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Applied Healthspan Engineering

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Abstract

According to the Homeric Hymn to Aphrodite, when Eos asked Zeus for Tithonus to be granted immortality, she forgot to ask for eternal youth. Applied Healthspan Engineering (AHE) seeks to address this problem. All organisms have a minimal level of functional reserve required to sustain life that eventually declines to a point incompatible with survival at death. AHE seeks to maintain or restore optimal functional reserve of critical tissues and organs. Tissue reserve correlates with well being. Diet, physical exercise, and currently available small-molecule-based therapeutics may attenuate the rate of decline of specific organs or organ systems, but are unlikely to restore lost reserve. Inherent evolutionary-derived limitations in tissue homeostasis and cell maintenance necessitate the development of therapies to enhance regenerative processes and possibly replace whole organs or tissues. AHE supports the study of cell, tissue, and organ homeostatic mechanisms to derive new regenerative and tissue replacement therapies to extend the period of human health.

Introduction

Tithonus indeed lived forever:

"but when loathsome old age pressed full upon him, and he could not move nor lift his limbs, this seemed to her in her heart the best counsel: she laid him in a room and put to the shining doors. There he babbles endlessly, and no more has strength at all, such as once he had in his supple limbs (Homeric Hymn to Aphrodite)."[1]

What is Applied Healthspan Engineering?

All organisms have a minimal level of functional reserve required to sustain life that declines to a point incompatible with survival at death. Applied Healthspan Engineering (AHE) seeks to maintain or restore optimal functional reserve of critical tissues and organs. A minimal outcome of successful AHE will be to "square" the human healthspan curve (Fig. 1). If this approach is successful, more people will live to a healthy "old age" with a shorter period of morbidity before death. We anticipate that the median lifespan will increase, although increases in maximum lifespan are less predictable.

Most multicellular organisms follow a familiar trajectory, the cycle of life. Young individuals grow to maturity, reproduce, and subsequently age and die. To assure successful reproduction, by its inherent mechanism natural selection can only maximize well being during the reproductive years. After cessation of reproduction, the body declines in function and survives only as long as sufficient physiological reserve is maintained. Even in some single-cell species (e.g., yeast), maximization of healthspan appears to correlate with reproductive fitness. For example, mother cells selectively accumulate damaged proteins, permitting more vital daughter cells a better chance of survival and reproduction.[2] Thus, inherent limitations to longevity and ultimately healthspan have been incorporated into the blueprints of each organism by natural selection. AHE seeks to overcome the inherent limitations introduced into the construction of the human body and its tissue and organs. We hypothesize that strategies to augment physiological reserve will increase the healthspan.

The human healthspan begins at conception, with many antecedent, parental, *in utero* influences,[3,4] and continues until death (see Fig. 1). The first two decades of life are characterized by growth and development. Maximum vitality is attained in the late teens and twenties, followed by gradual degeneration, accumulating morbidity and eventually death. In the United States, median life expectancy has increased from $\approx$45 years in 1900 to 80+ years in 2010. Graying of the baby boomers has created a large population of so-called "zoomers," people in relatively good health over the age of 50 (www.demko.com/zoomers.htm). A convenient model to consider and compare interventions employs the concept of functional reserve capacity. Although somewhat arbitrary (in the sense that optimal performance for an athlete might greatly exceed reserve capacity of a healthy sedentary

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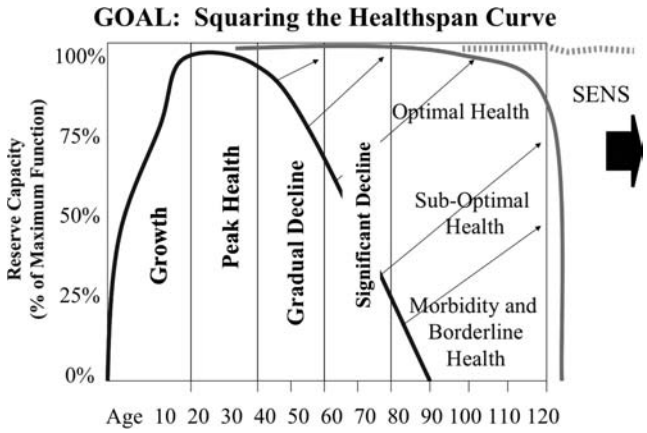

FIG. 1. Minimal goal of Applied Healthspan Engineering (AHE) is to square the healthspan curve. Well-being peaks at the end of growth phase and then declines. AHE seeks to stabilize well-being, possibly resulting in increased longevity. Strategies for Engineered Negligible Senescence (SENS) seeks to increase longevity by "defeating" aging.[5]

person), optimal health corresponds to perhaps 75–100% of maximal function. At <30% of maximal function, a person is likely in suboptimal health. Obviously this is a generalized schema wherein a given tissue or organ system may be limiting when the remainder of the body's functional reserve is adequate.

AHE will use behavioral, pharmacological, biomechanical, and regenerative means to maximize physiological reserve and wellness at increasing chronological age. The initial focus is to modulate pre-existing homeostatic mechanisms, such as blood pressure, heart rate, energy metabolism, etc. When AHE reaches hard limits (those that require genetic or cellular modification) imposed by natural selection, it will be necessary to switch emphasis to engineer new regenerative capacity. Like traditional disease-focused aging research, AHE will promote advances in medical treatments specific to organ systems and tissues. In contrast, traditional molecular aging research seeks to elucidate universal biochemical mechanisms of longevity that might be used to increase human lifespan. By focusing on maximizing organ/tissue functional reserve instead of longevity, AHE represents a practical, incremental alternative that will also likely increase longevity. However, AHE-mediated improvements in tissue reserve, especially in organs such as muscle or skin that are not usually the first point of failure, will not necessarily lead to increased lifespan. Furthermore, although AHE may provide increased well-being, decline due to cancer or accident may lead to rapid death (Fig. 1).

AHE has much in common with Strategies for Engineered Negligible Senescence (SENS), which seeks to "defeat aging" by repairing tissue damage.[5] The essential difference is that AHE seeks to maintain tissue homeostasis first by modulating existing homeostatic and regenerative mechanisms and then in the future by re-engineering the regenerative capacity of the tissue at the cellular level. SENS seeks to create new means to repair the cellular damage that underlies tissue dysfunction, including direct rejuvenation of aging differentiated cells. Importantly, AHE emphasizes what is available presently as well as future innovations.

