Aging and longevity science: Where are we in 2015?

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PeerJ PrePrints | https://dx.doi.org/10.7287/peerj.preprints.1015v1 | CC-BY 4.0 Open Access | rec: 29 Apr 2015, publ: 29 Apr 2015

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ABSTRACT

Aging has been defined as the loss of function and ability to interact with the environment. The social perception of aging involves the inability to live independently due to loss of mobility, cognition, and sensory functions. Aging is a risk factor for pathology including cancer, cardiovascular disease, metabolic disease, and neurodegenerative disease. Due to these accompanying conditions, the esteem and respect once regarded for old age has been replaced by efforts to postpone, stop, or even reverse the aging process. Anti-aging medicine has gained popularity through media and marketing as companies have promoted an approach to delaying the side effects of aging through diet, exercise, supplements and hormonal therapy. The following is a comprehensive review of current research into potential strategies for anti-aging therapy and longevity, supported by current scientific and clinical research.

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INTRODUCTION: THE MECHANISM OF AGING

Aging is thought to be a multifactorial process and many theories have attempted to explain the process. To date, the biological mechanism of aging is only partially understood. Oxidative stress, cellular senescence, and chronic inflammation are speculated to be leading mechanisms behind aging.

Free radicals are the basis of oxidative stress in the human cell. Free radicals are intermediate oxygen and nitrogen species that can damage cell membrane and machinery. These intermediate species are products of normal cellular metabolism and are eliminated by endogenous reducing agents, like glutathione. For example, in a process called the respiratory burst, neutrophils release large amounts of superoxide ions that become hydrogen peroxide and hypochlorous acid in an effort to neutralize infective agents in the body such as bacteria. One to two percent of oxygen used in mitochondrial respiration is transformed into reactive oxygen species (ROS).(Oliveira et al. 2010) In 1956, Harman proposed a potential role of free radical species in aging. A proportional increase in oxidizing species compared to reducing species can result in cellular damage, as is the case of during excessive or inappropriate activation of respiratory burst.(Reistad et al. 2005) This is due to an imbalance in signal transduction cascade mediators cGMP and cAMP that regulate generation of ROS by neutrophils. The pro-inflammatory molecule, cGMP, becomes overactive compared to the anti-inflammatory molecule, cAMP in individuals over the age of 50.(Coelho Horta et al. 2005) In addition, ROS can affect proteostasis, causing the accumulation of damaged proteins in cells which cause additional protein misfolding or aggregation.(Powers et al. 2009) Nerve cells seem to be protected from proteotoxicity by inhibitors of the insulin/IGF (insulin-like growth factor) signaling pathway.(El-Ami et al. 2014)

Cellular senescence, which was described by Hayflick in 1961, gained support as one of the contributing mechanisms of aging.(Harley et al. 1992) With each cycle of cell replication, a small segment of telomeric DNA is lost due to incomplete replication or degradation of DNA ends. When a certain length of telomere is reached, the cell evokes the Hayflick limit and arrests irreversibly.(Harley et al. 1992)

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Because of the lack of cell division, damaged cells cannot be replaced by new cells and cellular senescence takes place. In addition to telomeric shortening, DNA damage, oncogenesis, and tumor suppressor signals act as stressors that can trigger cellular senescence.(Rodier & Campisi 2011) Oncogenesis chronically activates p53 activity, which is a tumor suppressor gene; prior studies in mice indicate that p53 over-activation result in premature aging and shortened lifespan.(Maier et al. 2004; Tyner et al. 2002) The immune system can clear senescent cells in young individuals, but this process seems to stall or become overwhelmed by the production of new senescent cells in the elderly.(Rodier & Campisi 2011)

The aging immune system is less effective in resolving infection and responding to vaccinations, but triggers systemic inflammation more frequently leading to aggravation of autoimmune and degenerative diseases.(Cavanagh et al. 2012) The anomalous aging inflammatory system is tied to both the ROS as well as cell senescence. Studies indicate that anti-inflammatory prostaglandin levels (PGI2) decrease with age while pro-inflammatory prostaglandins (PGE2, TXA2, PGH2) increase with age.(Beharka et al. 1997; Hornych et al. 1991; Lee & Feldman 1994; Nakajima et al. 1997) ROS produced via the prostaglandin synthesis pathway during the conversion of PGG2 to PGH2 are thought to increase with age.(Chung et al. 2003) In addition, certain pro-inflammatory cytokines such as IL-1β and IL-6 may also increase, which substantiates the observation of heightened autoimmune diseases with old age.(Chung et al. 2003) Lastly, gene expression of pro-inflammatory transcription factors such as NF-κB may increase with aging. Increase in pro-inflammatory mediators seems to be associated with age-related diseases and functional decline in the elderly.

GENETIC TARGETS OF AGING

Klotho

Named after the goddess who spins the thread of life, klotho is a single-pass transmembrane protein that is mainly expressed in the choroid plexus of the brain, distal tubule of the kidney, and the parathyroid glands.(Kuro-o et al. 1997) The extracellular domain of klotho protein is released into the bloodstream and binds to an unidentified receptor.(Yamamoto et al. 2005) Studies suggest that klotho is an anti-aging protein that extends the mouse lifespan, suppresses insulin signaling, and increases resistance to oxidative stress in cells by indirectly activating FoxO forkhead transcriptional factors.(Kurosu et al. 2005) FoxO transcriptional factors gained attention due to their role in atrophy, autophagy, apoptosis, cell cycle arrest, stress resistance, and many other functions inside the cell.(Salih & Brunet 2008) Furthermore, the importance of klotho in aging was demonstrated in klotho knockout mice that showed symptoms akin to human aging: shortened lifespan, infertility, growth arrest, hypoactivity, and skin atrophy.(Kuro-o et al. 1997) Klotho's anti-senescence and longevity effect may result from inhibition of the insulin/IGF-1 cascade(Yamamoto et al. 2005) and the mitogen-activated protein kinases/extracellular signal-regulated kinase (MAPK/ERK) stimulation pathways.

