Part 1
The Aging Brain in Neurology

Chapter 1
The Biology of Aging: Implications for Diseases of Aging and Health Care in the Twenty-First Century

Douglas F. Watt

Department of Neuropsychology Cambridge City Hospital, Harvard Medical School, and Alzheimer's Center/Clinic for Cognitive Disorders, Quincy Medical Center, Quincy, MA, USA

Summary

- Aging demographics, increasing penetration of diseases of aging, and the heightening expense of high technology health-care interventions are creating exploding costs that are becoming economically unsustainable.
- Evolutionary theory suggests that aging is the fading out of adaptation once reproductive competence is achieved, and reflects the lack of selection for a sustained post-reproductive adaptation.
- If extrinsic mortality is high in the natural environment, selection effects are less likely to promote organism maintenance for extended periods. Alternatively, aging is simply change of the organism over time, and is primarily under the control of the hypothalamic pituitary gonadotropin axis. Although traditionally viewed as opposing theories, these may be simply different perspectives on the same process.
- Cellular and molecular theories attribute aging to a genetically modulated process, a consequence of "wear-and-tear", or a combination of both types of processes.
- Aging is probably a complex and recursive network of many changes.
- Molecular and cellular models of aging include: nuclear and mitochondrial and even ribosomal DNA damage, including genomic instability, loss of epigenetic regulation, and mitochrondrial DNA deletion.
  - Oxidative stress (OS) and associated mitochondrial dysfunction and decline
  - Inflammation which is progressively disinhibited ('inflammaging')
  - Glycation
  - Declining autophagy
  - Dysregulation of apoptosis
  - Sarcopenia
  - Cellular senescence
- Calorie or dietary restriction (CR/DR) has been shown to have positive effects in most but not all species on longevity and aging.
- A network of interacting molecular pathways has been implicated in CR physiology. Sirtuins, a class of transcription factors, are thought to play an important role in cell signaling and aging, in concert with mTOR, AMPK, PGC-1a, and insulin signaling pathways.
- The target of rapamycin (TOR) signaling network influences growth, proliferation, and lifespan. Rapamycin, an immunosuppressive macrolide, inhibits mammalian target of rapamycin (mTOR) and has been shown to increase lifespan.
- CR mimetics are substances that potentially mimic the molecular effects and physiology of CR. Resveratrol is the most well known CR mimetic but only extends lifespan in obese animals.
- Genetic manipulation of growth hormone, IGF-1, and insulin signaling pathways may mimic CR effects.
- Lifestyle factors such as sleep, diet, exercise, and social support may affect a shared set of cellular and molecular pathways.
  - Exercise: elicits an acute anti-inflammatory response and inhibits production of proinflammatory cytokines. Protective against disease associated with low grade systemic inflammation.
  - Obesity: abdominal fat may contribute to the disinhibition of inflammation.
- Polyphenols, often regarded as antioxidants, affect cell physiology and cell signaling in a wide variety of ways that are probably far more critical to their effects in mammalian physiology beyond any putative free radical scavenging.
- Healthy lifestyle practices match those of ancestral hunter gatherers (HGs), suggesting that diseases of aging may be potentated by a mismatch between our genes and the modern environment.

Do not go gentle into that good night,
Old age should burn and rave at close of day;
Rage, rage against the dying of the light.
Dylan Thomas

Aging is arguably the most familiar
yet least-well understood aspect of human biology.
Murgatroyd, Wu, Bockmuhl, and Spengler (2009)

Old age is no place for sissies.
Bette Davis

Dedication: To my Dad, Richard F. Watt, who believed that the best scholarship and the best social values would eventually reveal each other.

Introduction

Aging, now the focus of a rapidly expanding, if still immature, biological science, remains one of the most fundamental yet mysterious aspects of biology. The science of aging has explored the cellular and molecular basis of aging largely in three target organisms with fully sequenced genomes and short lifespans (yeast, roundworms, and fruit flies), as well as an increasing number of *in vivo* studies in mammalian animal models. Evidence argues that multiple pathways modulating aging in these three target organisms are well conserved in mammals, primates, and humans, although perhaps with additional modifications. The science of aging has made progress in describing and analyzing several critical phenotypes or components of aging, including sarcopenia, glycation, inflammation and oxidative stress (OS), endocrine dyscrasia, apoptosis, telomere loss and cellular senescence, genomic damage and instability, mitochondrial dysfunction and decline, and increasing junk protein and declining autophagy (removal of damaged or "junk" proteins). Although the relationships among these various aspects of aging remain incompletely mapped, evidence increasingly indicates that they are deeply interactive, perhaps reflecting the many linked "faces" or facets of aging. Increasing evidence links most, if not all, of these processes to the major diseases of aging and most neurodegenerative disorders.

Evolutionary perspectives argue that aging must be a process against which natural selection operates minimally, in a postreproductive animal. In other words, basic selection processes ensure that enough members of the species (absent predation or other accidental death) survive to a period of maximum reproductive competence (otherwise, a species would not exist), but selection does not and indeed cannot ensure longevity much past a peak reproductive period. *Aging is the result of this relative absence of selection for an extended postreproductive adaptation*. In this sense, evolution "does not care too much about aging", although partial exceptions to this principle in humans

may exist due to the likely contribution of tribal elders to an extended "group fitness," possibly helping to explain why humans are longer lived than almost all other mammals. Such evolutionary perspectives also suggest that aging (and its deceleration) is likely to be highly polygenetic and not easily radically modified, arguing strongly against any wild optimism about improvements to maximum human lifespan beyond its documented maxima (about 120 years). Current thinking also suggests that aging clearly reflects an "antagonistic pleiotropy"—genes beneficial to and even critically necessary for growth and reproduction "backfire" in older animals and contribute to aging, in part through "unexpected" interactions.