What Is Possible? The Upper Limits to Mammalian Healthspan and Longevity

Humans are among the longest-living mammals.[6] Although mice usually live less than 4 years, the naked mole rat lives >28 years and similar-sized individuals with very high metabolic rates such as bats have survived >35 years. Dogs live up to 21 years, cats as long as 36 years, and horses up to 62 years.[6] A French woman, supercentarian Jeanne Calment, lived for 122 years, 164 days, the longest documented human lifes pan. Among mammals, whales have the longest documented lifespans. Prof. Jeffrey Bada (Scripps Research Institute, La Jolla, CA) used the amount of racemized aspartic acid (L-asp → R-asp) obtained from the lens of a bowhead whale to estimate its age as 211 years ±16%.[7] Perhaps AHE can benefit from a better understanding of the metabolic basis for the long lives of these creatures with whom we share common ancestors. Consistent with recommendations of most healthcare professionals, whales abstain from smoking, live a low-stress lifestyle and exercise daily. In addition, cetaceans consume fewer glycotoxic and lipotoxic substances than is found in the typical Western diet!

Whales have the slowest heart rates among mammals, which supports a remarkable correlation between heart rate (a reflection of autonomic nervous system activity) and lifespan.[8,9] Mean life expectancy among mammals correlates with total lifetime heartbeats (Fig. 2). Perhaps this simple relationship instructs AHE to employ vagal stimulation or other means to reduce heart rate to maintain cardiopulmonary reserve.[10] In mammals, degenerative processes affecting the cardiovascular system with eventual pathological changes in parenchymal and the extracellular matrix/fibroconnective tissue takes place in virtually all organ systems.[11] For example, both quality of life and viability are linked to deterioration of the cardiovascular, pulmonary, and, less commonly, renal systems. In the United States, heart failure is the most common cause of hospitalization, and 93% of deaths in adults over age 55[12] and among centenarians, by definition individuals who have maximized lifetime fitness, result from failure of cardiopulmonary reserve (68% cardiovascular, 25% pulmonary).[13] Hence, if AHE can optimize cardiopulmonary functional reserve to permit a majority of the population to attain supercentarian health, it will be considered to be a success.

AHE Seeks to Identify Interventions That Augment Physiological Reserve: A Framework for Discussion of Healthspan Engineering

Wellness diminishes with age. Advancing age correlates with an increasing probability of lost vitality and function in all organ systems. The reserve and vitality of each tissue can be represented by a two-dimensional box the area of which shrinks over time (Fig. 3). At some future time, physiological reserve has diminished to a level incompatible with survival. Alternatively, an insult earlier in life can push one "outside the box" of physiological reserve. This can be lethal if the tissue is not returned to homeostasis within a reasonable period of time. For example, until the 1940s many people died of infectious diseases that are today routinely treated with widely available antibiotics. The potentially lethal streptococcal infection that affected Ann Miller in 1942 is a classic example of this model. At age 30, Ms. Miller sustained a life-threatening throat infection which left her deleterious and febrile to 107°F.

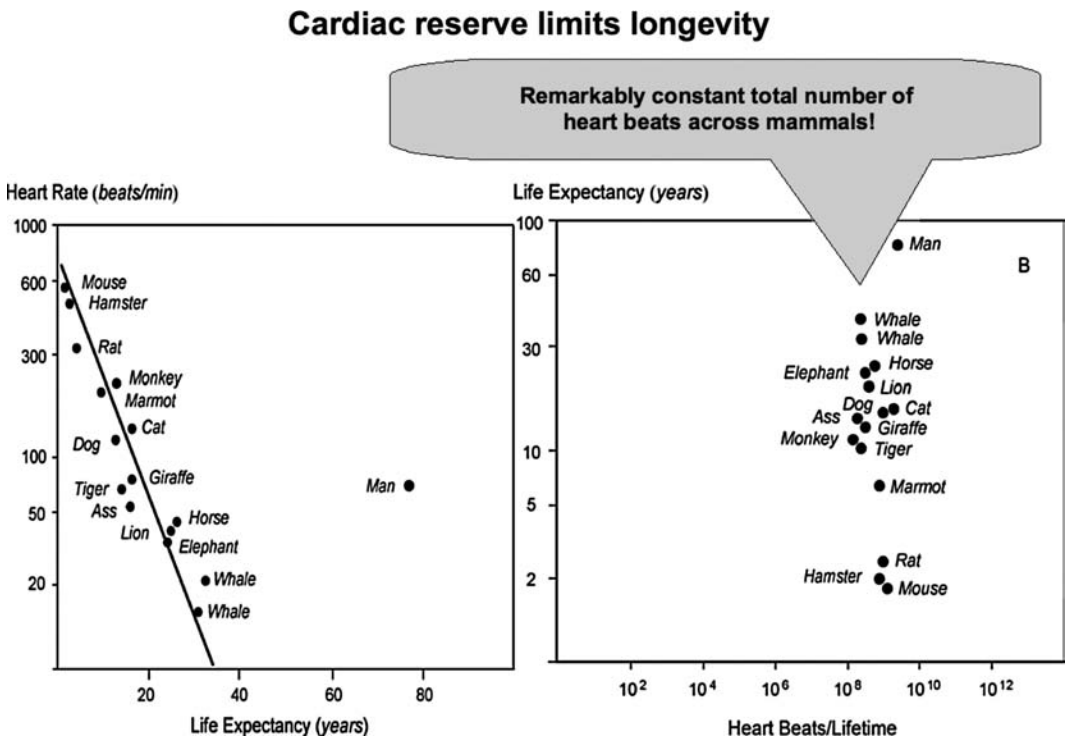

FIG. 2. Cardiac reserve limits longevity. Heart rate (*left*) as well as total number of heartbeats per lifetime (*right*) as well as heart rate correlate with life expectancy in mammals. (Figure modified from ref. 9).

Luckily, she was the first patient to receive penicillin, which saved her life.[14] By pushing her "back into the box," penicillin permitted her to live an additional 60 years! The success of conventional medical intervention is measured by its capacity to reduce the threat of injuries and conditions that push individuals "outside the box" of physiological reserve.

Solutions to the problem of inexorable loss of physiological reserve span a spectrum between two extreme cases. In the first case, an intervention slows decline of function in one or more organs. In the second case, function is restored or actually reversed. Restoration and reversal almost certainly involves replacement of cells, tissue, or organs. To date the replacement of hormones has provided short-term benefits with regard to well being with unclear and perhaps detrimental effects long term. Examples include growth hormone,[15] estrogen replacement therapy,[16] dehydroepiandrosterone (DHEA),[17,18] testosterone,[19,20] etc.

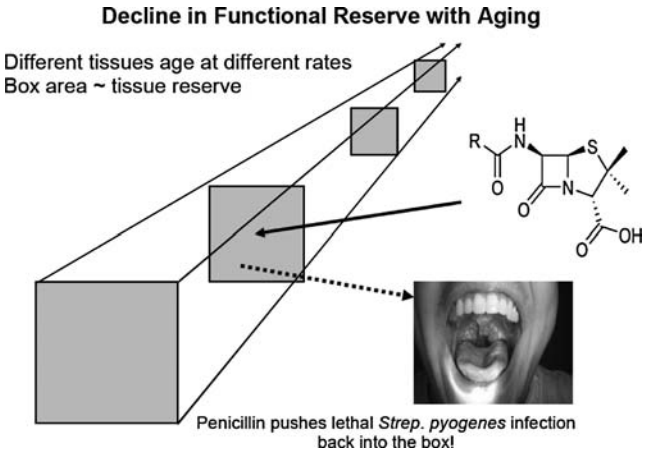

FIG. 3. Schematic depiction of decline in functional reserve with aging. Tissue reserve (illustrated by boxed area) decreases with age. Environmental stressors can push an individual (or an organ system) outside of the box. Therapy such as penicillin can restore well-being to a patient suffering from a life-threatening bacterial infection (inside the box).

