in yeast, flies, worms, and mice (Johnson *et al.*, 2013), and increased 4E-BP1 activity promotes longevity at least in flies. In *C. elegans*, the DAF-16/FOXO transcription factor is required for lifespan extension in S6 kinase-defective mutants (Seo *et al.*, 2013), as is autophagy (Hansen *et al.*, 2008). Interestingly, DAF-16/FOXO and autophagy are both required for impaired insulin/IGF-1 signaling to extend lifespan in worms (Kenyon *et al.*, 1993; Melendez *et al.*, 2003), further linking these two perturbations.

An appealing aspect of considering mTOR as a target for anti-aging interventions is the availability of rapamycin, a pharmacological agent that is a specific inhibitor of mTOR, and that has been shown to extend lifespan in mice, up to 30% in females at high doses and to a lesser extent in males (Harrison *et al.*, 2009; Miller *et al.*, 2014). Rapamycin is not an active site inhibitor, but rather creates a trimolecular complex between the mTOR kinase and an FKBP protein, most notably FKBP12 (Brown *et al.*, 1994; Sabatini *et al.*, 1994; Marz *et al.*, 2013). This action disrupts the mTORC1 complex leading to acute inhibition and prevents newly synthesized mTOR from entering either mTORC1 or

mTORC2, leading to chronic inhibition of both complexes (Sarbasov *et al.*, 2006).

Rapamycin has been studied for decades, tested in hundreds of clinical trials, and approved for use in several clinical conditions, including kidney cancers (Lamming *et al.*, 2013; Kennedy & Pennypacker, 2014). However, rapamycin has important side effects, which limits its consideration as an anti-aging therapy. The adverse effects of rapamycin include metabolic dysregulation (e.g., hyperglycemia, hyperinsulinemia, and insulin resistance), proliferative defects in hematopoietic lineages, and others (Soefje *et al.*, 2011). The metabolic side effects of rapamycin have been attributed to inhibition of mTORC2 (Lamming *et al.*, 2012), suggesting that it may be possible to enhance efficacy-to-adverse effect ratios by specifically inhibiting mTORC1. Safety studies using rapamycin have been not been conducted in healthy people, so it is not clear whether rapamycin is harmful in healthy adults. Currently, several trials testing rapamycin are ongoing in healthy older populations, which will help evaluate the importance of mTOR in human aging and determine whether mTOR should be considered a target for interventions to extend healthspan.

In summary, ongoing studies to address the role of mTOR in aging and to determine whether it is a valuable target for interventions to extend healthspan are extensive and will likely shed light on these important questions in the near future.

Inhibitors of glycolysis

Another area that is being actively explored as a strategy of mimicking DR is the inhibition of enzymes within the glycolytic pathway (Minor *et al.*, 2009). It has been suggested that this strategy could provide a more effective upstream targeting to produce DR-like effects rather than targeting single downstream pathways. The rationale is that a greater cascade of responses could be activated, although this could also lead to a broader range of adverse effects (Minor *et al.*, 2009). The first candidate drug in this category, 2-deoxy-D-glucose (2DG), which inhibits phosphoglucose isomerase, produced a remarkable phenotype similar to DR when fed to rats (Minor *et al.*, 2009); however, other long-term studies reported that the concentrations necessary to elicit DR-like physiological responses also had cardiotoxic effects (Ganapathy-Kanniappan & Geschwind, 2013).

Recent preliminary studies have reported promising results with a nutraceutical product made from unripened avocados containing a seven-carbon sugar, mannoheptulose, which inhibits hexokinase (Minor *et al.*, 2009). Studies in mice and dogs showed that the avocado extract improved insulin sensitivity as well as increased median lifespan in nematodes and mice. Additionally, there is currently great interest in applying glycolytic inhibitors in the treatment of cancer, as many tumor cells switch their metabolism to glucose as the major energy source (Ingram & Roth, 2010).

Mice with dysfunctional telomeres and senescent cells in culture display increases in energy demand and defects in mitochondrial biogenesis (Passos *et al.*, 2010). In this context, supplementation with glucose improves tissue and body weight maintenance, increasing their lifespan (Missios *et al.*, 2014). These results may appear counterintuitive as they suggest that an increase in dietary glucose can help maintain energy and tissue homeostasis at advanced age. Of note, however, malnutrition is present in more than 30% of geriatric patients. Furthermore, this requirement for additional glucose at older ages is analogous to that described above for protein, underscoring the need for a better understanding of optimal age-specific dietary composition and intake.

Inhibitors of the GH/IGF-1 axis

Although the lack of global IGF-1 signaling is lethal, data from studies conducted in animal models have shown that a reduction in IGF-1 levels or IGF-1 action can extend lifespan. Additionally, human IGF-1 receptor gene polymorphisms are associated with exceptional longevity (Suh *et al.*, 2008), and recently, Barzilai and colleagues have shown that low plasma IGF-1 concentrations predict survival in long-lived people (Milman *et al.*, 2014), specifically in women with a history of cancer. In animals, dwarf, long-lived mice lacking the growth hormone receptor (GHR^r) have reduced levels of IGF-1, are insulin sensitive despite obesity, and have decreased risk for cancer and diabetes (Zhou *et al.*, 1997; Shevah & Laron, 2007; Ikeno *et al.*, 2009). Importantly, similar results have been reported in growth hormone receptor-deficient Laron syndrome (LS) patients. In this regard, no formal aging studies have been performed on patients with LS; however, they are protected from diabetes and fatal neoplasms. (Guevara-Aguirre *et al.*, 2011; Steuerman *et al.*, 2011). Thus, pharmaceutical interventions that directly lower IGF-1 levels in adults could improve health and prolong lifespan.

Pharmacological targets for lowering IGF-1 action include those that act directly or indirectly on cells/tissues that produce or respond to GH and/or IGF-1. In this regard, human or humanized monoclonal antibodies and drugs directed against the IGF-1R have been used in clinical trials to treat several types of cancer (Warshamana-Greene *et al.*, 2005; Carboni *et al.*, 2009); however, none have been approved for clinical use. We are unaware of the development of any antibody against GH or IGF-1, but several classes of compounds that inhibit the GH/IGF-1 axis have been approved for use in patients with acromegaly. Recently, a consensus document has been developed for the use of this therapeutics (Giustina *et al.*, 2014). One of these drug classes, somatostatin analogues, lower serum GH levels by suppressing GH secretion by pituitary somatotrophs, thereby ultimately decreasing serum IGF-1 levels. Unfortunately, these compounds also suppress secretion of other endocrine hormones, including insulin. Furthermore, only 20-50% of patients with acromegaly respond to these drugs, and significant adverse events have been documented including gallstones, diarrhea, and anorexia. Thus, the use of somatostatin analogues to increase longevity or healthspan appears to be unwarranted at this time.

The second approved drug for treating acromegaly is the GH receptor antagonist pegvisomant (Trainer *et al.*, 2000; van der Lely *et al.*, 2001; Kopchick *et al.*, 2002; van der Lely & Kopchick, 2006). Pegvisomant is unique in that it does not inhibit GH secretion, but rather inhibits GH action by binding to and blocking the GHR (Kopchick *et al.*, 2002). Notably, a dose-dependent decrease of IGF-1 levels is seen in up to 90% of pegvisomant-treated patients (Trainer *et al.*, 2000; van der Lely *et al.*, 2001; Kopchick *et al.*, 2002; van der Lely & Kopchick, 2006). Additionally, pegvisomant is an insulin sensitizer that blocks the diabetogenic action of GH and thus produces beneficial effects on glucose metabolism. Pegvisomant, therefore, could have positive effects on both longevity and healthy aging by lowering serum IGF-1 and increasing insulin sensitivity. Regarding adverse effects, van der Lely *et al.* (2012) reported that *Long-term data on the efficacy and safety profile of pegvisomant are reassuring and few long-term serious adverse events have been reported but ongoing vigilance is required to monitor liver function and tumor size.* Thus, pegvisomant is an approved drug that should be tested for its effects on longevity and healthy aging. Future therapeutics targeted at inhibiting the GH/IGF-1 axis could include small inhibitory RNAs directed against the GHR or IGF-1 receptor mRNAs, monoclonal antibodies directed against GH or IGF-1, or novel GHR or IGF-1R tyrosine kinase inhibitors.

Another way to reduce global IGF-1 action may be to inhibit IGF-1 availability. For example, loss of PAPP-A, a protease that cleaves and inactivates the IGF-1 sequestering protein IGFBP-4, reduces IGF-1-induced signaling without affecting overall serum IGF-1 levels and not only extends mouse lifespan, but has many other beneficial effects on healthspan and age-related diseases (Conover, 2012).

In summary, reducing the activity of the GH/IGF-I somatotrophic axis is perhaps the most validated and consistent genetic intervention to extend mouse lifespan and healthspan. In addition GHR/IGF-I deficiency is also among the few phenotypes that is well characterized in humans (patients with Laron syndrome) with very few side effects in adults, even considering the extreme level of GH receptor deficiency and the resulting >80% reduction in circulating IGF-I. Notably, a pharmaceutical intervention targeting this pathway may or may not be designed to achieve such a low level of hepatic IGF-I secretion.

Activators of the sirtuin pathways

The deacetylases known as sirtuins (SIRT1 to 7) promote longevity in diverse species and could mediate many of the beneficial effects of DR (Sato *et al.*, 2013). Given their apparent role in mediating health benefits, sirtuins have attracted considerable interest as a drug target. The first potent sirtuin-activating compounds (STACs) to be identified included several classes of plant-derived metabolites, such as flavones, stilbenes, chalcones, and anthocyanidins. These phytochemicals directly activate SIRT1 *in vitro* through an allosteric mechanism that lowers substrate K_m (Howitz *et al.*, 2003). Resveratrol (3,5,4'-trihydroxystilbene) is still the most potent of these natural activators identified to date. The discovery of natural STACs prompted the production of synthetic SIRT1 activators that are considerably more potent, soluble, and bioavailable (Hubbard & Sinclair, 2014). In many studies, resveratrol and synthetic STACs have been shown to induce physiological and gene expression changes that are similar to DR, and improve function and extend the lifespan of numerous organisms including *S. cerevisiae*, *C. elegans*, *D. melanogaster*, *N. furzeri* (a short-lived fish), and *A. mellifera* (Hubbard & Sinclair, 2014).

An alternative approach to activating sirtuins, which raises the activity of the entire family of enzymes, is to exploit their common requirement for NAD⁺. NAD⁺ levels can be increased by providing NAD precursors (NMN or NR), by activating NAD biosynthetic enzymes (Wang *et al.*, 2014a), or by inhibiting the NAD hydrolase CD38 (Yoshino *et al.*, 2011; Canto *et al.*, 2012; Escande *et al.*, 2012; Gomes *et al.*, 2014).