In humans, the KL-VS variant of the gene KLOTHO increases klotho secretion and upregulates FGF23 signaling in vitro(Zhou et al. 2013), furthers longevity(Arking et al. 2005; Arking et al. 2002), suppresses insulin/insulin-like growth factor (IGF-1)(Chen et al. 2014), and decreases age-related cardiovascular disease(Arking et al. 2005). Recent studies have identified klotho's role in neurocognitive protection with aging: promoting oligodendrocyte differentiation(Chen et al. 2014), preserving the activities of daily living (ADL) in the elderly(Crasto et al. 2012), and enhancing cognition independent of age(Dubal et al. 2014). Hence, one current anti-aging medicinal approach attempts to modulate plasma klotho levels to achieve anti-senescence and longevity.

Sirtuin

Sirtuin 1 (SIRT1) is the mammalian analogue of Sir2p which has shown to increase longevity in "lower" organisms.(Frye 2000) SIRT1 is a NAD+ dependent type III histone/protein deacetylase that removes

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acetyl groups from histones and signaling proteins, indirectly modulating gene transcription. Through deacetylation of FOXO3, NF-κβ, p53, Wnt, and many other proteins, SIRT1 regulates signaling pathways that affect inflammation, cellular senescence, apoptosis, and metabolism.(Yao & Rahman 2012) Previous studies in senescent mouse fibroblasts, epithelial cells, and oxidatively damaged human endothelial cells demonstrated a decrease in SIRT1 levels, leading to the belief that SIRT1 plays an active role in aging.(Caito et al. 2010; Orimo et al. 2009; Sasaki et al. 2006; Yang et al. 2007) Recent studies revealed that SIRT1 downregulation causes the inhibition of telomerase activity and induces DNA damage, paralleling the process that occurs in cellular senescence.(Chen et al. 2005) Reversal of such a phenomenon has been witnessed in mesenchymal stem cells with SIRT1 over-expression.(Chen et al. 2005)

One of the main mechanisms by which SIRT 1 may prevent cellular senescence is through regulation of the FOXO3 transcription factor. In addition to FOXO3, signaling proteins that are involved in stress response such as Ku70/Ku80, Wnt/β-catenin, Notch, and Werner syndrome protein seem to be affected by SIRT1.(Guarani et al. 2011; Holloway et al. 2010; Li et al. 2008; Vaitiekunaite et al. 2007) Another potential anti-aging mechanism is the increased expression of telomerase transcriptase via upregulation of SIRT1, elongating telomeres.(Yamashita et al. 2012) Further facilitating DNA integrity, SIRT1 can deacetylate DNA repair proteins that will repair damaged DNA segments.(Yao & Rahman 2012) Recent theories propose that SIRT1's DNA repair activity may prevent an age-related decrease in mtDNA-encoded oxidative phosphorylation units, thus countering mitochondrial dysfunction.(Christian & Shadel 2014) Because of such beneficial effects, SIRT1 activators are being investigated for their potential anti-aging effect.

GDF11

A circulating protein in young animals, growth/differentiation factor 11 (GDF11) has been shown to rejuvenate cardiac muscle, skeletal muscle, and the brain when injected into older mice. While GDF11

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received immediate recognition as a component of the "Elixir of Youth," additional research and clinical trials are necessary to characterize GDF11's mechanisms of action.(Andersen & Lim 2014; Sinha et al. 2014)

NON-MEDICINAL STRATEGIES

Caloric Restriction and Nutrition

Cardiovascular disease is a principal cause of disability, morbidity, and mortality in developed countries.(Rizza et al. 2014) It is a well-accepted fact that nutrition plays a major role in the onset and course of cardiovascular disease, cancer, diabetes, and infections. A healthier diet will prolong lifespan by preventing premature death. However, whether a healthy diet is an anti-aging method is still unclear largely due to its overlapping studies with caloric restriction.

The only established method of delaying the aging process is caloric restriction (CR), or "under-nutrition" without malnutrition.(Lee et al. 1999) It also delays the onset of age-related diseases and helps maintain youthful physiologic functions.(Lee et al. 1999) Based on small animal studies, caloric restriction increases insulin sensitivity and lifespan.(Kenyon 2005; Longo & Finch 2003) Unfortunately, due to conflicting results, this cannot be as easily concluded in studies involving primates. One study, done by the Wisconsin National Primate Research Center, concluded that a 30% CR in monkeys led to significant lifespan extension, a 50% decrease in cancer and cardiovascular disease, and prevention of neurodegeneration.(Colman et al. 2009; Colman et al. 2012) But another study performed by the National Institute of Aging (NIA) concluded that long-term CR did not significantly increase lifespan even though it decreased the incidence of cancer, obesity, and diabetes.(Mattison et al. 2012)

Increasing data shows that men and women who practice long-term caloric restriction have less metabolic syndromes including atherosclerosis, hypertension, and diabetes(Fontana et al. 2004)—all chronic diseases that are significant risk factors to end-organ disease and adverse cardiac events. However, it's

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possible that CR practitioners choose to consume food with higher nutritional value, thus contributing to longevity. Caloric restriction seems to prevent cell senescence via cell proliferation and anti-apoptotic pathways, influenced by P13K/mTOR pathway and IGF-1.(Fontana et al. 2006; Fontana et al. 2008; Pollak et al. 2004)