Inhibition of problematic cellular circuits (e.g., fibrosis, inflammation, etc.) is accessible today. In the future, it may be possible to stimulate protective cellular circuits or cell-based repair pathways (e.g., endogenous stem cells). Augmentation of cell-based repair pathways with new cells, tissues, or organs using stem cells and bioengineering will most likely lead to huge advances. However, limited progress at the practical level has been made to date.

Biological systems have a hierarchical modular structure that instructs interventions at any of several levels: Nutrients → macromolecular → organelle → cellular → tissue → organism (Table 1). On the basis of design and repair of various human-designed hierarchical systems (e.g., computers, automated manufacturing, machinery, etc.), the fastest and most facile way to restore high function is to replace the highest-level module above the malfunction. This approach works even when understanding is limited, as is the situation with aging. Thus, ultimately organ transplants and/or advanced regeneration methodologies may predominate AHE, as described in more detail below.

The physiological reserve of each organ or tissue declines at a characteristic rate. Reliable measurements of organ reserve and function serve as benchmarks to evaluate the interventions of AHE. Age-related decline in pulmonary function is illustrative (Fig. 4, upper).[21–24] This figure demonstrates age-specific decline in the forced expiratory volume in 1 s (FEV1),

Table 1. Means of Intervention: An Artificial Hierarchy of Modularity

Category	*Intervention*
Spiritual?	Yoga, meditation, prayer, etc.
Physical	Temperature, electromagnetic, ultrasound
Nutrition/performance	Diet, calories, exercise,
Single molecules	Pharmaceuticals, nutraceuticals
Macromolecules	Proteins, fats, sugars
	Chaotic versus self-assembled (''nano-machines'')?
Gene therapy	Therapy with ''information''; genes, antisense, RNA interference
Organelle	Stimulation of autophagy, mitochondrial enhancement
Cellular	Therapy with ''modular automata''; stem cells
Tissue/organ	Organ or tissue transplants, artificial organs/devices

a standard measure of pulmonary function. Note that the rate of decline is accelerated by the habit of smoking cigarettes, a known insult to lung tissue. Although, cessation of smoking can reduce the loss of functional capacity with a major impact on public health, are there other interventions that can reduce the inexorable decline of pulmonary function and reserve?

Statins (drugs originally developed to inhibit 3-hydroxy-3-methyl-glutaryl-CoA reductase [HMG CoA reductase], the rate-limiting step in cholesterol biosynthesis) have been reported to slow age-related decline in pulmonary function.[25,26] Results of the Boston Veterans Administration Normative Study (Fig. 4, lower), which followed a cohort of over 800 $\approx$ 71-year-old men for 10 years, revealed that use of statins significantly slowed the loss of pulmonary function (both FEV1 and lung capacity) in nonsmokers as well as previous and current smokers. At present, no interventions are known to reverse pulmonary decline except for lung transplantation, an intervention that is not practical on a large scale or at advanced age. Thus, statins are a rational component of AHE, acting to lower low-density lipoprotein cholesterol (LDL-C), to impede the progression of cardiovascular disease as well as multiple effects on inflammation, tissue repair, and homeostasis.[27]

What Interventions If Any Can Slow End Organ Deterioration?

Biological systems are comprised of several levels or hierarchies of modularity, e.g., molecular, organelle, cellular, tissue, and organism (Table 1). Although functional "wellness" can be measured at the organismal and tissue levels, the underlying biochemical processes act across modules, complicating interventions. Although interventions to slow or reverse dysfunction can be made at any of several levels, there is an imperfect match between type of intervention and "module/level of action" (see Table 1). As noted above, two options are available: (1) modify native processes or (2) simply replace a particular module.

Extensive data support the benefits of diet and exercise to increase healthspan. Such interventions are easily and immediately accessible. Unfortunately, motivation may limit efficacy, hence the interest in an exercise pill.[28]

Prophylactic alteration of the activity of disease-associated physiological pathways is not without the possibility of tradeoffs in which suppression of inflammation, for example, may reduce wound healing and immunity. Even life-saving therapeutics may actually accelerate aging. For example, cancer chemotherapy may induce additional tumor mutations, increasing drug resistance, and contribute to development of subsequent secondary neoplasms. Chemotherapy may also cause the destruction of adult stem cell populations that help maintain the organism and delay aging.

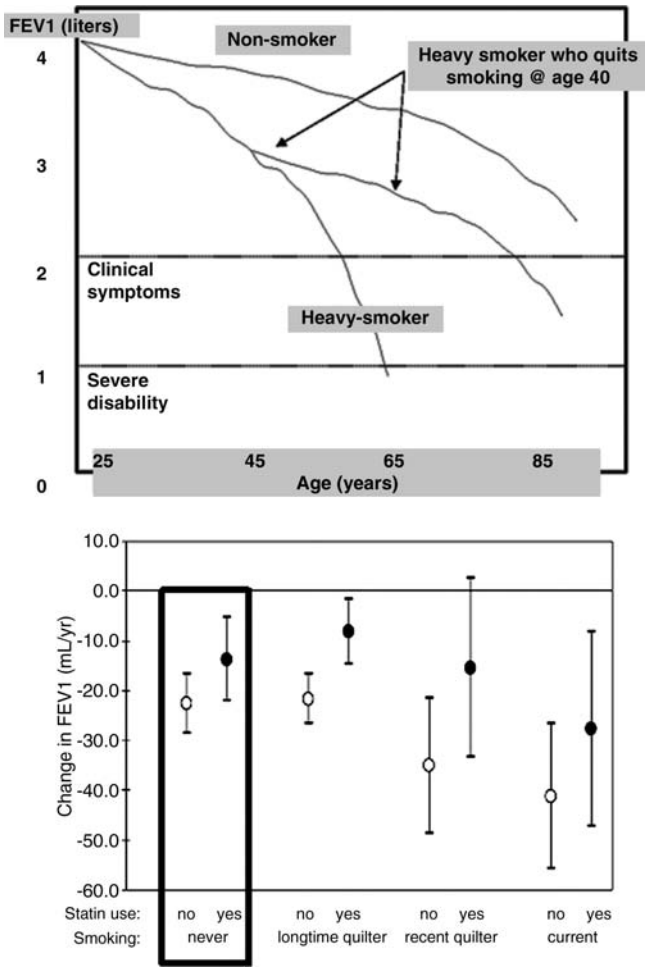

FIG. 4. Pulmonary reserve (forced expiratory volume, 1 s [FEV1]) declines with age (*upper*); however, decline can be slowed by statin therapy (*lower*). (*Top*) FEV, a measure of pulmonary function, decreases with age. (Based on data from ref. 20). (*Bottom*) Statin use slows decline in FEV in 803 men with an average age of 71. Forced vital capacity (FVC) and FEV1 were measured two to four times between 1995 and 2005. (Figure modified from ref. 25).