In mice, resveratrol extends lifespan when given to animals on a high-fat diet (Pearson *et al.*, 2008) and in combination with standard chow when fed every other day (Baur *et al.*, 2006; Pearson *et al.*, 2008), but not when provided daily with standard chow (Pearson *et al.*, 2008; Strong *et al.*, 2013). Synthetic activators SRT1720 and SRT2104 also extend the lifespan of mice fed either a high-calorie or a low-calorie diet, and both protect mice against age-related changes in multiple tissues including muscle loss (Minor *et al.*, 2011). In preclinical models, STACs have also shown considerable promise in treating diseases and complications associated with aging including cancer, type 2 diabetes, inflammation, cardiovascular disease, stroke, and hepatic steatosis (Hubbard & Sinclair, 2014).

Considerable progress has been made in the use of STACs to treat inflammatory and autoimmune disorders (Hubbard & Sinclair, 2014). For example, SRT1720 has beneficial effects in mouse models of chronic obstructive disease and asthma. In rhesus monkeys fed a high-fat, high-sugar diet, resveratrol exerts anti-inflammatory effects in visceral white adipose tissue (Jimenez-Gomez *et al.*, 2013). STACs could also be

beneficial in neurodegeneration (Zhao *et al.*, 2013) based on mouse models of Alzheimer's or Parkinson's disease and multiple sclerosis (Graff *et al.*, 2013; Hubbard & Sinclair, 2014). STACs prevent and reverse the effects of obesity and age-related metabolic decline. In mice fed a high-calorie diet, resveratrol and STACs protect against obesity, increase insulin sensitivity, increase mitochondrial function, and prevent liver steatosis (Baur & Sinclair, 2006; Baur *et al.*, 2006). These effects are also seen in nonhuman primates fed a high-fat, high-sugar diet (Fiori *et al.*, 2013; Jimenez-Gomez *et al.*, 2013). Data from studies conducted in mouse models of obesity and aging have demonstrated that increasing NAD⁺ levels protects mice from metabolic decline and aging (Yoshino *et al.*, 2011; Canto *et al.*, 2012; Escande *et al.*, 2012) and reverses mitochondrial decline, inflammation, and markers of muscle wasting (Gomes *et al.*, 2014).

A considerable amount of data has accumulated on treating humans with STACs (Hubbard & Sinclair, 2014). Resveratrol has had mixed efficacy, in that its insulin-sensitizing effects (Ghanim *et al.*, 2011; Smoliga *et al.*, 2011) and DR-like phenotypes have been observed in elderly and obese humans (Timmers *et al.*, 2011), but not in nonobese subjects with normal glucose tolerance (Yoshino *et al.*, 2012). This suggests that STACs may restore homeostasis preferentially in metabolically compromised individuals. A meta-analysis of six unique datasets, including a total of 196 patients with type II diabetes (104 resveratrol, 92 control/placebo) showed statistically significant ($P < 0.05$) positive effects of resveratrol supplementation compared to placebo/controls for systolic blood pressure, hemoglobin A1c, and creatinine, but not for fasting glucose, homeostatic model assessment of insulin resistance, diastolic blood pressure, insulin, triglycerides, LDL, or HDL cholesterol (Hausenblas *et al.*, 2014). Synthetic STACs have been tested in humans and are currently in clinical development (Venkatasubramanian *et al.*, 2013; Hubbard & Sinclair, 2014). Human clinical studies with NAD⁺ precursors are expected in the next couple of years.

In summary, sirtuins and sirtuin activators have been implicated in longevity extension as well as in the prevention and treatment of a wide range of diseases in rodent models. However, there was not full consensus among meeting participants about the ability of sirtuin activators to slow aging, given long-running controversies in this area (Ledford, 2010; Couzin-Frankel, 2011).

Activators of the AMPK pathway

AMP-activated protein kinase (AMPK) is a conserved, energy-sensing serine/threonine kinase that is activated when cellular energy levels are low, resulting in increasing levels of AMP (Ruderman & Prentki, 2004). AMPK activation generates insulin-sensitizing effects resulting in increased glucose uptake in skeletal muscles, and decreased hepatic glucose production and enhanced fatty acid oxidation in several tissues (Ruderman & Prentki, 2004; Ruderman *et al.*, 2013). Activators have been developed, such as 5-aminoimidazole-4-carboxamide riboside (AICAR), and some FDA-approved drugs such as biguanides, thiazolidinediones, glucagon-like peptide-1 receptor agonists, salicylates, and resveratrol have AMPK-activating properties (Coughlan *et al.*, 2014).

The biguanide, metformin, which is a first line therapy for type 2 diabetes mellitus (T2DM) activates AMPK in the liver (Rena *et al.*, 2013). Evidence from animal models and *in vitro* studies suggests that metformin changes metabolic and cellular processes (Cabreiro *et al.*, 2013) associated with the development of age-related conditions (Ruderman & Prentki, 2004). Notably, treatment of rats (Anisimov, 2010) mice (Martin-Montalvo *et al.*, 2013) and nematodes (Cabreiro *et al.*, 2013) with metformin extends lifespan. Exercise also stimulates

AMPK and causes stimulation of glucose uptake and mitochondrial biogenesis during and after exercise. AICAR administration closely mimics these effects (Hayashi *et al.*, 1998; Song *et al.*, 2002).

Numerous studies are now focused on whether anti-aging effects of metformin can be demonstrated in patients with T2DM. Notably, in the United Kingdom Prospective Diabetes Study (UKPDS), metformin use, compared with other antidiabetic drugs, decreased the risk of cardiovascular disease (Group, 1998), cancer incidence and overall mortality (Wu *et al.*, 2014), and possibly cognitive decline (Ng *et al.*, 2014). Safety has been established and the pro-longevity effects noted would argue for testing the use of metformin in humans. However, to consider metformin for clinical trials testing its potential anti-aging effect, it will be important to closely examine the known effects of long-term use on a wide range of subjects and particularly those who are relatively healthy, but also those with specific conditions for whom chronic metformin use may have been shown to be detrimental. For example, recent evidence indicates that metformin also promotes inhibition of mitochondrial glycerophosphate dehydrogenase and gluconeogenesis, suggesting that only a partial understanding of the mechanisms of action of this powerful drug is known, and that further studies are necessary to determine whether it should be considered for treating generally healthy or relatively healthy populations (Madiraju *et al.*, 2014). In fact, gluconeogenesis is required during fasting but also plays an important role in people consuming ketogenic diets, which may potentially make metformin dangerous for a subset of the population on diets that are relatively common and depend on gluconeogenesis.

Inhibitors of inflammatory pathways

Chronic, low-grade inflammation is recognized as a major characteristic of aging. This phenomenon is so pervasive that the term *inflammaging* (Franceschi *et al.*, 2000; Franceschi & Campisi, 2014) has been coined to emphasize that many major age-related disabilities, including cancers, susceptibility to infections, and dementia have immunopathogenic components (Franceschi & Campisi, 2014). Thus, as inflammation is associated with many age-related conditions, genes and pathways that regulate inflammation are candidate targets to combat them (Franceschi & Campisi, 2014). Inflammaging appears to be much more complex than we previously thought, and a variety of tissues and organs participate in producing inflammatory stimuli (Franceschi *et al.*, 2007; Cevenini *et al.*, 2012). The list is extensive and includes the immune system, but also adipose tissue, skeletal muscle, liver, and the gut. The gut is of unique importance, because it is the body's largest immune organ and contains trillions of bacteria that can release inflammatory stimuli into the portal and systemic circulation (Biagi *et al.*, 2010).

Despite its importance, mechanistic details for the most important stimuli that trigger inflammaging remain unknown, and much additional investigation is needed. Stimuli triggering inflammation can be exogenous (e.g., persistent cytomegalovirus infection) (Sansoni *et al.*, 2014), but most are probably endogenously produced, possibly coming from the 'self-debris' resulting from the continuous turnover of cells and tissues (Franceschi & Campisi, 2014). For example, circulating mitochondrial DNA (mtDNA) is recognized by immune sensors as a foreign nucleic acid, is a powerful inflammatory stimulus, and increases with age (Pinti *et al.*, 2014). Pro-inflammatory galactosylated N-glycans, which also represent one of the most powerful biomarkers of biological age in humans (Dall'Olio *et al.*, 2012), and pro-inflammatory circulating microRNA ('inflammaMIR') (Olivieri *et al.*, 2013) also increase in circulation with age and could contribute to inflammaging.

The most important drivers of age-dependent inflammation probably lie at the cellular and molecular levels. Cellular senescence is associated with a pro-inflammatory senescence-associated secretory phenotype (SASP), which is triggered by damaging agents (radiation, viruses) and possibly by continuous exposure to cellular debris (Coppe *et al.*, 2010). Cellular senescence can also spread to neighboring cells (Jurk *et al.*, 2014). Second, DNA and telomere damage caused by reactive oxygen species and other agents can trigger an inflammatory DNA damage response (Vitale *et al.*, 2013). Third, activation of inflammasomes and the NF- κ B pathway can be elicited by ROS and cellular debris (Youm *et al.*, 2013). These mechanisms provide many targets for therapies to decrease inflammaging either locally or systemically. Possible strategies to target them include elimination of senescent cells (e.g., by NK cell activation) (Tchkonia *et al.*, 2013), de-activation of inflammasomes (Youm *et al.*, 2013), diets enriched with omega3 fatty acids (the 'Mediterranean diet') (Berendsen *et al.*, 2013), and other nutritional strategies, such as DR discussed above.

In mouse studies, anti-inflammatory drugs showed a potential for extending lifespan, although their effects were relatively small (Strong *et al.*, 2008); for example, nordihydroguaiaretic acid (NDGA) and aspirin increased survival but did not extend maximum lifespan. Other anti-inflammatory drugs were not effective in lifespan extension, underlining the need for additional and larger studies to determine the potential of NSAID and other anti-inflammatory drugs in extending human healthspan.

Modulators of epigenetic pathways

The term epigenetics denotes heritable phenotypic alterations caused by postreplicative modifications of chromatin, rather than classical mutation-based genetic changes. Such covalent and noncovalent modifications of DNA and proteins (e.g., histones) alter the state of chromatin conformation and elicit corresponding changes in transcriptional activity (Jaenisch & Bird, 2003; Goldberg *et al.*, 2007; Baker *et al.*, 2008). Epigenetic effects can be elicited by three distinct principal means: (i) DNA methylation; (ii) post-translational histone modifications; and (iii) noncoding RNA interference (Goldberg *et al.*, 2007; Baker *et al.*, 2008). Twin studies suggest that genetics at birth determines only 25% of lifespan; therefore, it is proposed that epigenetic factors also contribute to aging. Such epigenetic factors are likely influenced by lifestyle, diet, and exogenous stress, raising the possibility that strategies can be developed to ameliorate age-associated cellular dysfunction (Imai *et al.*, 2000; Longo, 2009).