In addition to the PI3K/mTOR pathway, several theories attempt to explain the effect of CR on longevity. One hypothesis is that activation of autophagy in times of nutrient shortage might correlate with anti-aging effects.(Bergamini et al. 2007) Another study suggests that ad libitum fed mice may develop resistance to hormones and CR might prevent these changes.(Masoro 2002) Insulin and glucagon are two main hormones regulating autophagy; however, the regulatory effects of these hormones seem to be lost in aging cells. Caloric restriction is able to moderate the age-dependent loss in autophagy regulation mediated by glucagon and insulin.(Donati et al. 2008) Another theory supports the possibility of CR decreasing oxidative stress by downregulating age-related proteins such as NF-κβ, IL-1β, IL-6, TNF-α, cyclooxygenase-2, and inducible nitric oxidase.(Chung et al. 2003)

The effects of CR may not be directly proportional or universal. For instance, experiments with rodents have shown that 10 to 50% decrease in calorie consumption linearly increases lifespan, but mortality increased with CR that exceeds 50%.(Fontana et al. 2010; Lee et al. 1999) In addition, some mice did not show lifespan extension with 40% CR, and in fact, demonstrated higher mortality rates compared to ad libitum fed mice.(Harper et al. 2006; Liao et al. 2010)

Physical Activity

Diet and exercise have been highly recommended for the treatment and prevention of metabolic syndromes. In 2014, the American Heart Association recommended that a minimum of 150 minutes of moderate exercise or 75 minutes of vigorous-intensity exercise per week.(March 22 2013) Exercise improves mean longevity mainly through reducing mortality risk from cardiovascular disease, type 2 diabetes, and other age-related diseases.(Holloszy 1997; Manini et al. 2006) However, similar to a healthy

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diet, exercise is not proven to lengthen the maximum human lifespan.(Pekkanen et al. 1987) Controlled studies in mice also suggest that exercise does not increase maximum lifespan.(Holloszy 1997; Holloszy 1998) Compared to a weight-matched sedentary CR mouse, the physically active mouse was significantly leaner; however, the elongation in lifespan was still observed in the CR mouse.(Huffman et al. 2008) The explanations behind these findings are debated. One age-modifying aspect of physical activity is muscular function. By raising the citrate synthase activity and converting the myosin to a more oxidative form, exercise seems to restore age-related loss in muscle function.(Demirel et al. 1999)

Despite the health benefits of physical activity, the literature supports the notion that exercise induces oxidative stress.(Davies et al. 1982; Dillard et al. 1978) Many theories have been suggested to explain this phenomenon, but the most significant factor is thought to be leakage of free radicals from the inner mitochondrial membrane—notably, from complex II and III in the electron transport chain.(Boveris & Chance 1973) Even with an increased ROS production during exercise, there seems to be no scientific evidence for adverse health effects due to exercise-induced oxidative stress. Recent studies also found a decrease in number of neutrophils, lymphocytes, and leukocytes in athletes when compared to non-athletes—demonstrating the characteristics of an aging immune system.(Moro-García et al. 2013) On the other hand, some studies suggest that chronic exercise primes the immune system to compensate for exercise-induced oxidative stress and helps attain a high baseline level of antioxidants which prevent oxidative damage.(Radak et al. 2000) Based on these studies, there is no clear connection between exercise and anti-aging. Nonetheless, the benefits of exercise in preventing early mortality due to metabolic syndromes significantly outweigh any potential risks. Even though exercise is not a proven method of anti-aging, it is health-sustaining.

NUTRITIONAL SUPPLEMENTATION

Polyphenols

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Resveratrol is a well-known polyphenol found in red wine that has been the focus of anti-aging for the past decade. Studies have suggested that resveratrol extends longevity in yeast(Hall 2003), the nematode C. elegans(Greer & Brunet 2009), Drosophila(Bauer et al. 2004), short-lived fish(Valenzano et al. 2006), and mice(Baur et al. 2006). Resveratrol supplementation also produced effects of caloric restriction in obese humans as well.(Timmers et al. 2011)

However, more recent studies have cast doubt on these findings by demonstrating no significant changes in longevity in resveratrol-treated C. elegans and Drosophila.(Burnett et al. 2011) Resveratrol was thought to be a SIRT1 activator(Alarcon de La Lastra & Villegas 2007) until recent studies suggested that resveratrol only indirectly increases SIRT1 activity by augmenting NAD+ levels via the nicotinamide phosphoribosyltransferase (NAMPT) and cAMP-Epac1-AMP-activated kinase (AMPK) pathways.(Yao & Rahman 2012) Some experiments even demonstrated that resveratrol has no impact on SIRT1 levels during oxidative stress.(Chang et al. 2012; Pizarro et al. 2011) In 2010, the National Institute of Aging Interventions Testing Program (NIA-ITP) concluded that resveratrol does not benefit longevity based on murine studies(Miller et al. 2010); however, the study did suggest that resveratrol may improve age-related effects such as cataracts, osteopenia, and aortic stiffness.(Miller et al. 2010) Such studies have prompted researchers to shift away from resveratrol and explore other potential anti-aging modalities such as quecertin and catechin, which are new plant derived polyphenols.