Table 2. Representative Rational Healthspan Interventions—2010

Target/process	*Intervention*	*References*
Blood pressure	Multiple; exercise, dietary, sodium restriction, see RAS (below)	48
Heart rate	Exercise, vagal nerve stimulation	9
Dyslipidemia	Fish oil; flaxseed oil, olive oil niacin, statins	34–37, 48–51, 123–125
Renin–angiotensin system (RAS)	Exercise, dietary, sodium restriction, ACE inhibitors, ARBs, renin inhibitors	See Table 3
Medial elastocalcinosis	Vitamin K2	33
Glucose homeostasis	Exercise, metformin, dietary-caloric restriction	42, 47, 52, 53
mTOR pathway	Resveratrol, rapamycin, dietary-caloric restriction	41, 42, 43–46, 54
Inflammation	Aspirin, NF-κB inhibitors (e.g., EGCG, quercetin, etc.)	55, 58
Autophagy	Verapamil, trephalose, others	56, 57
Extracellular matrix cross-link	Alagebrium, ALT-711	59
Chemopreventive	Aspirin, bioflavonoids	38–40, 60

ACE, angiotensin converting enzyme; ARBs, angiotensin receptor blockers; EGCG, epigallocatechin 3-gallate; mTOR, mammalian target of rapamycin.

Elimination of exposure to stress and exogenous infection/inflammatory agents/stimuli is moderately controllable, especially by vaccination in some cases. However, substantial progress will be required to eliminate chronic viral infections that apparently contribute to long-term debilitation of the immune system. The continual evolution of pathogens and difficult development of new, engineered substances make this approach problematic.

Certain surgical procedures, although invasive, effectively eliminate, delay, or repair certain conditions, such as degenerative diseases of the heart or cardiovascular system. Improved surgical approaches employing micro- and nanodevices are under development and may play an important role in the not-so-near future.

Modulation of Physiological Processes to Extend Healthspan (Targeting Specific Critical Tissue/Organ Systems

Nutritional supplementation and/or pharmacological interventions may partially overcome genetic limitations of metabolism by attenuating or stimulating key pathways. Presently, despite substantial supplement industry advertising, there are no commercially available nutraceutical or pharmaceutical interventions proven to slow the aging process substantially in humans. However, a strong rationale may exist for some agents. Furthermore, in the near future, personalized medicine based on genome analysis may permit development of tailored prophylactic antiaging, prohealthspan regimens (Table 2).[29–32]

Increased understanding of the pathophysiology of mammalian/human diseases associated with, but probably secondary consequences of, fundamental aging processes provide a very good set of targets to extend healthspan. Although many components of these pathways are evolutionarily conserved and may be studied in invertebrates, the significant differences in metabolism, as well as their effects on the homeostasis of complex mammalian organ systems, will require development of mammalian/human model systems to advance AHE. Several pathways accessible today are described below.

Example #1: Renin–angiotensin system

The renin–angiotensin system (RAS) is among the most important physiological mechanisms maintaining homeostasis of the cardiopulmonary system. Although of paramount importance in regulation of blood pressure, the RAS has global effects, helping to regulate tissue maintenance in almost every organ (Table 3; Fig. 5).[85]

Readily available drugs (e.g., angiotensin converting enzyme [ACE] inhibitors and angiotensin receptor blockers

Table 3. Renin–Angiotensin System Blockade: Protective Actions in Multiple Organ Systems

Pathology	*Treatment*	*References*
Hypertension		
Vascular dysfunction	ACEi, ARB	61, 81
Atherosclerosis		
Excess adhesion molecules	ACEi	62
Arterial stiffness	ACEi, ARB	63–66
Arterial fibrosis	ACEi, ARB	67, 68
Arterial hypertrophy	ACEi, ARB	67, 68
Endothelial function	ACEi	69, 70
LDL oxidation	ACEi, ARB	71, 72
Heart		
Myocardial fibrosis	ACEi	73
Ventricular hypertrophy	ACEi	74, 75
Atrial fibrillation	ACEi, ARB	76–78
Diabetes		
Insulin resistance (type II diabetes)	ACEi, ARB	79, 80
Diabetic nephropathy	ACEi, ARB	81, 82
Inflammation		
Inflammatory cytokines	ACEi	69, 70
Low levels bradykinin, NO	ACEi	69
Monocyte activation	ARB	83
Autoimmune		
Autoimmune encephalomyelitis/ Multiple Sclerosis	ACEi	84

ACEi, Angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor blockers; LDL, low-density lipoprotein; NO, nitric oxide.

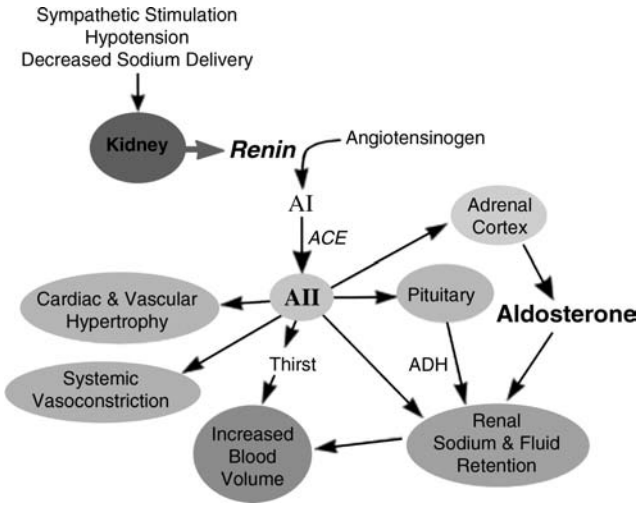

FIG 5. Renin–angiotensin system physiology. AI/AII, Angiotensin I and II; ADH, antidiuretic hormone; ACE, angiotensin-converting enzyme.

[ARBs]) that inhibit this pathway have been shown to extend rodent lifespan.[86] Recent work of Basso et al.[86] demonstrated a ≈20% increase in median and maximal lifespan in rats treated with enapril (ACE inhibitor) or losartan (ARB). Although these investigators documented a significant reduction in systolic blood pressure (≈144 mmHg in controls vs. ≈105 mmHg in treated), most impressive was the reduction in vascular fibrosis. Histology figures in their report (http://ajpheart.physiology.org/cgi/content-nw/full/293/3/H1351/F1-F3) show dramatic reductions in tissue fibrosis in the treated animals compared to controls. Presumably this reduction in tissue fibrosis contributes to increased physiological reserve and function in multiple tissues.