Although manipulation of enzymes (sirtuins, histone acetyltransferases, histone deacetylases) that regulate the (de)acetylation status of chromatin (and other targets) can prolong lifespan in yeast, flies, and worms, the role of histone modifications in lifespan regulation is poorly understood. A fly model has recently been introduced in which the impact of such histone mutations on aging and lifespan can be evaluated (Pengelly *et al.*, 2013).

A naturally occurring polyamine, spermidine, directly inhibits histone acetyltransferases (HATs), thereby maintaining histone H3 in a hypoacetylated state (Eisenberg *et al.*, 2009). Functionally, this results in higher resistance to heat and oxidative stress as well as markedly reduced rates of cell necrosis during aging in human and yeast cells. Strikingly, this mechanism extends chronological lifespan across species, including flies, nematodes, and human cells. These data support the existing body of knowledge regarding histone acetylation in lifespan maintenance, including the finding that deletion of *sas2*, encoding a histone acetyltransferase, extends the replicative lifespan in yeast (Dang

et al., 2009). Sas2 antagonizes Sir2, a prominent histone deacetylase involved in aging, and its deletion stabilizes Sir2 levels in aging cells, thereby allowing a low basal level of acetylation on specific histone residues associated with longevity regulation (Raisner & Madhani, 2008). Finally, a simple way to change age-related histone acetylation consists of dietary strategies that deplete cellular acetyl CoA, the sole donor for acetylation reactions. Indeed, depletion of acetyl CoA has been recently shown to be sufficient for autophagy induction and lifespan extension, although it is not known whether these effects are dependent on epigenetic changes (Eisenberg *et al.*, 2014; Marino *et al.*, 2014).

In humans, only nontoxic natural substances such as spermidine or resveratrol, which lead to deacetylation of chromatin, should be considered for clinical testing (Morselli *et al.*, 2011). As a caveat, mechanistic understanding of this strategy is highly challenging as the drugs could have many off-target effects and even at the epigenetic level, the integrated response of multiple histone sites might be needed to mediate anti-aging effects. However, data from mice and humans indicate that spermidine has the potential to be safe for testing its epigenetic-dependent and independent effects on human healthspan. In one human study, a polyamine-rich traditional Japanese food (fermented soybeans) showed significant enhancement of polyamine concentration in the blood of the participants without obvious adverse effects (Soda *et al.*, 2009).

Other promising potential drugs and drug targets

β 2-adrenergic receptor (β 2AR) signaling

Chronic administration of β 2AR agonists increases mortality and morbidity (Ho *et al.*, 2010). Conversely, β 2AR antagonists (β -blockers) decrease mortality after myocardial infarction and improve the health of individuals with heart failure (Bristow, 2000; Ellison & Gandhi, 2005). Oral administration of the β -blockers, metoprolol and nebivolol, beginning at 12 months of age, increased the mean and median lifespan of isocalorically fed male C3B6F1 mice by 10 and 6.4%, respectively (Spindler *et al.*, 2013). Neither drug affected body weight or food intake, eliminating DR or altered energy expenditure as explanations for these effects. The drugs also extended *Drosophila* lifespan without affecting food intake. The effects of long-term administration of β -blockers on human healthspan need to be investigated further in mice and humans before they can be considered for anti-aging interventions in healthy individuals.

Meso-nordihydroguaiaretic acid (NDGA)

NDGA is a lignin present at high concentrations in creosote bushes (V.E.Tyler, 1994). Oral administration of NDGA extends *Drosophila* and mouse lifespan (Spindler *et al.*, 2014). Studies *in vitro* show that NDGA inhibits intercellular inflammatory signaling, tumor cell proliferation, insulin-like growth factor-1 (IGF1R) and HER2 receptor activation, and oxidative phosphorylation (Pardini *et al.*, 1970; Lu *et al.*, 2010). NDGA reduces weight in a dose-dependent manner without change in food consumption, suggesting it either decreases absorption or increases caloric utilization (Spindler *et al.*, 2014). NDGA was not overtly toxic in mice, but was associated with increased liver, lung, and thymus tumors, as well as peritoneal hemorrhage (Spindler *et al.*, 2014). Less toxic derivatives of NDGA should be explored as anti-aging therapeutics in preclinical trials (Meyers *et al.*, 2009; Castro-Gamero *et al.*, 2013), although the associations with toxicities make it an unlikely candidate for human healthspan interventions.

Statins and angiotensin-converting enzyme (ACE) inhibitors

Statins (3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors) reduce age-related heart arrhythmias (Ludman *et al.*, 2009; Spindler *et al.*, 2012) and mortality from multiple types of cancer (Zeichner *et al.*, 2012; Nielsen *et al.*, 2013). The health benefits of statins stem from reduced protein isoprenylation (Spindler *et al.*, 2012) and reduced cholesterol biosynthesis (Ludman *et al.*, 2009). Statins increase the lifespan and healthspan of *Drosophila* by decreasing protein isoprenylation (Spindler *et al.*, 2012). ACE inhibitors are antihypertensives (Crowley *et al.*, 2012) that reduce AT₁R activity, thereby reducing mortality and morbidity secondary to myocardial infarction (Corvol *et al.*, 2004; Gradman, 2009; Hoogwerf, 2010; Crowley *et al.*, 2012), and mitigating adverse cardiac events in patients with congestive heart failure (Corvol *et al.*, 2004; Gradman, 2009; Hoogwerf, 2010; Crowley *et al.*, 2012). Combined oral administration of statins and ACE inhibitors extends mouse lifespan by approximately 9% without affecting serum cholesterol or food intake. However, monotherapy with either drug is not effective. Statins and ACE inhibitors are generally well tolerated, and together, they could increase the lifespan of normotensive, normocholesterolemic individuals. Further studies and the understanding of their mechanisms of action and potential effects on aging are necessary before they can be considered anti-aging drugs. Considering the very wide use of statins in many countries, we should be able to begin to investigate its broader healthspan effects on the relatively healthy populations being treated for mildly elevated cholesterol.

Hexosamine pathway and glycobiochemistry

The hexosamine pathway produces the metabolite uridinediphosphate N-acetylglucosamine (UDP-GlcNAC), which is the precursor for N-linked glycosylation in the endoplasmic reticulum, and O-linked glycosylation in cytosol and other compartments. Activation of this pathway through gain-of-function mutations in the enzyme GFAT-1 (glutamine fructose 6-phosphate amino transferase) results in excess UDP-GlcNAC, and extension of *C. elegans* lifespan (Denzel *et al.*, 2014). Activation of the hexosamine pathway or upregulation of GlcNAC enhances several aspects of protein quality control, including proteasome activity, autophagy, and ER-associated degradation pathways, which collectively lead to alleviation of phenotypes in models of proteotoxic disease. In mice, ischemia/reperfusion of the heart triggers the ER stress response, with upregulation of GFAT as one of the consequences. GFAT upregulation or GlcNAC treatment was reported to protect against ischemic challenge (Wang *et al.*, 2014b). Additionally, the related metabolite glucosamine has been shown to extend murine lifespan (Weimer *et al.*, 2014). These studies point to the potential significance of the hexosamine pathway in regulating protein quality control and longevity. Previous work in this area also suggested that overactivation of the hexosamine pathway could produce diabetic-like symptoms (Hawkins *et al.*, 1996). Thus, the activity and tissue responses of this pathway will need to be optimized to achieve beneficial effects. Interestingly, the long-lived naked mole rat harbors extensive hyaluronan glycoconjugates (comprised of GlcNAC and glucuronic acid subunits), thought to be protective against cancer and perhaps other age-related diseases (Tian *et al.*, 2013). Glycoconjugates are also suggested to serve as predictive biomarkers of human aging (Dall'Olio *et al.*, 2013). Clearly, the hexosamine pathway could provide novel targets to treat age-related disease and further work in this area is merited.

DNA damage signaling

Studies in mice provided the proof of principle that the deletion of upstream DNA damage responses (Exo1-dependent end resection) and downstream DNA damage checkpoints (p21-dependent cell cycle arrest, Puma-dependent apoptosis) can prolong tissue maintenance and increase the lifespan of aging telomerase-deficient mice (Choudhury *et al.*, 2007; Schaetzlein *et al.*, 2007; Sperka *et al.*, 2011; Wang *et al.*, 2012). This is a potential anti-aging target as telomere dysfunction and DNA damage accumulate in aging human stem cells and tissues (Jiang *et al.*, 2008). Another connection between DNA damage signaling, telomeres and aging discussed at the meeting was the noncanonical activation of DNA damage pathways (e.g., ATM) by mitochondrial ROS (Schroeder *et al.*, 2013). This extends yeast chronological lifespan by epigenetically silencing subtelomeric transcription, suggesting that mitochondrial adaptive ROS signaling pathways could potentially be targeted to extend healthspan.

Stem cells

During aging, adult tissue stem cells exhibit impairments in functionality and exponential increases in premalignant mutations driven by cell-intrinsic defects and alterations in the stem cell niche and the blood circulatory environment (Ju *et al.*, 2007; Behrens *et al.*, 2014). Emerging data indicate that the reversal of age-associated defects in stem cell stability and function could help to improve tissue maintenance and to prevent stem cell-derived carcinogenesis during aging (Patel & Demontis, 2014). Both stem cell-based interventions and other dietary and pharmacological interventions which induce stem cell-based regeneration and rejuvenation are likely to be very important for healthspan in the future, but are currently only beginning to be tested as modulators of lifespan in model organisms.

Retrotransposable elements

Evidence from multiple model systems, including yeast, *Drosophila*, and more recently, mouse and human cell culture demonstrate that retrotransposable elements become active during cellular senescence and aging (De Cecco *et al.*, 2013a,b; Sedivy *et al.*, 2013). Active retrotransposition is mutagenic and potentially highly destabilizing to genomes. Given the importance of genome integrity as it relates to cancer and aging, this raises the interesting and novel possibility that activation of retrotransposition could contribute to some age-associated pathologies. Some nucleoside reverse transcriptase inhibitors currently used clinically to treat HIV infection, such as lamivudine or adefovir, block retrotransposition of several endogenous elements, including LINE1, the only known active retrotransposition family in human genomes (Dai *et al.*, 2011). While the current reverse transcriptase inhibitors have adverse effects arguing against their long-term use in humans as an anti-aging intervention, if studies in mice show beneficial effects, new drugs could be developed specifically to target LINE1 elements.