Antioxidants

Oxygen radicals occur within organelles as natural by-products of cellular function.(Eaton & Qian 2002) Subsequently, these reactive species cause oxidative damage to target molecules of mitochondria and lysosomes within the heart, liver, and pancreatic cells.(Eaton & Qian 2002) Antioxidants assist enzymes such as superoxide dismutase, a natural antioxidant enzyme, with preventing reactive species from damaging cellular structures. In humans, iron derived from heme is an important source of potent antioxidant bilirubin Ixα.(Maines 2005) The attainment of iron from heme is facilitated by heme

oxygenase isozymes, HO-1 and HO-2, which degrade heme into CO and biliverdin. Then, biliverdin is almost immediately converted to bilirubin Ixα by bilirubin reductase.(Maines 2005) Free radical oxygen inactivates bilirubin and deprives the cell of its natural antioxidants.(Stocker 2004) In addition, deficiency of antioxidants or other micronutrients prevent the formation of correct intermediaries during heme biosynthesis, resulting in cellular damage caused by an accumulation of oxygen-reactive species.(Scapagnini et al. 2006) A well-known antioxidant prevalent in vegetables and supplements is vitamin C. It is an inhibitor of lipid peroxidation and helps regenerate vitamin E in lipoprotein and membranes. Vitamin E is understood to assist in the prevention of atherosclerosis by interfering with LDL oxidation. Vitamin E supplementation has been shown to decrease the levels of isoprostane plasma concentrations in humans, a biomarker of free radical LDL oxidation.(Roberts II et al. 2007) A recent study on cardiomyocytes derived from human pluripotent stem cells showed increased beating frequency, mitochondrial membrane potential, and telomere-related gene expression when the cells were given vitamin C.(Kim et al. 2013) These are all qualities usually observed in younger cardiomyocytes.

Another class of antioxidant that gained much attention are carotenoids—pigments formed by certain species of algae, bacteria, and fungi.(Franceschelli et al. 2014) Carotenoids' antioxidant properties originate from their ability to eliminate reactive oxygen species through reduction and isomerization.(Franceschelli et al. 2014) Astaxanthin is a red carotenoid pigment that reacts with various free radical species due to the double bonds in its polyene backbone.(Franceschelli et al. 2014) Some evidence indicate that the antioxidant effect of astaxanthin help re-establish superoxide dismutase and catalase activities, which further promote reduction of free radicals.(Franceschelli et al. 2014) Despite recent studies demonstrating the promise of antioxidants in anti-aging, fifteen clinical trials conducted in the past on antioxidants such as tocopherol, beta-carotene, vitamin C, vitamin E, retinol, and folic acid have failed to show statistically significant effects on aging.(Howes 2006)

Bioactive Compounds in Food

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"Nutraceutical" is a term coined by Stephen DeFelice in 1989 from the words "nutrition" and "pharmaceutical".(Kalra 2003) According to DeFelice, its definition is "a food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease."(Brower 1998) Numerous accounts of bioactive compounds and nutraceuticals providing potential anti-aging effects have been published. One such that has gained much attention is curcumin. Curcumin is mainly found in the South Asian spice turmeric, an essential component of many South Asian delicacies. Curcumin has been reported to increase longevity, and decrease reactive oxygen species and lipofuscin in aging C. elegans nematodes.(Liao et al. 2011) It is hypothesized that the potential anti-aging effect of curcumin is derived from its anti-inflammatory and antioxidant activities.(Sikora et al. 2010) Further studies of signal transduction pathways in Drosophila Melanogaster have suggested that curcumin may affect the Notch, Wnt, p53, cell cycle regulation, and riboprotein synthesis pathways.(Xiao 2013)

L-Theanine is an amino acid in green tea that seems to increase the potency of chemotherapeutic drugs.(Sadzuka et al. 1996) It has also demonstrated benefits in cognition, mentation, and protection against beta-amyloid formation in Alzheimer's murine models.(Kim et al. 2009) Furthermore, studies indicate that L-theanine decreases body mass(Zheng et al. 2004) and blood pressure(Yokogoshi et al. 1995), and increases the lifespan of C. elegans.(Zarse et al. 2012)

Persimmon is yet another food that has been reported to be effective in age prevention. Persimmons contain oligomeric proanthocyanidins that enhance phosphorylation on vascular endothelial growth factor (VEGFR-2) and have neuroprotective effects in mice.(Yokozawa et al. 2011) These oligomers also increased SIRT1 expression in mice which is an important regulator gene of aging.(Yokozawa et al. 2011)

Cranberry extract is rich in polyphenols that are thought to have anti-aging effects. When exposed to cranberry extract, C. elegans becomes more resistant to heat shocks, V. cholera infection, and mortality.(Guha et al. 2014) The study also indicated that an earlier intervention with cranberry extract

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resulted in greater benefits for the C. elegans than later intervention.(Guha et al. 2014) Another extract consumed in various countries is the *Ludwigia octovalvis* extract. *Ludwigia octovalvis* is a type of water primrose that grows in tropical countries and has been used as herbal medicine for diverse medical conditions.(Lin et al. 2014) Fruit flies and mice exposed to this extract were found to have extended lifespan, perhaps because *Ludwigia octovalvis* acts in ways similar to dietary restriction.(Lin et al. 2014) Further analysis of this extract revealed antioxidants, polyphenols, phytosterols, and squalenes--all of which could prevent oxidative stress.(Lin et al. 2014)

Chinese Medicine

Chinese medicine is used in many East Asian countries as both a remedy and prevention of ailments. One of its components, red ginseng, is reported to have positive effects against obesity(Kim et al. 2005), hyperglycemia(Yun et al. 2004), and thrombotic plaques(Jin et al. 2007). A study performed in rats demonstrated ginseng's anti-inflammatory and protective effects against atherosclerosis.(Lee et al. 2014) Due to its role as a potential anti-cancer supplement, ginseng is one of the better known Chinese medicine ingredients to the western world.(Helms 2004) Aside from red ginseng, other Chinese herbal ingredients have been mentioned in reports as promoting anti-aging: *Platycladus orientalis* promotes ROS scavenging activity and extension of the nematode lifespan(Liu et al. 2013), *Lycium Barbarum* has neuroprotective effects(Yang et al. 2012a), *Damnacanthus officinarum* has neuroprotective activity and extends the lifespan in C. elegans(Yang et al. 2012b) and yam tuber has antioxidative effects in mice(Han et al. 2014).