Consistent with these findings, mice lacking the AT1A receptor, the target of ARBs, live longer than littermate controls.[87] Given the significance of cancer in mouse longevity, the primary mechanism of action may be on reduced tumor angiogenesis and growth.[88,89] Alternatively, kidney function is also responsible for age-associated mouse[90] and rat deaths,[91] and lower blood pressure associated with inhibiting the RAS may also reduce kidney damage. Recent data suggest that cardiac function, which is also adversely affected by angiotensin signaling,[73] may be partially responsible for mouse deaths.[89,92] AT1 angiotensin receptor inhibitors have been reported to inhibit the formation of circulating MDA-modified LDLs in human diabetic patients and the modification by MDA of lungs in rats affected with bleomycin-induced pulmonary fibrosis.[93] Table 3 summarizes the benefits of RAS blockage that, in addition to those already described, include antiatherosclerotic, antiinflammatory, antiautoimmune, and procardiac activities.

In summary, the RAS, so critical for mammalian homeostasis, cannot be studied in models of aging in yeast or invertebrates, yet is quite important to mammalian healthspan and lifespan.

Example #2: Medial elastocalcinosis

Medial elatocalcinosis is a significant process within vascular tissue that contributes to cardiovascular decline with aging.[94–96] This process is modulated by several vitamin K–dependent, gamma-carboxylated (Gla) proteins, e.g., matrix Gla protein (MGP), osteocalcin, growth arrest specific gene 6 (*Gas6*), and four transmembrane Gla proteins (TMGPs). Recent work of Schurgers et al. demonstrated a significant reduction in medial elastocalcinosis of rodents given pharmacological doses of vitamin K2 (menaquinone).[33] Figures in their original report (http://bloodjournal.hematologylibrary.org/cgi/content/full/109/7/2823/F2-F6) show reversal and up to 50% reduction in vessel calcification. Supplementation with vitamin K2 is a relatively benign means to reduce vascular calcification.

Example #3: Lipid metabolism

Lipid metabolism plays a critical role in organismal homeostasis, particularly the cardiovascular system. Statins are the frontline drugs to reduce LDL-C to slow or reverse atherosclerosis and maintain cardiovascular health.[34] Many beneficial effects result from the pleiotropic effects of statins: Protection of endothelial function via augmentation of nitric oxide, increasing the number of endothelial progenitor cells, decreasing oxidative stress via reduction of nicotinamide adenine dinucleotide phosphate (NAPDH) oxidase, reduced thrombogenic response via decreased platelet function, decreased lymphocyte activation/adhesion and decreased inflammation as measured by reduced C-reactive protein (see Fig. 1 in ref. 45).[36] Statins also have modest beneficial effects on atrial fibrillation, the most common arrhythmia of the elderly.[97] Benefits for heart failure[98,99] and complications of type II diabetes[100] are under investigation. Substantial evidence supports using statins to prevent cardiovascular disease and augment cardiovascular reserve.[101]

Fish oil containing docosahexaenoic acid (DHA) is now well known to benefit cardiovascular health by reducing blood pressure, heart rate,[37] and function of platelets.[102] Other benefits include attenuation of the activity of immune-mediated inflammatory pathways that contribute to increased incidence of age-associated diseases from arthritis, atherosclerosis, and cancer to Alzheimer disease.

Example #4: Antiinflammation

Chronic systemic inflammation increases after middle age in humans, resulting in tissue damage that reduces reserve and favors tumor formation.[103,104] Many diseases associated with aging, such as Alzheimer disease,[105] rheumatoid arthritis,[106] chronic obstructive pulmonary disease,[107] and atherosclerosis[108] have significant immunoinflammatory components. Increased serum levels of inflammatory cytokines tumor necrosis factor-α (TNF-α, interleukin-1β (IL-1β, as well as the associated antiinflammatory cytokine IL-6, are often found elevated in older people.[103] Body mass index increases with age[103] and increased adipose tissue may explain the increased cytokine production.[109] Therefore, a balanced diet and exercise to control weight can provide significant benefit to control inflammation. Many of the secondary benefits of statins, fish oil, ARBs, and ACE (Examples 2 and 3) are hypothesized to be due to suppression of inflammation.[110,111] Numerous studies support the use of low-dose acetyl salicylic acid (ASA, aspirin) to attenuate augmented platelet function that contributes to vascular disease.[112] ASA at higher doses may be chemopreventive for colonic tumors.[38–40] Nonsteroidal antiin-

flammatory drugs (NSAIDs) such as ibuprofen have been reported to decrease the incidence of Alzheimer disease in some studies, although further study is needed.[113,114] Reactive oxygen species (ROS) play an important role in the induction and maintenance of systemic inflammation, suggesting that antioxidant therapy may be of use as well (see below). Preventing or ameliorating potential tissue damage caused by activation of inflammatory pathways is an important goal of AHE. The reduction of body mass index may play at least as important role as pharmaceutical or nutraceutical intervention.

Other Metabolic and Physiological Pathways Suitable for AHE

For ultimate success, AHE requires an inventory of the pathways involved in organ maintenance and decline. What processes limit physiological reserve? A deep understanding of the limits of cellular and tissue homeostatic mechanisms is necessary. Unfortunately, for integrated organ–tissue systems, maintenance requires continuous high-level function from specialized, often postmitotic cells. A tension will arise pitting the limits of pathway augmentation against the need to replace aged, malfunctioning cells *in toto*, i.e., bona fide regenerative medicine.

Growth/stress pathways: Universal maintenance/longevity pathways?

Alterations of specific pathways that control growth, homeostasis, and stress response have been shown to increase longevity in yeast, *Caenorhabditis elegans, Drosophila melanogaster*, and mice. Inhibition of the evolutionarily conserved insulin/insulin-like growth factor-1 (IGF-1)/phosphoinositide-3 (PI3) kinase/Akt pathway or TOR pathway results in increased longevity, presumably through an incompletely understood combination of growth suppression by decreased ribosomal biogenesis and protein translation and increased repair by autophagy.[41] Dietary or caloric restriction (CR), which appears to act through these two pathways, increases lifespan and healthspan in all of these organisms. It has been hypothesized that CR will similarly increase healthspan and lifespan in humans and other long-lived primates. Although data do not yet support lifespan extension, CR has significant healthspan benefit in primates and humans, including insulin sensitization and improved cardiopulmonary function.[42,115] The benefits of CR points to the existence of cellular maintenance mechanisms that may not only be ubiquitous in eukaryotes, but likely can affect all organ systems to some extent. However, the existence of universal mechanisms of cellular maintenance contributing to longevity does not obviate the need to discover and reverse defects in homeostasis associated with specialized tissues, e.g., kidney, eye, brain, heart, etc.