Conclusions

Accumulating scientific evidence from studies conducted in various organisms and species suggests that targeting aging will not just postpone chronic diseases but also prevent multiple age-associated metabolic alterations while extending healthy lifespan. A number of pathways affecting metabolism, growth, inflammation, and epigenetic

modifications that alter the rate of aging and incidence of age-related diseases have been identified. Interventions with the potential to target these pathways safely and to induce protective and rejuvenating responses that increase human healthspan are becoming available. These include intermittent or prolonged fasting, mild CR combined with a low glycemic index diet and protein restriction, inhibition of the GH/IGF-I axis, inhibition of TOR-S6K signaling, and activation of sirtuins or AMPK. Additional pharmacological interventions such as treatments with metformin, acarbose, spermidine, statins, and β -blockers should also be evaluated. While not yet ready for human trials, novel strategies including drugs that affect epigenetic modifications or inhibit retrotransposition deserve additional research and attention. Given the logistical issues of clinical studies aimed primarily at prolonging lifespan or healthspan, the participants of the workshop concluded that initial trials should be first designed to treat age-related diseases and conditions (not specifically aging), and should start with smaller cohorts, relatively short time periods, and a primary focus on safety and tolerability. This approach is likely to provide early clues for especially promising potential candidates that would then merit lengthier or more detailed studies potentially focused on aging.

In agreement with the title of this workshop *Interventions to Slow Aging in Humans: Are We Ready?*, we, the members of this workshop, believe that the time has come not only to consider several therapeutic options for the treatment of age-related comorbidities, but to initiate clinical trials with the ultimate goal of increasing the healthspan (and perhaps longevity) of human populations, while respecting the guiding principle of physicians *primum non nocere*.

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Conflict of interest

Longo, VD. has equity interest in L-Nutra, a company that develops medical food.

Barzilai, N. is a founder and on the board of Cohbar inc. Kenyon, C. is Vice President for Aging Research at Calico Life Sciences, a company focused on aging and age-related disease. Klein, S. is a stockholder and consultant of Aspire Bariatrics Shareholder and Officer:

Ingram, D. is a shareholder and officer at GeroScience, Inc. Prolongevity Technologies, Inc.

References

- Anisimov VN (2010) Metformin for aging and cancer prevention. *Aging (Albany NY)*. **2**, 760–774.
- Anton S, Leeuwenburgh C (2013) Fasting or caloric restriction for healthy aging. *Exp. Gerontol.* **48**, 1003–1005.
- Baker LA, Allis CD, Wang GG (2008) PHD fingers in human diseases: disorders arising from misinterpreting epigenetic marks. *Mutat. Res.* **647**, 3–12.
- Baur JA, Sinclair DA (2006) Therapeutic potential of resveratrol: the in vivo evidence. *Nat. Rev. Drug Discov.* **5**, 493–506.

- Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, Prabhu VV, Allard JS, Lopez-Lluch G, Lewis K, Pistell PJ, Poosala S, Becker KG, Boss O, Gwinn D, Wang M, Ramaswamy S, Fishbein KW, Spencer RG, Lakatta EG, Le Couteur D, Shaw RJ, Navas P, Puigserver P, Ingram DK, de Cabo R, Sinclair DA (2006) Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* **444**, 337–342.
- Behrens A, van Deursen JM, Rudolph KL, Schumacher B (2014) Impact of genomic damage and ageing on stem cell function. *Nat. Cell Biol.* **16**, 201–207.
- Berendsen A, Santoro A, Pini E, Cevenini E, Ostan R, Pietruszka B, Rolf K, Cano N, Caille A, Lyon-Belgy N, Fairweather-Tait S, Feskens E, Franceschi C, de Groot CP (2013) A parallel randomized trial on the effect of a healthful diet on inflammation and its consequences in European elderly people: design of the NU-AGE dietary intervention study. *Mech. Ageing Dev.* **134**, 523–530.
- Biagi E, Nylund L, Candela M, Ostan R, Bucci L, Pini E, Ninkila J, Monti D, Satokari R, Franceschi C, Brighi D, De Vos W (2010) Through ageing, and beyond: gut microbiota and inflammatory status in seniors and centenarians. *PLoS One* **5**, e10667.
- Bristow MR (2000) beta-adrenergic receptor blockade in chronic heart failure. *Circulation* **101**, 558–569.
- Brown EJ, Albers MW, Shin TB, Ichikawa K, Keith CT, Lane WS, Schreiber SL (1994) A mammalian protein targeted by G1-arresting rapamycin-receptor complex. *Nature* **369**, 756–758.
- Brown-Borg HM, Rakoczy SG, Wonderlich JA, Rojanathammanee L, Kopchick JJ, Armstrong V, Raasakka D (2014) Growth hormone signaling is necessary for lifespan extension by dietary methionine. *Ageing Cell* **13**, 1019–1027.
- Cabreiro F, Au C, Leung KY, Vergara-Irigaray N, Cocheme HM, Noori T, Weinkove D, Schuster E, Greene ND, Gems D (2013) Metformin retards aging in *C. elegans* by altering microbial folate and methionine metabolism. *Cell* **153**, 228–239.
- Canto C, Houtkooper RH, Pirinen E, Youn DY, Oosterveer MH, Cenn Y, Fernandez-Marcos PJ, Yamamoto H, Andreux PA, Cettour-Rose P, Gademann K, Rinsch C, Schoonjans K, Sauve AA, Auwerx J (2012) The NAD(+) precursor nicotinamide riboside enhances oxidative metabolism and protects against high-fat diet-induced obesity. *Cell Metab.* **15**, 838–847.
- Carboni JM, Wittman M, Yang Z, Lee F, Greer A, Hurlburt W, Hillerman S, Cao C, Cantor GH, Dell-John J, Chen C, Discenza L, Menard K, Li A, Trainor G, Vyas D, Kramer R, Attar RM, Gottardis MM (2009) BMS-754807, a small molecule inhibitor of insulin-like growth factor-1R/IR. *Mol. Cancer Ther.* **8**, 3341–3349.
- Castro-Gamero AM, Borges KS, Moreno DA, Suazo VK, Fujinami MM, de Paula Gomes Queiroz R, de Oliveira HF, Carlotti CG Jr, Scrideli CA, Tone LG (2013) Tetra-O-methyl nordihydroguaiaretic acid, an inhibitor of Sp1-mediated survivin transcription, induces apoptosis and acts synergistically with chemo-radiotherapy in glioblastoma cells. *Invest. New Drugs* **31**, 858–870.
- Cava E, Fontana L (2013) Will calorie restriction work in humans? *Ageing (Albany NY)* **5**, 507–514.
- Cavuto P, Fenech MF (2012) A review of methionine dependency and the role of methionine restriction in cancer growth control and life-span extension. *Cancer Treat. Rev.* **38**, 726–736.
- Cevenini E, Monti D, Franceschi C (2012) Inflamm-ageing. *Curr. Opin. Clin. Nutr. Metab. Care* **16**, 14–20.
- Cheng CW, Adams GB, Perin L, Wei M, Zhou X, Lam BS, Da Sacco S, Mirisola M, Quinn DI, Dorff TB, Kopchick JJ, Longo VD (2014) Prolonged fasting reduces IGF-1/PKA to promote hematopoietic-stem-cell-based regeneration and reverse immunosuppression. *Cell Stem Cell* **14**, 810–823.
- Choudhury AR, Ju Z, Djojosebrotto MW, Schienke A, Lechel A, Schaetzlein S, Jiang H, Stepczynska A, Wang C, Buer J, Lee HW, von Zglinicki T, Ganser A, Schirmacher P, Nakauchi H, Rudolph KL (2007) Cdkn1a deletion improves stem cell function and lifespan of mice with dysfunctional telomeres without accelerating cancer formation. *Nat. Genet.* **39**, 99–105.
- Christensen K, Doblhammer G, Rau R, Vaupel JW (2009) Ageing populations: the challenges ahead. *Lancet* **374**, 1196–1208.
- Colman RJ, Beasley TM, Kemnitz JW, Johnson SC, Weindruch R, Anderson RM (2014) Caloric restriction reduces age-related and all-cause mortality in rhesus monkeys. *Nat. Commun.* **5**, 3557.
- Conover CA (2012) Role of PAPP-A in aging and age-related disease. *Exp. Gerontol.* **48**, 612–613.
- Coppe JP, Despres PY, Krtolica A, Campisi J (2010) The senescence-associated secretory phenotype: the dark side of tumor suppression. *Annu. Rev. Pathol.* **5**, 99–118.
- Corvol PE, Eyries M, Soubrier F. (2004). Peptidyl-dipeptidase A/angiotensin I-converting enzyme. In *Handbook of Proteolytic Enzymes* (Barrett A., ed). Elsevier Academic Press 525 B Street, Suite 1900 San Diego, California 92101-4495, USA: Elsevier Academic Press, **1**, 332–346.
- Coughlan KA, Valentine RJ, Ruderman NB, Saha AK (2014) AMPK activation: a therapeutic target for type 2 diabetes? *Diabetes Metab. Syndr. Obes.* **7**, 241–253.
- Couzin-Frankel J (2011) Genetics. Aging genes: the sirtuin story unravels. *Science* **334**, 1194–1198.
- Crowley MJ, Powers BJ, Myers ER, McBroom AJ, Sanders GD (2012) Angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers for treatment of ischemic heart disease: future research needs prioritization. *Am. Heart J.* **163**, 777–782, e778.
- Dai L, Huang Q, Boeke JD (2011) Effect of reverse transcriptase inhibitors on LINE-1 and Ty1 reverse transcriptase activities and on LINE-1 retrotransposition. *BMC Biochem.* **12**, 18.
- Dall'Olio F, Vanhooren V, Chen CC, Slagboom PE, Wuhrer M, Franceschi C (2012) N-glycomic biomarkers of biological aging and longevity: a link with inflammation. *Ageing Res. Rev.* **12**, 685–698.
- Dall'Olio F, Vanhooren V, Chen CC, Slagboom PE, Wuhrer M, Franceschi C (2013) N-glycomic biomarkers of biological aging and longevity: a link with inflammation. *Ageing Res. Rev.* **12**, 685–698.
- Dang W, Steffen KK, Perry R, Dorsey JA, Johnson FB, Shilatfard A, Kaeberlein M, Kennedy BK, Berger SL (2009) Histone H4 lysine 16 acetylation regulates cellular lifespan. *Nature* **459**, 802–807.
- De Cecco M, Criscone SW, Peckham EJ, Hillenmeyer S, Hamm EA, Manivannan J, Peterson AL, Kreiling JA, Neretti N, Sedivy JM (2013a) Genomes of replicatively senescent cells undergo global epigenetic changes leading to gene silencing and activation of transposable elements. *Ageing Cell* **12**, 247–256.
- De Cecco M, Criscone SW, Peterson AL, Neretti N, Sedivy JM, Kreiling JA (2013b) Transposable elements become active and mobile in the genomes of aging mammalian somatic tissues. *Ageing (Albany NY)* **5**, 867–883.
- Denzel MS, Storm NJ, Gutschmidt A, Baddi R, Hinze Y, Jarosch E, Sommer T, Hoppe T, Antebi A (2014) Hexosamine pathway metabolites enhance protein quality control and prolong life. *Cell* **156**, 1167–1178.
- Eisenberg T, Knauer H, Schauer A, Buttner S, Ruckenstein C, Carmona-Gutierrez D, Ring J, Schroeder S, Magnes C, Antonacci L, Fussi H, Deszcz L, Hartl R, Schraml E, Criollo A, Megalou E, Weiskopf D, Laun P, Heeren G, Breitenbach M, Grubbeck-Loebenstein B, Herker E, Fahrenkrog B, Frohlich KU, Sinner F, Tavernarakis N, Minois N, Kroemer G, Madeo F (2009) Induction of autophagy by spermidine promotes longevity. *Nat. Cell Biol.* **11**, 1305–1314.
- Eisenberg T, Schroeder S, Andryushkova A, Pendl T, Kuttner V, Bhukel A, Marino G, Pietroccola F, Harger A, Zimmermann A, Moustafa T, Sprenger A, Jany E, Buttner S, Carmona-Gutierrez D, Ruckenstein C, Ring J, Reichelt W, Schimmel K, Leeb T, Moser C, Schatz S, Kamolz LP, Magnes C, Sinner F, Sedej S, Frohlich KU, Juhasz G, Pieber TR, Dengjel J, Sigrist SJ, Kroemer G, Madeo F (2014) Nucleocytosolic depletion of the energy metabolite acetyl-coenzyme a stimulates autophagy and prolongs lifespan. *Cell Metab.* **19**, 431–444.
- Ellison KE, Gandhi G (2005) Optimising the use of beta-adrenoceptor antagonists in coronary artery disease. *Drugs* **65**, 787–797.
- Escande C, Nin V, Price NL, Capellini V, Gomes AP, Barbosa MT, O'Neil L, White TA, Sinclair DA, Chini EN (2012) Flavonoid Apigenin Is an Inhibitor of the NAD+ase CD38: implications for Cellular NAD+ Metabolism, Protein Acetylation, and Treatment of Metabolic Syndrome. *Diabetes* **2013**; **62**, 1084–1093.
- Fiori JL, Shin YK, Kim W, Krzysik-Walker SM, Gonzalez-Mariscal I, Carlson OD, Sanghvi M, Moaddel R, Farhang K, Gadkaree SK, Doyle ME, Pearson KJ, Mattison JA, de Cabo R, Egan JM (2013) Resveratrol Prevents beta-cell Dedifferentiation in Non-Human Primates Given a High Fat/High Sugar Diet. *Diabetes* **2013**; **62**, 3500–3513.
- Fontana L, Adelaye RM, Rastelli AL, Miles KM, Ciamporcero E, Longo VD, Nguyen H, Vessella R, Pili R (2013) Dietary protein restriction inhibits tumor growth in human xenograft models. *Oncotarget*. **2013**; **4**, 2451–2461.
- Fontana L, Partridge L, Longo VD (2010) Extending healthy life span—from yeast to humans. *Science* **328**, 321–326.
- Franceschi C, Campisi J (2014) Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *J. Gerontol. A Biol. Sci. Med. Sci.* **69**(Suppl 1), S4–S9.
- Franceschi C, Bonafe M, Valensin S, Olivieri F, De Luca M, Ottaviani E, De Benedictis G (2000) Inflamm-aging. An evolutionary perspective on immunosenescence. *Ann. N. Y. Acad. Sci.* **908**, 244–254.
- Franceschi C, Capri M, Monti D, Giunta S, Olivieri F, Sevini F, Panourgia MP, Invidia L, Celani L, Scurti M, Cevenini E, Castellani GC, Salvioli S (2007) Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. *Mech. Ageing Dev.* **128**, 92–105.
- Gallinetti J, Harputlugi E, Mitchell JR (2013) Amino acid sensing and translational control in dietary restriction-mediated longevity and stress resistance: contrasting roles of signal transducing kinases Gcn2 and mTOR. *Biochem. J.* **449**, 1–10.