Studies on nutraceuticals and Chinese medicine are compilations of individual case reports or experiments conducted on non-primate animals. Based on current data and literature, there is insufficient information to conclusively state whether these nutritional supplements are conducive to anti-aging. The NIA-ITP conducted studies on anti-aging dietary supplements such as resveratrol, green tea extract,

curcumin and oxaloacetic acid on genetically heterogeneous mice and failed to discover statistically significant effects on longevity.(Strong et al. 2013)

WESTERN MEDICINE

Rapamycin

A natural antifungal medicine with low nanomolar concentration, rapamycin is commonly used in post-renal transplant patients to prevent organ rejection.(Kahan 2004) Rapamycin selectively inhibits the mammalian (mechanistic) target of rapamycin (mTOR), which is an evolutionarily conserved protein kinase that controls cell proliferation, growth, and survival.(Ballou & Lin 2008) Due to its evolutionary conservation, rapamycin is thought to be present in many species ranging from yeasts to humans.(Blagosklonny 2007) Over-activation of mTOR can induce inflammation and rapamycin has been used as an anti-inflammatory drug in settings of chronic kidney disease(Liu 2006), atherosclerosis(Chen et al. 2009), and lung infection(Abdulrahman et al. 2011). Rapamycin is antagonistically pleiotropic—it slows both yeast senescence and yeast growth.(Blagosklonny 2007) Developmental growth is not necessary in later life which makes mTOR an ideal target for therapy and potential anti-aging in the elderly.(Blagosklonny 2007) Rapamycin has also been shown to prevent tumors and osteoporosis(Kahan 2004), promote hyperlipidemia(Mohsin et al. 2007), and prevent atherosclerosis by enhancing adipose tissue lipase activity.(Morrisett et al. 2002)

In cell biology, the most notable feature of cell senescence is cell cycle arrest relating to telomere p53, p16, and p21.(Blagosklonny 2007) In a normal cell, mitogens activate the Raf-1/MEK/ERK and phosphatidylinositol 3-kinase (PI3K)/Akt kinase signaling pathways.(Blagosklonny 2007) They subsequently activate mTOR, and stimulate cell growth and protein synthesis.(Sabatini 2006) Simultaneously, these pathways govern the cell cycle by maintaining a balance of growth and division.(Sherr 2004) On the contrary, in senescent cells, this balance is disrupted by a blocked cell cycle

and an active growth-promoting pathway.(Blagosklonny 2006) In environments favorable for growth, mTOR increases mRNA translation and protein synthesis. Regulation of such mRNA expression through the mutation or inhibition of the mTOR pathway has shown to increase lifespan of both mice and invertebrates like C. elegans, yeast, and Drosophila.(Kaeberlein & Kennedy 2011) Furthermore, recent studies in aged mice indicated rapamycin's potential role in enhancing stem-cell function.(Yilmaz et al. 2012)

Reduced insulin/IGF-1 signaling and increased sensitivity to insulin are associated with longevity in small mammals.(Kenyon 2005; Longo & Finch 2003) These have both been shown to have similar effects in human longevity as well.(Van Heemst et al. 2005) Another physiological effect of mTOR is insulin resistance; therefore, insulin sensitivity is a marker of genetically reduced mTOR activity(Um et al. 2004) possibly contributing to the age-modulating effects of rapamycin. In 2009, the NIA-ITP reported rapamycin had statistically significant increases in lifespan for both male and female mice.(Harrison et al. 2009) Long-term rapamycin use can predispose to cataracts, diabetes, and decreased testicular function, but rapamycin also holds promise as an anti-aging medicine that can prevent age-related disease. Further studies are being conducted to determine its effects in humans.

Aspirin

Aspirin is a well-recognized, non-steroidal, anti-inflammatory drug that produces antithrombotic and antioxidant effects(Shi et al. 1999) by modulating inflammatory molecules such as PDGF and TGF-β.(Redondo et al. 2003) In addition to rapamycin, the NIA-ITP has reported high-dose aspirin to increase lifespan in male mice(Strong et al. 2008) (although the effect on longevity was not observed in female mice even when the mean plasma concentrations of female mice aspirin levels were not significantly higher than their male counterparts).(Strong et al. 2008) The mechanism behind aspirin's effect on longevity is elusive. Perhaps it is due to anti-inflammatory and antioxidant effects(Strong et al. 2008), or it may be due to an attenuation of insulin/IGF-1 signaling via the DAF-16/FOXO transcription

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factor(Ayyadevara et al. 2013). Additionally, as a part of the same study, a 3-fold increase in glutathione S-transferase was observed in C. elegans with aspirin treatment. This may be a contributing mechanism in nematode lifespan extension.(Ayyadevara et al. 2013)

In addition to the discovery of its potential longevity promoting effect as reported in Centenarians(Agüero-Torres et al. 2001), aspirin has been well-studied to reduce all-cause mortality of humans by preventing various pathologies including atherosclerosis(Khaidakov et al. 2010), myocardial infarction and stroke(Schreinemachers & Everson 1994), breast cancer(Johnson et al. 2002), prostate cancer(Jacobs et al. 2005), colorectal cancer(Stark et al. 2007), insulin resistance in type 2 diabetes(Hundal et al. 2002), and Alzheimer's disease(Thomas et al. 2001). Based on such numerous proposed benefits of aspirin, the age-prolonging effect is most likely a combination of various physiologic mechanisms. Further clinical trials are necessary to elucidate its risks and benefits.