Recent interest in the development of CR mimetics to increase healthspan/lifespan has skyrocketed. Headlines have been captured by two substances. The mTor inhibitor rapamycin increased lifespan in mice, although at the possible cost of increased rates of lymphoma.[43] Resveratrol, a bioflavonoid available as a dietary supplement, activates Sirt1, a downstream effector of the CR pathways. Resveratrol has been reported to protect mice from damage due to consumption of high fat diets.[44,45] Many of the same patterns of gene expression seen with CR are found after exposure to resveratrol.[46]

What about antioxidants?

Accumulation of damaged macromolecules is hypothesized to underlie loss of cell function with aging. Such damage has been linked to highly reactive chemical species, such as free radicals of oxygen (ROS) or nitrogen. Harman first postulated, the "Free Radical" and later the "Oxidative Stress" theory of aging in 1956.[116] These concepts led to the conjecture that substances with antioxidant activities, such as free radical scavengers, would limit the generation and damage mediated by free radicals. These interventions were expected to help maintain normal cellular function and thereby slow aging. This catechism has become a dogma among the general population with >40% consuming antioxidant supplements of one type or another.[117] A huge industry has grown up around this idea.

However, there is limited evidence that antioxidant supplementation, outside of preventing deficiencies, provides any positive effect on longevity or the maintenance of healthspan in healthy individuals.[118,119] In fact, it appears that in some cases antioxidants may actually inhibit normal ROS and damage protective mechanisms by eliminating the oxidative stress that would normally trigger their induction. For example, Ristow et al.[120] showed that supplementation with antioxidant vitamins C and E blocked the beneficial increase in insulin sensitivity that is associated with moderate exercise, a known enhancer of healthspan.[47]

Many other reports question the validity of the free radical theory of aging (reviewed in ref. 122). With the exceptions of *Drosophila*[121] and mitochondrial localization of catalase in mice,[89,92,123] neither antioxidant vitamin supplementation nor individual ectopic expression of protective enzymes in their endogenous subcellular locations (e.g., catalase and superoxide dismutase) have been shown to increase longevity in rodents and *C. elegans*, although these organisms may be better protected from exogenous oxidative stress, e.g., paraquat.[122] Remarkably even combined endogenous expression of a superoxide dismutase and catalase does not extend longevity in mice.[124] Despite exhibiting increased oxidative stress, $Mclk^{+/-}$ mice that are partially coenzyme Q (CoQ7) deficient live longer than normal littermate controls.[125] Increased oxidative damage is also observed in the long-lived naked mole rat, compared with short-lived rodents such as mice or rats.[126] Consistent with these studies, Van Raamsdonk et al. demonstrated that the *sod-2* mutant of *C. elegans*, has increased oxidative stress, but lives longer.[127]

There could be many reasons for the failure of antioxidants to produce a significant increase of healthspan or lifespan. The simplest explanation may be that the most studied antioxidants either lack enough broad-spectrum activity or exhibit counterproductive activities that interfere with potential benefits. For example, many antioxidants, such as ascorbic acid, are redox compounds that when oxidized can act as prooxidants. For example, transport of increased antioxidant vitamin C to the murine lens actually causes increased protein cross-linking and cataract formation.[128]

Alternatively, failure of antioxidants to provide significant benefit may be much more fundamental. ROS are not only a potential source of damage, but are also employed by evolutionary processes in eukaryotic development and homeostasis. ROS signaling through conserved NADPH oxidases promotes cell proliferation, maintenance of the

undifferentiated state in pluripotent stem cells, new blood vessel formation, and developmental processes such as cardiogenesis. Furthermore, the innate immune system uses ROS generation by NADPH oxidases to destroy pathogens.[129] Most significantly, activation of NADPH oxidases plays a significant evolutionarily conserved role in wound repair.[129] Inappropriate activation of wound-healing pathways by age-associated disease processes activate angiotensin II and proinflammatory cytokines that in turn stimulates NADPH oxidase activity. Resulting inflammation can exacerbate incipient diseases associated with aging such as arthritis, fibrosis, cancer, and reperfusion injury due to stroke. Although antioxidants may not provide systemic relief of age-associated decline in organ reserves, they have proven useful in attenuating inflammatory signaling associated with NADPH oxidase activation in several diseases. Two antioxidants are used clinically. *N*-Acetyl cysteine is used to treat acetaminophen toxicity. The free radical scavenger edaravone is used clinically to reduce damage caused by activation of the arachidonic acid cascade associated with reperfusion injury of the brain.[130] Because of the wide spectrum of inflammatory diseases, antioxidants may still have an important role to play in medicine and AHE.

Activation of inflammatory pathways by NADPH oxidase signaling provides a relatively simple framework to reinterpret experiments that seem to support the free radical theory of aging. For example, we hypothesize that mitochondrial-directed catalase expression in transgenic mice[123] suppresses inappropriate activation of the RAS/NADPH oxidase/fibrotic axis. Reduced RAS activity may make a greater contribution to the increased longevity of these mice than the observed decreased oxidative damage,[89,92,123] although it is difficult to distinguish these possibilities given that damage can lead to a ROS-based positive feedback signaling loop. In this explanation, increased angiotensin II in the cardiovascular system of aging mammals[131] stimulates fibrosis/hypertrophy by activating cytoplasmic NADPH oxidase,[132] which in turn stimulates mitochondrial ROS by opening the mitochondrial permeability transition pore. Mitochondrial ROS then activates p38/ERK1/2/MEK1/2 kinases[133] and profibrotic pathways resulting in cardiac dysfunction.[134] Although the resulting oxidative damage is secondary to RAS signaling, this damage probably plays a role in the reduced cardiac function, possibly by inducing DNA oxidation and subsequent stress-mediated cell senescence.[135] Consistent with this interpretation is that knockout of angiotensin II receptor AT1A not only increases murine longevity but also decreases oxidative markers in many organs, including the heart.[87]

Another way to reconcile the relatively poor efficacy of antioxidants is to invoke a more generalized version of the oxidative stress model of aging. In this model, generalized accumulation of "molecular garbage" is postulated to be responsible for reduced cell function that may eventually result in a "garbage catastrophe."[136] This hypothesis incorporates more potential sources of damage than the simple ROS theory. For example, unfolded proteins and inappropriate macromolecular modification (e.g., advanced glycation end products) are included (where ROS may not be the root cause). Substances that promote reduced garbage accumulation or better garbage disposal would be the target of new small molecular drug/nutraceutical development. A key function may be to stimulate autophagy or induce a stronger unfolded protein response in addition to any antioxidant function.