- Ganapathy-Kanniappan S, Geschwind JF (2013) Tumor glycolysis as a target for cancer therapy: progress and prospects. *Mol. Cancer* **12**, 152.
- Gems D (2014) What is an anti-aging treatment? *Exp. Gerontol.* **58**, 14–18.
- Ghanim H, Sia CL, Korzeniewski K, Lohano T, Abuaysheh S, Marumganti A, Chaudhuri A, Dandona P (2011) A resveratrol and polyphenol preparation suppresses oxidative and inflammatory stress response to a high-fat, high-carbohydrate meal. *J. Clin. Endocrinol. Metab.* **96**, 1409–1414.
- Giustina A, Chanson P, Kleinberg D, Bronstein MD, Clemmons DR, Klibanski A, van der Lely AJ, Strasburger CJ, Lamberts SW, Ho KK, Casanueva FF, Melmed S (2014) Expert consensus document: a consensus on the medical treatment of acromegaly. *Nat. Rev. Endocrinol.* **10**, 243–248.
- Goldberg AD, Allis CD, Bernstein E (2007) Epigenetics: a landscape takes shape. *Cell* **128**, 635–638.
- Gomes AP, Price NL, Ling AJ, Moselehi J, Montgomery M, Rajman L, DeCabo R, Rolo AP, Turner N, Bell E, Sinclair DA (2014) Declining NAD⁺ induces a pseudohypoxic state disrupted by nuclear-mitochondrial communication during aging. *Cell* **155**, 1624–1638.
- Gradman AH (2009) Evolving understanding of the renin-angiotensin-aldosterone system: pathophysiology and targets for therapeutic intervention. *Am. Heart J.* **157**, S1–S6.
- Graff J, Kahn M, Samiei A, Gao J, Ota KT, Rei D, Tsai LH (2013) A Dietary Regimen of Caloric Restriction or Pharmacological Activation of SIRT1 to Delay the Onset of Neurodegeneration. *J. Neurosci.* **33**, 8951–8960.
- Group U (1998) Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* **352**, 854–865.
- Guevara-Aguirre J, Balasubramanian P, Guevara-Aguirre M, Wei M, Madia F, Cheng CW, Hwang D, Martin-Montalvo A, Saavedra J, Ingles S, de Cabo R, Cohen P, Longo VD (2011) Growth hormone receptor deficiency is associated with a major reduction in pro-aging signaling, cancer, and diabetes in humans. *Sci. Transl. Med.* **3**, 70ra13.
- Hansen M, Chandra A, Mitic LL, Onken B, Driscoll M, Kenyon C (2008) A role for autophagy in the extension of lifespan by dietary restriction in *C. elegans*. *PLoS Genet.* **4**, e24.
- Harputlugil E, Hine C, Vargas D, Robertson L, Manning BD, Mitchell JR (2014) The TSC Complex Is Required for the Benefits of Dietary Protein Restriction on Stress Resistance In Vivo. *Cell Rep.* **8**, 1160–1170.
- Harrison DE, Strong R, Sharp ZD, Nelson JF, Astle CM, Flurkey K, Nadon NL, Wilkinson JE, Frenkel K, Carter CS, Pahor M, Javors MA, Fernandez E, Miller RA (2009) Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature* **460**, 392–395.
- Harvie MN, Pegington M, Mattson MP, Frystyk J, Dillon B, Evans G, Cuzick J, Jebb SA, Martin B, Cutler RG, Son TG, Maudsley S, Carlson OD, Egan JM, Flyvbjerg A, Howell A (2010) The effects of intermittent or continuous energy restriction on weight loss and metabolic disease risk markers: a randomized trial in young overweight women. *Int. J. Obes. (Lond)*. **35**, 714–727.
- Hausenblas HA, Schoulda JA, Smoliga JM (2014) Resveratrol treatment as an adjunct to pharmacological management in type 2 diabetes mellitus-systematic review and meta-analysis. *Mol. Nutr. Food Res.* **59**, 147–159.
- Hawkins M, Barzilai N, Chen W, Angelov I, Hu M, Cohen P, Rossetti L (1996) Increased hexosamine availability similarly impairs the action of insulin and IGF-1 on glucose disposal. *Diabetes* **45**, 1734–1743.
- Hayashi T, Hirshman MF, Kurth EJ, Winder WW, Goodyear LJ (1998) Evidence for 5' AMP-activated protein kinase mediation of the effect of muscle contraction on glucose transport. *Diabetes* **47**, 1369–1373.
- Heilbronn LK, Smith SR, Martin CK, Anton SD, Ravussin E (2005) Alternate-day fasting in nonobese subjects: effects on body weight, body composition, and energy metabolism. *Am. J. Clin. Nutr.* **81**, 69–73.
- Ho D, Yan L, Iwatsubo K, Vatner DE, Vatner SF (2010) Modulation of beta-adrenergic receptor signaling in heart failure and longevity: targeting adenylyl cyclase type 5. *Heart Fail. Rev.* **15**, 495–512.
- Hoogerwerf BJ (2010) Renin-angiotensin system blockade and cardiovascular and renal protection. *Am. J. Cardiol.* **105**, 30A–35A.
- Howitz KT, Bitterman KJ, Cohen HY, Lamming DW, Lavu S, Wood JG, Zipkin RE, Chung P, Kisilewsky A, Zhang LL, Scherer B, Sinclair DA (2003) Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature* **425**, 191–196.
- Hubbard BP, Sinclair DA (2014) Small molecule SIRT1 activators for the treatment of aging and age-related diseases. *Trends Pharmacol. Sci.* **35**, 146–154.
- Hung WW, Ross JS, Boockvar KS, Siu AL (2011) Recent trends in chronic disease, impairment and disability among older adults in the United States. *BMC Geriatr.* **11**, 47.
- Ikeno Y, Hubbard GB, Lee S, Cortez LA, Lew CM, Webb CR, Berryman DE, List EO, Kopchick JJ, Bartke A (2009) Reduced incidence and delayed occurrence of fatal neoplastic diseases in growth hormone receptor/binding protein knockout mice. *J. Gerontol. A Biol. Sci. Med. Sci.* **64**, 522–529.
- Imai S, Armstrong CM, Kaeberlein M, Guarente L (2000) Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature* **403**, 795–800.
- Ingram DK, Roth GS (2010) Glycolytic inhibition as a strategy for developing calorie restriction mimetics. *Exp. Gerontol.* **46**, 148–154.
- Jaenisch R, Bird A (2003) Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat. Genet.* **33**(Suppl), 245–254.
- Jiang H, Schiffer E, Song Z, Wang J, Zurbig P, Thedieck K, Moes S, Bantel H, Saal N, Jantos J, Brecht M, Jenö P, Hall MN, Hager K, Manns MP, Hecker H, Ganser A, Dohner K, Bartke A, Meissner C, Mischak H, Ju Z, Rudolph KL (2008) Proteins induced by telomere dysfunction and DNA damage represent biomarkers of human aging and disease. *Proc. Natl Acad. Sci. USA* **105**, 11299–11304.
- Jimenez-Gomez Y, Mattison JA, Pearson KJ, Martin-Montalvo A, Palacios HH, Sossong AM, Ward TM, Younts CM, Lewis K, Allard JS, Longo DL, Belman JP, Malagon MM, Navas P, Sanghvi M, Moaddel R, Tilmont EM, Herbert RL, Morrell CH, Egan JM, Baur JA, Ferrucci L, Bogan JS, Bernier M, de Cabo R (2013) Resveratrol improves adipose insulin signaling and reduces the inflammatory response in adipose tissue of rhesus monkeys on high-fat, high-sugar diet. *Cell Metab.* **18**, 533–545.
- Johnson JE, Johnson FB (2014) Methionine restriction activates the retrograde response and confers both stress tolerance and lifespan extension to yeast, mouse and human cells. *PLoS One* **9**, e97729.
- Johnson SC, Rabinovitch PS, Kaeberlein M (2013) mTOR is a key modulator of ageing and age-related disease. *Nature* **493**, 338–345.
- Ju Z, Jiang H, Jaworski M, Rathinam C, Gompf A, Klein C, Trumpp A, Rudolph KL (2007) Telomere dysfunction induces environmental alterations limiting hematopoietic stem cell function and engraftment. *Nat. Med.* **13**, 742–747.
- Jurk D, Wilson C, Passos JF, Oakley F, Correia-Melo C, Greaves L, Saretzki G, Fox C, Lawless C, Anderson R, Hewitt G, Pender SL, Fullard N, Nelson G, Mann J, van de Sluis B, Mann DA, von Zglinicki T (2014) Chronic inflammation induces telomere dysfunction and accelerates ageing in mice. *Nat. Commun.* **2**, 4172.
- Kennedy BK, Pennypacker JK (2014) Drugs that modulate aging: the promising yet difficult path ahead. *Transl. Res.* **163**, 456–465.
- Kenyon C, Chang J, Gensch E, Rudner A, Tabtiang R (1993) A *C. elegans* mutant that lives twice as long as wild type. *Nature* **366**, 461–464.
- Kim SG, Buel GR, Blenis J (2013) Nutrient regulation of the mTOR complex 1 signaling pathway. *Mol. Cells* **35**, 463–473.
- Kopchick JJ, Parkinson C, Stevens EC, Trainer PJ (2002) Growth hormone receptor antagonists: discovery, development, and use in patients with acromegaly. *Endocr. Rev.* **23**, 623–646.
- Laeger T, Henagan TM, Albarado DC, Redman LM, Bray GA, Noland RC, Munzberg H, Hutson SM, Gettys TW, Schwartz MW, Morrison CD (2014) FGF21 is an endocrine signal of protein restriction. *J. Clin. Invest.* **124**, 3913–3922.
- Lamming DW, Ye L, Katajisto P, Goncalves MD, Saitoh M, Stevens DM, Davis JG, Salmon AB, Richardson A, Ahima RS, Guertin DA, Sabatini DM, Baur JA (2012) Rapamycin-induced insulin resistance is mediated by mTORC2 loss and uncoupled from longevity. *Science* **335**, 1638–1643.
- Lamming DW, Ye L, Sabatini DM, Baur JA (2013) Rapalogs and mTOR inhibitors as anti-aging therapeutics. *J. Clin. Invest.* **123**, 980–989.
- Laplante M, Sabatini DM (2012) mTOR signaling in growth control and disease. *Cell* **149**, 274–293.
- Ledford H (2010) Ageing: much ado about ageing. *Nature* **464**, 480–481.
- Lee BC, Kaya A, Ma S, Kim G, Gerashchenko MV, Yim SH, Hu Z, Harshman LG, Gladyshev VN (2014) Methionine restriction extends lifespan of *Drosophila melanogaster* under conditions of low amino-acid status. *Nat. Commun.* **5**, 3592.
- van der Lely AJ, Kopchick JJ (2006) Growth hormone receptor antagonists. *Neuroendocrinology* **83**, 264–268.
- van der Lely AJ, Hutson RK, Trainer PJ, Besser GM, Barkan AL, Katznelson L, Klibanski A, Herman-Bonert V, Melmed S, Vance ML, Freda PU, Stewart PM, Friend KE, Clemmons DR, Johannsson G, Stavrou S, Cook DM, Phillips LS, Strasburger CJ, Hackett S, Zib KA, Davis RJ, Scarlett JA, Thorner MO (2001) Long-term treatment of acromegaly with pegvisomant, a growth hormone receptor antagonist. *Lancet* **358**, 1754–1759.
- van der Lely AJ, Biller BM, Brue T, Buchfelder M, Ghigo E, Gomez R, Hey-Hadavi J, Lundgren F, Rajcic N, Strasburger CJ, Webb SM, Koltowska-Hagstrom M (2012) Long-term safety of pegvisomant in patients with acromegaly: compre-