Nordihydroguaiaretic Acid (NDGA)

Nordihydroguiaretic Acid (NDGA) is an antioxidant derived from the creosote bush whose derivative masoprocol, is used as an anti-neoplastic drug for the skin. It is a polyphenol that is thought to activate sirtuins; thereby prolonging the lifespan of metazoan species.(Wood et al. 2004) NDGA is the third drug after rapamycin and aspirin that the NIA-ITP reported to have a significant effect on mice longevity. Similar to aspirin, NDGA increased survival in male mice but not females.(Strong et al. 2008) However, unlike aspirin the plasma concentration of NDGA in male mice was significantly higher than that of their female counterparts—suggesting peak or steady state concentration between the genders may explain the observed difference.(Strong et al. 2008)

As mentioned, the anti-neoplastic effect of NDGA is under intense scrutiny and is thought to involve the 5-lipoxygenase inhibitor.(Hoferová et al. 2004) It has also been observed to inhibit breast cancer growth by down-regulating the tyrosine kinases IGF-1R and HER2/neu.(Youngren et al. 2005) Aside from NDGA's anticancer activities, it has been noted to prevent ischemia-induced neuron death(Shishido et al.

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2001) as well as decrease insulin resistance and triglycerides levels in rats(Reed et al. 1999). All three drugs mentioned by the NIA-ITP have promise for future use in humans; however, it is apparent in these studies that preventing age-associated disease may be more important than merely focusing on longevity.

Klotho Upregulators

As mentioned, klotho is a transmembrane protein that is thought to promote anti-aging; hence, upregulation of klotho expression may potentially prolong longevity and prevent age-related disease. Angiotensin II type 1 receptor blockers (ARB) are drugs commonly used for blood pressure management, which were recently reported to have potential anti-aging effects. Over-activation of the angiotensin II type 1(AT1) receptor stimulates the renin-angiotensin-aldosterone system which promotes water and salt retention as well as vasoconstriction, leading to an increase in blood pressure. Hypertension in turn is associated with various cardiovascular and renal pathologies. Mice with mutated AT1 receptors have increased longevity and less oxidative stress when compared to their wild-type counterparts.(Benigni et al. 2009) A recent study demonstrated that cyclosporine-induced renal injury decreases klotho expression in the mouse kidney dose-dependently.(Yoon et al. 2012) However, simultaneous treatment with cyclosporine and losartan, an ARB, normalized klotho expression and improved histological appearance.(Yoon et al. 2011) A study conducted by the same author suggested that statin administration in mice upregulated klotho and the FoxO signaling pathway, thus increasing resistance to oxidative stress and slowing apoptosis.(Yoon et al. 2012) Agents targeting klotho upregulation are slowly being discovered, but they need large-scale multicenter testing to isolate their effective mechanisms in anti-aging.

Sirtuin Activators

Also mentioned previously, SIRT1 is a gene that regulates DNA repair, inflammation, apoptosis, cell senescence, and potentially, aging. Sirtuin activators derived from resveratrol have been developed to modulate sirtuin activity and manage age-associated disease.(Vu et al. 2009) Among these, the most

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potent activator is SRT1720, which is three times more effective than resveratrol in SIRT1 activation.(Milne et al. 2007) Consequently, studies report that SRT1720 administration decreased insulin resistance in type 2 diabetic animals(Milne et al. 2007), attenuated lung inflammation in smoke-induced cellular senescence(Yao et al. 2012), and increased the lifespan of obese mice(Minor et al. 2011). Development of selective and specific sirtuin activators may not only improve longevity but also mitigate age-associated disease, although more studies need to be performed to elucidate their risks and benefits.

Metformin

Metformin is the first-line drug of choice when treating insulin resistance in type 2 diabetes. Studies in mammals have shown that hyperglycemia and hyperinsulinemia are contributing factors to aging and cancer development.(Anisimov et al. 2005) Because of these findings, anti-diabetic drugs such as metformin have become candidates for potential anti-aging agents. Various studies have demonstrated the life-prolonging effects of metformin in mice.(Anisimov et al. 2005; Dilman & Anisimov 1980) Another experiment conducted in rat soleus, red gastrocnemius, and white gastrocnemius muscle also suggests that biguanide enhances the peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α).(Suwa et al. 2006) PGC-1α is a central coactivator that regulates cellular metabolism. Additionally, metformin augmented the citrate synthase activity in all three muscles, hexokinase activity in white gastrocnemius, and β-hydroxyacyl-CoA dehydrogenase activity in the soleus.(Suwa et al. 2006) A recent study suggested that metformin upregulates SIRT1 activity(Caton et al. 2010), which may be the mechanism behind its positive effects. Further studies demonstrated that metformin targets the AMP-activated protein kinase (AMPK) pathway, an activator of SIRT 1.(Caton et al. 2010; Zheng et al. 2012) Based on these experimental findings, metformin seems to be a promising candidate for restoring muscle activity.

Fish Oil/ Omega-3Fatty Acid

Fish oil is commonly used in the outpatient setting to increase omega-3 fatty acid levels in the body. Fish oil changes mitochondrial membrane properties and gene expression.(Afshordel et al. 2014) For example,

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fish oil increases Bcl-2, an anti-apoptotic protein, in aged mice brains that may enhance mitochondrial function.(Ajami et al. 2011) It also significantly increases mitochondrial activity and ATP levels in the aged mice brains.(Afshordel et al. 2014) Another protein upregulated with fish oil is neuroprotectin D1 metabolite (NPD-1), which is important for normal brain function(Bazan et al. 2011) and cell survival via anti-apoptotic and neuroprotective gene expression.(Bazan 2009) Such increase in NPD-1 may compensate for age-related oxidative stress and mitochondrial dysfunction.(Lukiw & Bazan 2008) From these studies, it seems that fish oil and omega-3 fatty acid may potentially help prevent age-related cognitive decline.