The loss of homeostasis with resultant compromise of physiological reserve after the end of the reproductive period is more than simple garbage accumulation. We hypothesize that there are limitations of design inherent in somatic homeostasis. Two examples are dysregulated gene expression with time ("developmental drift") or absence of sufficient rejuvenative and/or regenerative mechanisms. An example of the former is the increased activity of GATA transcription factors *elt5* and *elt6* in aged *C. elegans*, leading to decreased expression of the master regulator *elt3*. Inhibiting *elt5* and *elt6* activity restores *elt3* function and extends worm lifespan by up to 50%.[137] Adult *C. elegans* are postmitotic, with limited ability to replace damaged or aging cells.[138] This compromised regenerative capacity may be fundamental to the observed reduction in physiological reserve and proximate demise of elderly worms. Our hypothesis not only explains the inability of antioxidants to significantly enhance lifespan/healthspan, but also suggests the importance of such design limits on longevity itself. The conclusion may mandate cell/tissue/organ replacement strategies as discussed below.

Future Directions: Enhanced Regeneration and Replacement Strategies for AHE

Natural selection also makes extensive use of replacement in organ maintenance in long-lived organisms such as humans. Replacement involves proliferation of progenitor or stem cells. In humans, there is a great amount of tissue-specific cell turnover. For example, new skin cells, gastrointestinal epithelial cells, and hematopoietic cells are produced continually. At the other extreme, new skeletal muscle, cardiomyocytes, and neurons are rarely formed, although in each case a limited capacity for renewal results from embedded stem cells in adult tissue. Although both progenitor and stem cells may be involved in organ homeostasis, a distinction can be made in tissues that have high cell turnover rates, such as tissue composed of epithelial cells. In the lung, progenitor cells maintain homeostasis, with an overall proliferation rate of less than 1% per day.[139,140] Clara cell secretory protein expressing stem cells are responsible for resistance to pollutants and regenerate specialized epithelial cell types in the bronchioles. Such cells function to repair more serious injuries that result in local depletion of epithelial progenitor cells.[141] Moreover, some tissues, such as pancreatic islet cells, are believed to lack stem cells and solely use progenitor cells for maintenance.[142]

Conversely, natural selection of organisms that lack replacement mechanisms are largely postmitotic as adults and are short-lived, as is the case of *C. elegans.*[138] Because organs are not needed after the period of successful reproduction, mechanisms to maintain organ function past this time are not likely to have been selected. This likely explains the small number of cardiac stem cells in the adult mammalian heart and the low level of cardiomyocyte turnover during the human lifespan.[143]

Enhanced regeneration appears to be the mechanism used by natural selection to maintain viability in the few examples in the animal kingdom in which the soma is not disposable. Asexual species of planarians and hydra lack germ cells, but instead have pluripotent somatic cells to replace aging, postmitotic tissue. Hydra eliminate aging cells by sloughing off differenti-

ated cells and are considered essentially immortal.[144] Asexual planaria detach their tails and regenerate two halves of their body, a process that requires their pluripotent somatic stem cells, neoblasts, to not only differentiate, but to recreate the positional information inherent in their body plan.[145] Although the planarian's method of maintaining healthspan is not practical for humans, the idea that it may be possible to significantly enhance tissue regeneration has significant merit.

AHE seeks replacement strategies that include enhanced rejuvenation/regeneration, stem cell–based tissue/organ repair, tissue/organ replacement, and the development of durable artificial organs.

Enhanced regeneration

Stimulation of endogenous tissue maintenance pathways to remove poorly functioning cells and replace them with new cells derived from progenitor or stem cells is the least disruptive strategy for tissue rejuvenation. Successful development of this strategy necessitates avoidance of dysfunction cellular immortalization (cancer) and replicative senescence (Hayflick limit).

Enhanced regeneration is likely to be highly tissue specific. Blastema formation, a hallmark of limb regeneration in newts and axolotls, has been observed in punctured ears of MRL mice[146] as well as other strains.[147] Thus, mammals may possess far more extensive regenerative capacity than is usually believed, although it appears that critical organs such as the heart and brain possess very limited regenerative capacity.

Until recently, the idea that the heart had significant regenerative potential or the capacity to generate new cardiomyocytes was controversial. Recent evidence suggests the human heart does have limited regenerative capacity that might be exploited for AHE. Hsieh et al. have provided evidence for the existence of at least a small population of adult cardiomyocyte stem cells that replace damaged cardiomyocytes after injury.[148] Furthermore, human cardiomyocytes are not a static postmitotic population. Bergmann et al. used carbon-14 (originating from 1950s atmospheric nuclear bomb tests) levels in DNA of human cardiomyocytes to demonstrate cell proliferation ranging from 1% in 25 year olds to 0.45% in 75 year olds.[143] This implies that almost 50% of cardiomyocytes may be renewed during a human lifetime.

The first promising step in the development of pharmaceutical rejuvenation techniques to increase new cardiomyocyte formation has been recently described. Stimulation of mononucleated cardiomyocytes (most cardiomyocytes are binucleated) by neuregulin 1[149] causes up to 6.5% of mononucleated cells to undergo DNA synthesis. Sufficient cardiomyocyte replacement prevented cardiac dilatation in a murine congestive heart failure model (following ischemia reperfusion induced by ligated left anterior descending artery [LAD]). Cell cycle reentry of postmitotic cells may be possible in skeletal muscle as well. A small synthetic molecule, reversine, stimulates skeletal muscle to dedifferentiate into a mesenchymal-like stem cell capable of forming chondrocytes and adipocytes.[150]

Development of new drugs that enhance preexisting regenerative capacity are an important goal of AHE, although serious hurdles persist. At a minimum, tissue specific/localized delivery may be required to contain the potentially deleterious activities of such compounds.

Stem cells and artificial organs

Stem cells provide a potential means to replace or augment old or malfunctioning tissue. In principle, physiological reserve and maintenance of homeostasis could result. However, four basic problems need to be solved for optimal use of stem cells: (1) Means to introduce the cells into a target tissue, (2) means to insure correct cell differentiation, (3) means to remove damaged or dysfunctional cells, and (4) means to insure integration into the target tissue. Replacement of damaged or diseased tissue is already being attempted with a variety of adult stem cells. Sources include hematopoietic, bone-marrow derived mesenchymal cells, and adipose cells.

Given the heart's limited regenerative capacity, a variety of attempts have been made to treat heart failure resulting from myocardial infarction using injection of either hematopoietic or bone marrow-derived mesenchymal autologous stem cells. Improvement of up to 10% of left ventricular ejection fraction has been observed. It appears that these adult stem cells mainly increase blood supply to the heart by differentiating into endothelial cells. The problem of delivery and retention of stem calls in the area of injury can be addressed by bispecific antibodies, one binding to the stem cell and the other binding an injury antigen such as myosin light chain.[151] Unfortunately, this approach does not solve the cell differentiation problem, i.e., provide viable replacement cardiomyocytes.