- hensive review of 1288 subjects in ACROSTUDY. *The Journal of clinical endocrinology and metabolism*. **97**, 1589–1597.
- Levine ME, Suarez JA, Brandhorst S, Balasubramanian P, Cheng CW, Madia F, Fontana L, Mirisola MG, Guevara-Aguirre J, Wan J, Passarino G, Kennedy BK, Wei M, Cohen P, Crimmins EM, Longo VD (2014) Low protein intake is associated with a major reduction in IGF-1, cancer, and overall mortality in the 65 and younger but not older population. *Cell Metab.* **19**, 407–417.
- Li W, Li X, Miller RA (2014) ATF4 activity: a common feature shared by many kinds of slow-aging mice. *Aging Cell* 2014; **13**, 1012–1018.
- Longo VD (2009) Linking sirtuins, IGF-I signaling, and starvation. *Exp. Gerontol.* **44**, 70–74.
- Longo VD, Mattson MP (2014) Fasting: molecular mechanisms and clinical applications. *Cell Metab.* **19**, 181–192.
- Lu JM, Nurko J, Weakley SM, Jiang J, Kougiyas P, Lin PH, Yao Q, Chen C (2010) Molecular mechanisms and clinical applications of nordihydroguaiaretic acid (NDGA) and its derivatives: an update. *Med. Sci. Monit.* **16**, RA93–RA100.
- Ludman A, Venugopal V, Yellon DM, Hausenloy DJ (2009) Statins and cardioprotection—more than just lipid lowering? *Pharmacol. Ther.* **122**, 30–43.
- Madiraju AK, Erion DM, Rahimi Y, Zhang XM, Braddock DT, Albright RA, Prigaro BJ, Wood JL, Bhanot S, MacDonald MJ, Jurczak MJ, Camporez JP, Lee HY, Cline GW, Samuel VT, Kibbey RG, Shulman GI (2014) Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase. *Nature* **510**, 542–546.
- Marino G, Pietroccola F, Eisenberg T, Kong Y, Malik SA, Andryushkova A, Schroeder S, Pendl T, Harger A, Niso-Santano M, Zamzami N, Scoazec M, Durand S, Enot DP, Fernandez AF, Martins I, Kepp O, Senovilla L, Bauvy C, Morselli E, Vacchelli E, Bennetzen M, Magnes C, Sinner F, Pieber T, Lopez-Otin C, Maiuri MC, Codogno P, Andersen JS, Hill JA, Madeo F, Kroemer G (2014) Regulation of autophagy by cytosolic acetyl-coenzyme A. *Mol. Cell* **53**, 710–725.
- Martin-Montalvo A, Mercken EM, Mitchell SJ, Palacios HH, Mote PL, Scheibye-Knudsen M, Gomes AP, Ward TM, Minor RK, Blouin MJ, Schwab M, Pollak M, Zhang Y, Yu Y, Becker KG, Bohr VA, Ingram DK, Sinclair DA, Wolf NS, Spindler SR, Bernier M, de Cabo R (2013) Metformin improves healthspan and lifespan in mice. *Nat. Commun.* **4**, 2192.
- Marz AM, Fabian AK, Kozany C, Bracher A, Hausch F (2013) Large FK506-binding proteins shape the pharmacology of rapamycin. *Mol. Cell. Biol.* **33**, 1357–1367.
- Mattison JA, Roth GS, Beasley TM, Tilmont EM, Handy AM, Herbert RL, Longo DL, Allison DB, Young JE, Bryant M, Barnard D, Ward WF, Qi W, Ingram DK, de Cabo R (2012) Impact of caloric restriction on health and survival in rhesus monkeys from the NIA study. *Nature* **489**, 318–321.
- Mattson MP (2014) Interventions that improve body and brain bioenergetics for Parkinson's disease risk reduction and therapy. *J. Parkinsons Dis.* **4**, 1–13.
- Mauro CR, Tao M, Yu P, Treviño-Villerreal JH, Longchamp A, Kristal BS, Ozaki CK, Mitchell JR (2014) Preoperative dietary restriction reduces intimal hyperplasia and protects from ischemia-reperfusion injury. *J. Vasc. Surg.* doi: 10.1016/j.jvs.2014.07.004 [Epub ahead of print].
- Melendez A, Tallozy Z, Seaman M, Eskelinen EL, Hall DH, Levine B (2003) Autophagy genes are essential for dauer development and life-span extension in *C. elegans*. *Science* **301**, 1387–1391.
- Meyers RO, Lambert JD, Hajicek N, Pourpak A, Kalaitzis JA, Dorr RT (2009) Synthesis, characterization, and anti-melanoma activity of tetra-O-substituted analogs of nordihydroguaiaretic acid. *Bioorg. Med. Chem. Lett.* **19**, 4752–4755.
- Michalsen A, Riegert M, Ludtke R, Backer M, Langhorst J, Schwickert M, Dobos GJ (2005) Mediterranean diet or extended fasting's influence on changing the intestinal microflora, immunoglobulin A secretion and clinical outcome in patients with rheumatoid arthritis and fibromyalgia: an observational study. *BMC Complement Altern. Med.* **5**, 22.
- Miller RA, Harrison DE, Astle CM, Fernandez E, Flurkey K, Han M, Javors MA, Li X, Nadon NL, Nelson JF, Pletcher S, Salmon AB, Sharp ZD, Van Roekel S, Winkelman L, Strong R (2014) Rapamycin-mediated lifespan increase in mice is dose and sex dependent and metabolically distinct from dietary restriction. *Aging Cell* **13**, 468–477.
- Milman S, Atzmon G, Huffman DM, Wan J, Crandall JP, Cohen P, Barzilai N (2014) Low insulin-like growth factor-1 level predicts survival in humans with exceptional longevity. *Aging Cell* **13**, 769–771.
- Minor RK, Smith DL Jr, Sossong AM, Kaushik S, Poosala S, Spangler EL, Roth GS, Lane M, Allison DB, de Cabo R, Ingram DK, Mattison JA (2009) Chronic ingestion of 2-deoxy-D-glucose induces cardiac vacuolization and increases mortality in rats. *Toxicol. Appl. Pharmacol.* **243**, 332–339.
- Minor RK, Baur JA, Gomes AP, Ward TM, Csiszar A, Mercken EM, Abdelmohsen K, Shin YK, Canto C, Scheibye-Knudsen M, Krawczyk M, Irusta PM, Martin-Montalvo A, Hubbard BP, Zhang Y, Lehmann E, White AA, Price NL, Swindell WR, Pearson KJ, Becker KG, Bohr VA, Gorospe M, Egan JM, Talan MI, Auwerx J, Westphal CH, Ellis JL, Ungvari Z, Vlasuk GP, Elliott PJ, Sinclair DA, de Cabo R (2011) SRT1720 improves survival and healthspan of obese mice. *Sci. Rep.* **1**, 70.
- Mirisola MG, Taormina G, Fabrizio P, Wei M, Hu J, Longo VD (2014) Serine- and threonine/valine-dependent activation of PDK and Tor orthologs converge on Sch9 to promote aging. *PLoS Genet.* **10**, e1004113.
- Mirzaei H, Suarez JA, Longo VD (2014) Protein and amino acid restriction, aging and disease: from yeast to humans. *Trends Endocrinol. Metab.* 2014; **25**, 558–566.
- Missios P, Zhou Y, Guachalla LM, von Figura G, Wegner A, Chakkarappan SR, Binz T, Gompf A, Hartleben G, Burkhalter MD, Wulff V, Gunes C, Sattler RW, Song Z, Illig T, Klaus S, Bohm BO, Wenz T, Hiller K, Rudolph KL (2014) Glucose substitution prolongs maintenance of energy homeostasis and lifespan of telomere dysfunctional mice. *Nat. Commun.* **5**, 4924.
- Morselli E, Marino G, Bennetzen MV, Eisenberg T, Megalou E, Schroeder S, Cabrera S, Benit P, Rustin P, Criollo A, Kepp O, Galluzzi L, Shen S, Malik SA, Maiuri MC, Horio Y, Lopez-Otin C, Andersen JS, Tavernarakis N, Madeo F, Kroemer G (2011) Spermidine and resveratrol induce autophagy by distinct pathways converging on the acetylproteome. *J. Cell Biol.* **192**, 615–629.
- Ng TP, Feng L, Yap KB, Lee TS, Tan CH, Winblad B (2014) Long-term metformin usage and cognitive function among older adults with diabetes. *J. Alzheimers Dis.* **41**, 61–68.
- Nielsen SF, Nordestgaard BG, Bojesen SE (2013) Statin use and reduced cancer-related mortality. *N. Engl. J. Med.* **368**, 576–577.
- Olivieri F, Rippon MR, Monsurro V, Salvioli S, Capri M, Procopio AD, Franceschi C (2013) MicroRNAs linking inflamm-aging, cellular senescence and cancer. *Ageing Res. Rev.* **12**, 1056–1068.
- Pardini RS, Heidker JC, Fletcher DC (1970) Inhibition of mitochondrial electron transport by nor-dihydroguaiaretic acid (NDGA). *Biochem. Pharmacol.* **19**, 2695–2699.
- Parrella E, Maxim T, Maialeffi F, Zhang L, Wan J, Wei M, Cohen P, Fontana L, Longo VD (2013) Protein restriction cycles reduce IGF-1 and phosphorylated Tau, and improve behavioral performance in an Alzheimer's disease mouse model. *Aging Cell* **12**, 257–268.
- Passos JF, Nelson G, Wang C, Richter T, Simillion C, Proctor CJ, Miwa S, Olijslagers S, Hallinan J, Wipat A, Saretzki G, Rudolph KL, Kirkwood TB, von Zglinicki T (2010) Feedback between p21 and reactive oxygen production is necessary for cell senescence. *Mol. Syst. Biol.* **6**, 347.
- Patel VK, Demontis F (2014) GDF11/myostatin and aging. *Aging (Albany NY)*. **6**, 351–352.
- Pearson KJ, Baur JA, Lewis KN, Peshkin L, Price NL, Labinskyy N, Swindell WR, Kamara D, Minor RK, Perez E, Jamieson HA, Zhang Y, Dunn SR, Sharma K, Pleshko N, Woollett LA, Csiszar A, Ikeno Y, Le Couteur D, Elliott PJ, Becker KG, Navas P, Ingram DK, Wolf NS, Ungvari Z, Sinclair DA, de Cabo R (2008) Resveratrol delays age-related deterioration and mimics transcriptional aspects of dietary restriction without extending life span. *Cell Metab.* **8**, 157–168.
- Peng W, Robertson L, Gallinetti J, Mejia P, Vose S, Charlip A, Chu T, Mitchell JR (2012) Surgical stress resistance induced by single amino acid deprivation requires Gcn2 in mice. *Sci. Transl. Med.* **4**, 118ra111.
- Pengelly AR, Copur O, Jackle H, Herzog A, Muller J (2013) A histone mutant reproduces the phenotype caused by loss of histone-modifying factor Polycomb. *Science* **339**, 698–699.
- Pinti M, Cevenini E, Nasi M, De Biasi S, Salvioli S, Monti D, Benatti S, Gibellini L, Cotichini R, Stazi MA, Trenti T, Franceschi C, Cossarizza A (2014) Circulating mitochondrial DNA increases with age and is a familiar trait: implications for "inflamm-aging". *Eur. J. Immunol.* **44**, 1552–1562.
- Raisner RM, Madhani HD (2008) Genomewide screen for negative regulators of sirtuin activity in *Saccharomyces cerevisiae* reveals 40 loci and links to metabolism. *Genetics* **179**, 1933–1944.
- Rena G, Pearson ER, Sakamoto K (2013) Molecular mechanism of action of metformin: old or new insights? *Diabetologia* **56**, 1898–1906.
- Ruckenstuhl C, Netzerberger C, Entfellner I, Carmona-Gutierrez D, Kickenweiz T, Stekovic S, Gleixner C, Schmid C, Klug L, Sorgo AG, Eisenberg T, Buttner S, Marino G, Koziel R, Jansen-Durr P, Frohlich KU, Kroemer G, Madeo F (2014) Lifespan extension by methionine restriction requires autophagy-dependent vacuolar acidification. *PLoS Genet.* **10**, e1004347.
- Ruderman N, Prentki M (2004) AMP kinase and malonyl-CoA: targets for therapy of the metabolic syndrome. *Nat. Rev. Drug Discovery* **3**, 340–351.
- Ruderman NB, Carling D, Prentki M, Cacicedo JM (2013) AMPK, insulin resistance, and the metabolic syndrome. *J. Clin. Invest.* **123**, 2764–2772.
- Sabatini DM, Erdjument-Bromage H, Lui M, Tempst P, Snyder SH (1994) RAFT1: a mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. *Cell* **78**, 35–43.