Lipid-Lowering Fibrates

Fibrates, such as the well-known gemfibrozil, are amphiphilic carboxylic acids that are clinically used to treat hypercholesterolemia and hypertriglyceridemia. One study reported that three fibrates, bezafibrate, clofibrate, and finofibrate, increased longevity in C. elegans.(Brandstädt et al. 2013) Fibrates exert their pharmacological effect through increasing the peroxisome proliferator-activated receptor alpha (PPAR-α) expression, which controls fatty acid metabolism. In C. elegans, an analogous gene to PPAR-α called NHR-49 induces fatty acid oxidation.(Brandstädt et al. 2013) However, during the beta oxidation process of fatty acid synthesis, increases in reactive oxygen species may occur which may induce an adaptive response and increase oxidative stress resistance in a compensatory manner, thus increasing lifespan.(Brandstädt et al. 2013) Although an interesting theory, there is scant literature supporting the life-prolonging effects of fibrates, and more animal model studies must be performed.

HORMONAL APPROACHES

Human Growth Hormone (GH)

Human growth hormone levels are high early in life corresponding to somatic growth, but decrease soon after physical and sexual maturation.(Bartke 2008) Age-related decline in GH levels are known to occur,

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primarily due to reduced hypothalamic secretion of GH-releasing hormone (GHRH) with decline in GH synthesis and release by anterior pituitary.(Kuwahara et al. 2004) This decline induces a fall in circulating levels of insulin-like growth factor-1 (IGF-1), the key mediator of GH action.(Maggio et al. 2006) Past studies showed that administration of GH in adults for 6 months increased lean body mass, decreased adipose-tissue mass, increased lumbar vertebral bone density, and increased skin thickness.(Papadakis et al. 1996; Rudman et al. 1990) Unlike GH treatment in GH-deficient adults, the treatment in adults over 60 years old resulted in an insignificant increase in bone mineral density after 1 year of GH treatment.(Rudman et al. 1990) Recent evidence suggests that IGF-1 treatment in mice has anti-atherogenic effects by modulating macrophage function—reducing atherosclerosis and stabilizing existing plaques.(Higashi et al. 2014)

Although GH treatment may increase lean body mass and decrease adipose tissue, adverse effects accompany such hormonal supplementation. Studies indicated that normally healthy, elderly individuals with GH treatment produced a number of side effects such as carpal tunnel syndrome, insulin resistance, edema, and arthralgia.(Blackman et al. 2002; Papadakis et al. 1996) The most concerning consequence of GH administration is the association between IGF-1 levels and cancer.(Cohen et al. 2000) Subjects with elevated IGF-1 levels have a significantly higher risk of developing malignancies later in life. This had previously been observed in men over the age of 60—significantly higher IGF-1 levels correlated with a higher number of prostate cancer cases.(Cohen et al. 2000) Thus it seems difficult to reproduce the therapeutic benefits of GH from treating young or middle-aged adults who develop deficiencies as a result of injury vs. disease in elders with diminished GH levels due to somatopause.(Bartke 2008)

On the contrary, studies in mice suggest that mice with mutations which cause GH deficiency or GH resistance live longer than mice that have normal GH levels.(Coschigano et al. 2003; Flurkey et al. 2001) The mutations in these mice range from lack of GH producing to a mutation in the receptor for hypothalamic GH-releasing hormone.(Coschigano et al. 2003; Flurkey et al. 2001) The increase in

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lifespan between mice with normal GH levels and mice with GH-related mutations is astounding: 25% to over 60%.(Bonkowski et al. 2006; Coschigano et al. 2003) Most importantly, these long-lived, mutant rodents display vigor and cognitive abilities at ages where mice typically display a decline in function and vitality.(Flurkey et al. 2001) This age-prolonging phenomenon in mice is replicated in invertebrates by knocking out the DAF-2 receptor, an analogue of IGF-1 receptor.(Sattler 2013) If these effects on mice and invertebrates are applicable to humans, subtle and long-term reduction in GH release and/or activity may retard the aging process.(Bartke 2008) However, symptoms of congenital or acquired GH-deficiency argue against severe or complete suppression of GH for human longevity.(Bartke 2008)

Growing experimental data indicate that men and women over 60 are not GH deficient. Comparison of 24 healthy adults to 24 patients with pituitary disease, all over 60 years old, showed that the latter have significantly decreased levels of GH secretion.(Toogood et al. 1998) The results obtained from mice and invertebrate GH experiments, along with many known undesirable side effects indicate that GH supplementation is currently not a reliable approach to anti-aging.

Melatonin

Melatonin is an indolamine that is produced and secreted by the pineal gland.(Cheng et al. 2006) Its levels are monitored by the hypothalamic suprachiasmatic nucleus and varies with the circadian rhythm: melatonin is highest during night time and lowest during day time.(Nishino et al. 1976) Melatonin determines the quality of sleep and the speed of falling asleep, which are both problems in old age.(Tzischinsky & Lavie 1994) Studies have shown that melatonin confers a free radical eliminating effect(Reiter et al. 2000), protection against oxidative stress(Allegra et al. 2003), immunoenhancing effects(Maestroni et al. 1994), protection from cytotoxic apoptosis(Maestroni et al. 1994), and prevention of induced tumors.(Tamarkin et al. 1981) Progressive lowering of nocturnal melatonin secretion peaks are observed with increased age, attributed to either a decrease in secretion of melatonin or a decrease in sensitivity and/or the number of noradrenergic receptors.(Magri et al. 2004)