The recent development of techniques to reprogram somatic cells to become pluripotent (induced pluripotent stem [iPS] cells) may be the breakthrough needed to generate sufficient numbers of specialized autologous cells for repair and replacement of damaged tissue. Takahashi[152,153] and colleagues found that ectopic expression of four transcription factors, Oct4, Sox2, Klf-4, and c-Myc, could reprogram somatic mouse and human tissue into cells that resemble embryonic stem (ES) cells. The original methodology used retroviral and lentiviral gene therapy techniques that are currently problematic for human therapy. Presently there is no gene therapy treatment approved as a therapeutic in the United States. However, recent work suggests a combination of small molecule drugs and cell-transducible reprogramming proteins will increase the safety of iPS production.[154–156] Techniques to differentiate iPS cells into many cell types, including cardiomyocytes, are under active development,[157] and clinical trials are on the immediate horizon.

Despite substantial progress, outstanding problems remain. Among these are: How best to remove dead or damaged cells without scar formation, how best to remove aged cells, and how best to promote cellular integration without disrupting tissue function. The latter problems are likely to be more difficult than the former, because the human body has mechanisms for removal of dead cells that can be co-opted.

Haphazard cellular integration may be problematic because appropriate structural and electrical connections must be established in the heart. Unfortunately delivery of skeletal muscle cells, originally thought to be a ready source of large numbers of autologous repair cells, failed to revive damaged hearts due to associated serious arrhythmias.[158] Transplanted cells can apparently sense potential problems with tissue integration; as a result, cardiomyocytes directly injected into rodent hearts have a strong tendency to die before successful integration.[159]

iPS cells and ES cells are also being used to create whole organs *ex vivo* for transplantation. Although this goal remains in the realm of science fiction, various hybrid organs are being developed using a combination of differentiated cells derived from ES or iPS cells, bioengineered microenvironments, scaffolds, and encapsulation materials. For example, substantial progress has been made toward an artificial liver.[160]

A serious drawback for AHE is that organ replacement requires transplantation surgery. Although iPS cells solve the immune rejection problem associated with current techniques, incomplete reenervation and the risks of surgery, especially on the elderly, make such a strategy impractical. Delivery of new tissue by catheterization may reduce this risk, although organ size limits this approach. Perhaps cells and tissues can be programmed for self-assembly *in vivo.* For example, heart valve engineers are designing possible *in vivo* assembly strategies in conjunction with catheter-based delivery.[161]

Bioengineered mechanical organs

Although AHE seeks to optimize biologically based tissues and organs ("carbon-organic-based solutions"), it is necessary to mention that competing biodevice technology ("silicon-inorganic based solutions") may ultimately prove superior. For example, the implementation of an implantable artificial heart has been a holy grail of bioengineering for quite some time.[162] Successful development of left ventricle assist devices (LVAD) represents an important milestone with survival of some patients for months to years. Current problems include strokes, unwieldy power connections, anticoagulation, and increased risk of potentially fatal infections. Interestingly, implantation of LVADs actually restored native heart function in some cases where a transplantable heart was not identified (reviewed by Mountis and Starling[163]).

Problems of integration of artificial biomaterials with biological systems are likely to be solved by hybrid approaches. We hypothesized that repair is best implemented by replacing the highest-level module above the identified malfunction (Table 1). This approach requires the least understanding of the underlying system components. Thus, stem cell–based and biomechanical organ transplantation may be predicted to advance more rapidly than technologies impinging at lower levels (e.g., pharmaceuticals).

Potential problems with regenerating the brain

The brain poses a special problem for AHE replacement strategies. Whole brain replacement is obviously nonsensical (although head transplants have been patented!; e.g., US Patent 4,666,425), and even replacement of individual neurons by regenerative therapies may be problematic for maintenance of memory and identity, depending upon the role some individual neurons play in distributed memory networks.[164] The creation and maintenance of memory remains a topic of active research, and the degree to which loss of specific neurons or addition of new neurons will disrupt brain memory can only be crudely estimated from brain injury patients.

Although the brain possesses limited homeostatic regenerative capacity, neurogenesis occurs in the hippocampal dentate gyrus and the subventricular zone of the lateral ventricles. The function of this regenerative capacity in adults is not completely understood, but may play a role in spatial memory formation (reviewed in Lee and Son[165]). Neurogenesis can be stimulated by a variety of compounds in mammals, including dietary supplements such as curcumin.[166] However, it is especially interesting that conventional aerobic exercise is among the most effective means to stimulate neurogenesis.[167] Although it is unclear to what extent these cells contribute to maintenance of brain homeostasis, exercise may provide a low-tech means to achieve increased maintenance of brain function. Certainly the cardiopulmonary benefits of aerobic exercise on brain function cannot be ignored.

Conclusion: "Meet Me at the Salad Bar after We Go for a Jog"

Unfortunately, a large gap separates potentially useful AHE interventions and *bona fide* evidence of significant healthspan benefit. Improved perinatal health, sanitation, and elimination of many serious life-shortening infectious diseases were most responsible for the increased healthspan of the 20th century. Ironically, despite huge advances in our understanding of fundamental metabolic processes and billions of dollars spent on discovery and development of new drugs, only modest pharmaceutical recommendations can be made presently. Consuming a modest balanced diet (forestalling type II diabetes and atherosclerosis) and partaking in daily exercise (causing new blood vessel formation, neurogenesis, reduced heart rate by vagal nerve tone, reduced inflammation, etc.) presently have the greatest potential to maintain critical organ reserves. Unfortunately, worldwide "overnutrition" coupled with modern sedentary habits are a significant roadblock to successful AHE. The present generation may be the first in almost 200 years to have a shorter median lifespan than its parents!

Fundamental questions remain regarding tissue homeostasis and maintenance of organ reserve. What is the universe of pathways that control homeostasis? What is the hierarchy of pathways? Which pathways are most critical? What are the limits of intervention? Development of novel biomarkers of aging are needed to guide future studies. However, inherent limitations of mammalian cellular and tissue design create barriers to highly successful AHE. Molecular interventions will likely provide only partial, incomplete solutions for AHE, hence the need for regenerative medicine.

Regenerative medicine promises to revolutionize AHE. We advocate an AHE-oriented research approach focused on the mechanisms of organ/tissue maintenance with age. Unfortunately, numerous pathways important in human healthspan are not well modeled in short-lived species used to study longevity. New model systems will need to be developed to measure functional decline in the absence of disease for critical systems such as the heart and lungs. Perhaps the decline in cardiac function seen in old C57Bl6 mice will make a good starting point to model the heart.[92] These model systems could be used to elucidate the limits of tissue maintenance by progenitor and stem cells. They would be useful to discover new targets for intervention and the limits to *in situ* stimulation of regeneration versus wholesale cellular or organ replacement. By whatever means, we anticipate that successful AHE will square the healthspan curve and greatly enlarge the population of youthful centenarians.

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Author Disclosure Statement

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