- Safdie FM, Dorff T, Quinn D, Fontana L, Wei M, Lee C, Cohen P, Longo VD (2009) Fasting and cancer treatment in humans: a case series report. *Aging (Albany NY)* **1**, 988–1007.
- Sansoni P, Vescovini R, Fagnoni FF, Akbar A, Arens R, Chiu YL, Cicin-Sain L, Dechanet-Merville J, Derhovanessian E, Ferrando-Martinez S, Franceschi C, Frasca D, Fulop T, Furman D, Gkrania-Klotsas E, Goodrum F, Grubeck-Loebenstien B, Hurme M, Kern F, Lilleri D, Lopez-Botet M, Maier AB, Marandu T, Marchant A, Mathei C, Moss P, Muntasell A, Remmerswaal EB, Riddell NE, Rothe K, Sauce D, Shin EC, Simanek AM, Smithy MJ, Soderberg-Naucler C, Solana R, Thomas PG, van Lier R, Pawelec G, Nikolich-Zugich J (2014) New advances in CMV and immunosenescence. *Exp. Gerontol.* **55**, 54–62.
- Sarbasov DD, Ali SM, Sengupta S, Sheen JH, Hsu PP, Bagley AF, Markhard AL, Sabatini DM (2006) Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. *Mol. Cell* **22**, 159–168.
- Satoh A, Brace CS, Rensing N, Cliften P, Wozniak DF, Herzog ED, Yamada KA, Imai S (2013) Sirt1 Extends Life Span and Delays Aging in Mice through the Regulation of Nk2 Homeobox 1 in the DMH and LH. *Cell Metab.* **18**, 416–430.
- Schaetzlein S, Kodandamireddy NR, Ju Z, Lechel A, Stepczynska A, Lilli DR, Clark AB, Rudolph C, Kuhnel F, Wei K, Schlegelberger B, Schirmacher P, Kunkel TA, Greenberg RA, Edelmann W, Rudolph KL (2007) Exonuclease-1 deletion impairs DNA damage signaling and prolongs lifespan of telomere-dysfunctional mice. *Cell* **130**, 863–877.
- Schroeder EA, Raimundo N, Shadel GS (2013) Epigenetic silencing mediates mitochondria stress-induced longevity. *Cell Metab.* **17**, 954–964.
- Sedivy JM, Kreiling JA, Neretti N, De Cecco M, Criscione SW, Hofmann JW, Zhao X, Ito T, Peterson AL (2013) Death by transposition - the enemy within? *BioEssays* **35**, 1035–1043.
- Seo K, Choi E, Lee D, Jeong DE, Jang SK, Lee SJ (2013) Heat shock factor 1 mediates the longevity conferred by inhibition of TOR and insulin/IGF-1 signaling pathways in *C. elegans*. *Aging Cell* **12**, 1073–1081.
- Shevah O, Laron Z (2007) Patients with congenital deficiency of IGF-I seem protected from the development of malignancies: a preliminary report. *Growth Horm. IGF Res.* **17**, 54–57.
- Smoliga JM, Baur JA, Hausenblas HA (2011) Resveratrol and health—a comprehensive review of human clinical trials. *Mol. Nutr. Food Res.* **55**, 1129–1141.
- Soda K, Kano Y, Sakuragi M, Takao K, Lefor A, Konishi F (2009) Long-term oral polyamine intake increases blood polyamine concentrations. *J. Nutr. Sci. Vitaminol. (Tokyo)* **55**, 361–366.
- Soefje SA, Karnad A, Brenner AJ (2011) Common toxicities of mammalian target of rapamycin inhibitors. *Target. Oncol.* **6**, 125–129.
- Solon-Biet SM, McMahon AC, Ballard JW, Ruohonen K, Wu LE, Cogger VC, Warren A, Huang X, Pichaud N, Melvin RG, Gokarn R, Khalil M, Turner N, Cooney GJ, Sinclair DA, Raubenheimer D, Le Couteur DG, Simpson SJ (2014) The ratio of macronutrients, not caloric intake, dictates cardiometabolic health, aging, and longevity in ad libitum-fed mice. *Cell Metab.* **19**, 418–430.
- Song XM, Fiedler M, Galuska D, Ryder JW, Fernstrom M, Chibalin AV, Wallberg-Henriksson H, Zierath JR (2002) 5-Aminoimidazole-4-carboxamide ribonucleoside treatment improves glucose homeostasis in insulin-resistant diabetic (ob/ob) mice. *Diabetologia* **45**, 56–65.
- Soukas AA, Kane EA, Carr CE, Melo JA, Ruvkun G (2009) Rictor/TORC2 regulates fat metabolism, feeding, growth, and life span in *Caenorhabditis elegans*. *Genes Dev.* **23**, 496–511.
- Sperka T, Song Z, Morita Y, Nalapareddy K, Guachalla LM, Lechel A, Begus-Nahrmann Y, Burkhalter MD, Mach M, Schlaudraff F, Liss B, Ju Z, Speicher MR, Rudolph KL (2011) Puma and p21 represent cooperating checkpoints limiting self-renewal and chromosomal instability of somatic stem cells in response to telomere dysfunction. *Nat. Cell Biol.* **14**, 73–79.
- Spindler SR (2009) Caloric restriction: from soup to nuts. *Ageing Res. Rev.* **9**, 324–353.
- Spindler SR, Li R, Dhahbi JM, Yamakawa A, Mote P, Bodmer R, Ocorr K, Williams RT, Wang Y, Ablao KP (2012) Statin treatment increases lifespan and improves cardiac health in *Drosophila* by decreasing specific protein prenylation. *PLoS One* **7**, e39581.
- Spindler SR, Mote PL, Li R, Dhahbi JM, Yamakawa A, Flegel JM, Jeske DR, Lublin AL (2013) beta1-Adrenergic receptor blockade extends the life span of *Drosophila* and long-lived mice. *Age (Dordr)* **35**, 2099–2109.
- Spindler SR, Mote PL, Lublin AL, Flegel JM, Dhahbi JM, Li R (2014) Nordihydroguaiaretic acid extends the lifespan of *drosophila* and mice, increases mortality-related tumors and hemorrhagic diathesis, and alters energy homeostasis in mice. *J. Gerontol. A Biol. Sci. Med. Sci.* [Epub ahead of print].
- Steuerman R, Shevah O, Laron Z (2011) Congenital IGF1 deficiency tends to confer protection against post-natal development of malignancies. *Eur. J. Endocrinol.* **164**, 485–489.
- Strong R, Miller RA, Astle CM, Floyd RA, Flurkey K, Hensley KL, Javors MA, Leeuwenburgh C, Nelson JF, Ongini E, Nadon NL, Warner HR, Harrison DE (2008) Nordihydroguaiaretic acid and aspirin increase lifespan of genetically heterogeneous male mice. *Aging Cell* **7**, 641–650.
- Strong R, Miller RA, Astle CM, Baur JA, de Cabo R, Fernandez E, Guo W, Javors M, Kirkland JL, Nelson JF, Sinclair DA, Teter B, Williams D, Zaveri N, Nadon NL, Harrison DE (2013) Evaluation of resveratrol, green tea extract, curcumin, oxaloeacetic acid, and medium-chain triglyceride oil on life span of genetically heterogeneous mice. *J. Gerontol. A Biol. Sci. Med. Sci.* **68**, 6–16.
- Suh Y, Atzmon G, Cho MO, Hwang D, Liu B, Leahy DJ, Barzilai N, Cohen P (2008) Functionally significant insulin-like growth factor I receptor mutations in centenarians. *Proc. Natl Acad. Sci. USA* **105**, 3438–3442.
- Tchkonina T, Zhu Y, van Deursen J, Campisi J, Kirkland JL (2013) Cellular senescence and the senescent secretory phenotype: therapeutic opportunities. *J. Clin. Invest.* **123**, 966–972.
- Tian X, Azpurua J, Hine C, Vaidya A, Myakishev-Rempel M, Ablaeva J, Mao Z, Nevo E, Gorbunova V, Seluanov A (2013) High-molecular-mass hyaluronan mediates the cancer resistance of the naked mole rat. *Nature* **499**, 346–349.
- Timmers S, Konings E, Bilet L, Houtkooper RH, van de Weijer T, Goossens GH, Hoeks J, van der Krieken S, Ryu D, Kersten S, Moonen-Kornips E, Hesselink MK, Kunz I, Schrauwen-Hinderling VB, Blaak EE, Auwerx J, Schrauwen P (2011) Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. *Cell Metab.* **14**, 612–622.
- Trainer PJ, Drake WM, Katznelson L, Freda PU, Herman-Bonert V, van der Lely AJ, Dimaraki EV, Stewart PM, Friend KE, Vance ML, Besser GM, Scarlett JA, Thorner MO, Parkinson C, Klibanski A, Powell JS, Barkan AL, Sheppard MC, Malsonado M, Rose DR, Clemmons DR, Johannsson G, Bengtsson BA, Stavrou S, Kleinberg DL, Cook DM, Phillips LS, Bidlingmaier M, Strasburger CJ, Hackett S, Zib K, Bennett WF, Davis RJ (2000) Treatment of acromegaly with the growth hormone-receptor antagonist pegvisomant. *N. Engl. J. Med.* **342**, 1171–1177.
- Trepanowski JF, Canale RE, Marshall KE, Kabir MM, Bloomer RJ (2011) Impact of caloric and dietary restriction regimens on markers of health and longevity in humans and animals: a summary of available findings. *Nutr. J.* **10**, 107.
- Tyler VE (1994) *The Honest Herbal, a Sensible Guide to the Use of Herbs and Related Remedies*. 1993 Published by Haworth Press, Incorporated, The, Binghamton, NY.
- Vellai T, Takacs-Vellai K, Zhang Y, Kovacs AL, Orosz L, Muller F (2003) Genetics: influence of TOR kinase on lifespan in *C. elegans*. *Nature* **426**, 620.
- Venkatasubramanian S, Noh RM, Daga S, Langrish JP, Joshi NV, Mills NL, Hoffmann E, Jacobson EW, Vlasuk GP, Waterhouse BR, Lang NN, Newby DE (2013) Cardiovascular Effects of a Novel SIRT1 Activator, SRT2104, in Otherwise Healthy Cigarette Smokers. *J. Am. Heart Assoc.* **2**, e000042.
- Vitale G, Salvioli S, Franceschi C (2013) Oxidative stress and the ageing endocrine system. *Nat. Rev. Endocrinol.* **9**, 228–240.
- Wang J, Sun Q, Morita Y, Jiang H, Gross A, Lechel A, Hildner K, Guachalla LM, Gompf A, Hartmann D, Schambach A, Wuestefeld T, Dauch D, Schrenzenmeier H, Hofmann WK, Nakauchi H, Ju Z, Kestler HA, Zender L, Rudolph KL (2012) A differentiation checkpoint limits hematopoietic stem cell self-renewal in response to DNA damage. *Cell* **148**, 1001–1014.
- Wang G, Han T, Nijhawan D, Theodoropoulos P, Naidoo J, Yadavalli S, Mirzaei H, Pieper AA, Ready JM, McKnight SL (2014a) P7C3 neuroprotective chemicals function by activating the rate-limiting enzyme in NAD salvage. *Cell* **158**, 1324–1334.
- Wang ZV, Deng Y, Gao N, Pedrozo Z, Li DL, Morales CR, Criollo A, Luo X, Tan W, Jiang N, Lehrman MA, Rothermel BA, Lee AH, Lavandero S, Mammen PP, Ferdous A, Gillette TG, Scherer PE, Hill JA (2014b) Spliced X-box binding protein 1 couples the unfolded protein response to hexosamine biosynthetic pathway. *Cell* **156**, 1179–1192.
- Warshamana-Greene GS, Litz J, Buchdunger E, Garcia-Echeverria C, Hofmann F, Krystal GW (2005) The insulin-like growth factor-I receptor kinase inhibitor, NVP-ADW742, sensitizes small cell lung cancer cell lines to the effects of chemotherapy. *Clin. Cancer Res.* **11**, 1563–1571.
- Wei M, Fabrizio P, Hu J, Ge H, Cheng C, Li L, Longo VD (2008) Life span extension by calorie restriction depends on Rim15 and transcription factors downstream of Ras/PKA, Tor, and Sch9. *PLoS Genet.* **4**, e13.
- Weimer S, Priebs J, Kuhlrow D, Groth M, Priebe S, Mansfeld J, Merry TL, Dubuis S, Laube B, Pfeiffer AF, Schulz TJ, Guthke R, Platzer M, Zamboni N, Zarse K, Ristow M (2014) D-Glucosamine supplementation extends life span of nematodes and of ageing mice. *Nat. Commun.* **5**, 3563.