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Even though decreasing melatonin levels are observed with aging, the physiological circadian periodicity of melatonin secretion is maintained in centenarians.(Magri et al. 2004) Several clinical studies have shown that patients with Alzheimer's disease show decreased levels of melatonin(Cheng et al. 2006); melatonin seems to have neuroprotective effects against the neurotoxic Amyloid-β protein.(Cheng et al. 2006) In addition, experiments with cultured neurons in vitro show that melatonin is capable of upregulating sirtuin 1 protein levels in aged primary neurons, thereby decreasing the acetylation levels of SIRT1 substrates such as p53, NF-κβ, PGC-1α, and FoxO1 in aged primary neurons.(Tajes et al. 2009)

Several experiments indicate that caloric reduction increases the production of melatonin inside the gastrointestinal tract.(Bubenik & Konturek 2011) Food restricted mice had night time melatonin levels twice as high as mice fed ad libitum.(Stokkan et al. 1991) Also, the number of adrenergic receptors in the pineal gland was twice as high in mice with caloric restriction as that of the controls.(Henden et al. 1992) Addition of melatonin to the drinking water of aged rats reduces intra-abdominal fat, decreases non-fasted plasma insulin and leptin levels, improves immunocompetence(Pierpaoli & Bulian 2001), increases thymus weight(Pierpaoli & Bulian 2001), and elevates blood concentration of testosterone and thyroid hormones(Pierpaoli & Bulian 2001). Recent data suggests that melatonin upregulates the expression of run-related transcription factor 2 (Runx2) and bone morphogenic proteins, increasing bone density and volume in older rats.(Tresguerres et al. 2014)

The mechanism behind melatonin's effect on aging is equivocal: melatonin may resynchronize disturbed bodily rhythms(Bubenik & Konturek 2011), attenuate free radical damage(Reiter et al. 2000), or ameliorate age-related mitochondrial dysfunction(Song et al. 2012). Despite the evidence behind potential benefits of melatonin, some studies suggest that melatonin can increase the incidence and lifespan of tumors as well.(Reiter 1998; Savaskan 2002) Because of such grave potential adverse effect, melatonin needs further investigation before being used for anti-aging.

Testosterone

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Male aging is accompanied by a gradual decline of serum testosterone levels, which manifests such symptoms as decrease in muscle mass and strength, cognitive decline, lowered bone density, and increase in abdominal adipose tissue.(Harman et al. 2001) Due to such correlations, interest in testosterone as a source of anti-aging therapy has increased in the past decade.(Emmelot-Vonk et al. 2008) Clinical trials report mixed results regarding testosterone's effect on the human body, most likely due to differences in experimental designs, health of the subjects, and gonadal status.(Kenny et al. 2001; Sih et al. 1997; Snyder et al. 1999) A double-blind randomized placebo control study revealed that testosterone made no significant difference in muscle strength, bone density, or cognitive abilities; however, the experimental group did experience an increase in lean body mass and decrease in body fat percentage.(Emmelot-Vonk et al. 2008) This decrease in body fat mass naturally resulted in an increase in insulin sensitivity and decreased plasma glucose levels.(Emmelot-Vonk et al. 2008) In addition, a significant decrease in both total and HDL cholesterol were seen in the variable group, and no changes in triglyceride levels were observed.(Emmelot-Vonk et al. 2008) The testosterone group contracted more metabolic syndromes such as coronary artery diseases and strokes, most likely due to the decline in HDL.(Emmelot-Vonk et al. 2008) From these studies, it can be assumed that testosterone is not a reliable anti-aging medication and can lead to detrimental side-effects. Recently, the FDA questioned the efficacy of testosterone replacement therapy and is re-assessing the associated risks.(2014)

DHEA

DHEA is a hormone precursor of testosterone and estrogen that is made in the mature adrenal and gonadal glands using the steroidogenic enzyme cytochrome P450.(Nawata et al. 2002) Cytochrome P450c17 has two enzymatic activities: 17-α-hydroxylase and 17, 20-lyase. Cortisol is synthesized by the former and DHEA by the latter.(Nawata et al. 2002) Plasma concentrations of DHEA begin to increase linearly during adrenarche, peak at puberty, and then decrease linearly with age.(Nawata et al. 2002) One of the regulators of 17, 20-lyase, cytochrome P450 reductase, significantly decreases in activity with age

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which accounts for the steady decline in DHEA synthesis.(Nawata et al. 2002) According to studies in mice, DHEA has anti-obesity, anti-diabetic, anti-atherosclerotic, anti-osteoporotic, and anti-amnesic effects.(Maggio et al. 2014; Nawata et al. 2002) However, a two year double-blind experiment in 2006 suggests that DHEA does not have any significant effect on human muscle strength, insulin sensitivity, or oxygen consumption rate.(Nair et al. 2006)

CONCLUSION

Although anti-aging strategies have gained popularity in recent decades, scientists have yet to discover the solution to age-related morbidity and senescence. Anti-aging genes, caloric restriction, nutritional supplements, medications, and hormonal therapies have been explored, but most if not all approaches have yet to yield scientifically reproducible results in humans, especially the widely marketed testosterone therapies (the medical literature illustrates formidable risks of heart attack, stroke, and death in men using testosterone therapy for anti-aging or "andropause"). Even though some medications such as rapamycin, GDF11, aspirin, and NDGA hold promise as potential anti-aging modalities, they are far from ready for prescription use in senescence due to their unknown efficacy and side effects. Rather than seeking a pill or ingredient that can prevent age-related changes, daily physical activity and healthy diet (occasionally skipping a meal) remain the only proven methods of reducing all-cause mortality and improving quality of life.

The authors declare that they have no commercial interests and received no research funding.

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