- Wu Z, Song L, Liu SQ, Huang D (2013) Independent and additive effects of glutamic acid and methionine on yeast longevity. *PLoS One* **8**, e79319.
- Wu JW, Boudreau DM, Park Y, Simonds NI, Freedman AN (2014) Commonly used diabetes and cardiovascular medications and cancer recurrence and cancer-specific mortality: a review of the literature. *Expert Opin. Drug Saf.* **13**, 1071–1099.
- Yoshino J, Mills KF, Yoon MJ, Imai S (2011) Nicotinamide mononucleotide, a key NAD(+) intermediate, treats the pathophysiology of diet- and age-induced diabetes in mice. *Cell Metab.* **14**, 528–536.
- Yoshino J, Conte C, Fontana L, Mittendorfer B, Imai S, Schechtman KB, Gu C, Kunz I, Rossi Fanelli F, Patterson BW, Klein S (2012) Resveratrol supplementation does not improve metabolic function in nonobese women with normal glucose tolerance. *Cell Metab.* **16**, 658–664.
- Youm YH, Grant RW, McCabe LR, Albarado DC, Nguyen KY, Ravussin A, Pistell P, Newman S, Carter R, Laque A, Munzberg H, Rosen CJ, Ingram DK, Salbaum JM, Dixit VD (2013) Canonical Nlrp3 inflammasome links systemic low-grade inflammation to functional decline in aging. *Cell Metab.* **18**, 519–532.
- Zeichner S, Mihos CG, Santana O (2012) The pleiotropic effects and therapeutic potential of the hydroxy-methyl-glutaryl-CoA reductase inhibitors in malignancies: a comprehensive review. *J. Cancer. Res. Ther.* **8**, 176–183.
- Zhao YN, Li WF, Li F, Zhang Z, Dai YD, Xu AL, Qi C, Gao JM, Gao J (2013) Resveratrol improves learning and memory in normally aged mice through microRNA-CREB pathway. *Biochem. Biophys. Res. Commun.* **435**, 597–602.
- Zhou Y, Xu BC, Maheshwari HG, He L, Reed M, Lozykowski M, Okada S, Cataldo L, Coschigamo K, Wagner TE, Baumann G, Kopchick JJ (1997) A mammalian model for Laron syndrome produced by targeted disruption of the mouse growth hormone receptor/binding protein gene (the Laron mouse). *Proc. Natl Acad. Sci. USA* **94**, 13215–13220.