However, aging research has extensively probed highly conserved protective effects associated with dietary or calorie restriction (DR/CR), the gold standard in terms of a basic environmental manipulation that slows aging in virtually every species in which it has been closely studied, from yeast to mammals. CR/DR functions as a global metabolic "reprogramming" for most organisms, reflecting a shift of biological priorities from growth and reproduction toward stasis and conservation. CR physiology was presumably selected by allowing organisms to survive in times of nutrient shortage and then resume the critical business of growth and procreation when again in environments more supportive of fecundity. CR extends lifespan and reduces penetration of the diseases of aging significantly, if not dramatically, in almost every species in which it has been studied, but does not appear to be a viable health-care strategy for the vast majority of individuals (due to the intrinsic stresses of chronic hunger). CR mimetics (substances offering at least some of the physiology of CR without the stress of chronic hunger) may offer some or many of the benefits of CR, protective effects of enormous relevance to Western societies as they undergo progressive demographic shifts in the direction of a larger percentage of elderly citizens than at any point in human history, with an impending tsunami of diseases of aging. However, clinical and long-term data on CR mimetics is badly lacking beyond animal models, where they show

impressive protective effects. CR mimetics are currently being studied in multiple diseases of aging, including cancer, heart disease, Alzheimer's disease (AD), diabetes, and several others.

Last but not least, accumulating evidence also indicates that Western lifestyles and an associated pandemic of obesity, reflecting a radical departure from our evolutionary environment, will expose us to increased penetration by the diseases of aging, despite (or perhaps because of) increasing life expectancy. These multifactorial lifestyle changes (poorer sleep, little exercise, complex dietary shifts, increased social isolation) may increase many of the phenotypes or components of aging, including OS, inflammation, glycation, insulin resistance, telomere loss, disordered cell cycling and aberrant growth signals, increased junk proteins, and DNA damage. Fundamental shifts in health-care strategy and priorities will be needed in the coming decades, away from high-technology interventions aimed at an advanced disease of aging (often one at which little real prevention was ever aimed) and toward a reprioritizing of meaningful prevention via substantive lifestyle modifications. Such a shift in health-care priorities is likely to be politically contentious, but the current (and unsustainable) escalation of health-care spending will eventually force basic changes in both health-care policy and clinical practice. The science of aging may eventually heuristically integrate much of our currently fragmented approach to the diseases of aging and thus merits much more attention and review not only in medical school curriculums, but also in basic biomedical research initiatives.

Aging and mortality

All complex organisms age and eventually die[1], with highly variable limits to their typical lifespans, a variability still poorly understood. The outer biological limit to the human lifespan is generally thought to be approximately 120 years. The oldest carefully verified human known was Jeanne Calment of France (1875–1997), who died at age 122 years, 164 days (Robine and Allard, 1995). As far as we know, we are the only species with a vivid awareness of and preoccupation with our own mortality (and perhaps, at other times, an equally great denial). Cultures from the earliest recorded history have been preoccupied with themes of dying and immortality, along with whether it would be possible to escape death or find a true "fountain of youth." Wishes for and even expectations of immortality are a powerful driver in many

organized religions and spiritual traditions. Yet despite such perennial and fundamental human wishes, no way of truly preventing aging or achieving any version of biological immortality has ever been achieved in human history. Aging and our eventual demise from it both seem as unavoidable as the next sunrise. Benjamin Franklin is credited with the famous quote, "The only thing certain in life are death and taxes." More humorous perspectives on these existential challenges include George Bernard Shaw's lament that youth was a wonderful thing and a shame that it had to be wasted on the young. When I was too young to fully appreciate the humor, my own father, who passed away during the writing of this chapter at the age of 93, offered, "Aging is *vastly overrated*, but most of the time, it beats the alternative." But ultimately, aging is no joking matter, exposing humans to slow and inevitable degradation of virtually every organ system, progressive disability, and eventual outright physiological failure of one sort or another, with inevitably fatal consequences. Yet if we did not age and die, humans and their progeny would quickly overrun the planet and totally exhaust its ecology and resources, causing mass extinctions not only for many other species, but potentially for our own as well. Thus, any true "fountain of youth" for humans might prove to be a seductive but ultimately deadly Faustian bargain. Yet who does not want more life, particularly if in decent health and with preserved functional capacities? Such primordial motivation and longing was surely captured in Dylan Thomas's haunting poem "Do Not Go Gentle into That Good Night," tapping universal sentiments in the face of aging and mortality.

In this context, one might ask why a chapter on the biology of aging appears in a textbook of geriatric neurology. Trivially, the obvious answer is that aging has everything to do with all things geriatric. However, less trivially and less obviously, one might argue that an understanding of the basic biology of aging could function as a "touchstone" or integrative "hub" around which much of the science of geriatric neurology might eventually be organized. Central questions here could include: What is aging? What drives the progressive deterioration of the human organism over time? Why does it lead to what have been called the "diseases of aging?" These diseases would include not just classic neurodegenerative disorders (most paradigmatically, AD, but also Parkinson's disease (PD), frontotemporal dementias, and motor neuron diseases—all core clinical concerns for geriatric neurologists, neuropsychologists, and psychiatrists), but also coronary artery and cerebrovascular disease, other forms of age-related vascular disease, diabetes, cancers, macular degeneration and glaucoma, arthritis, failing immunocompetence, and perhaps many, if not most, forms of end-stage organ disease.

Additional central questions potentially addressed by the science of aging include the following: what can we

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[1] Only in organisms in which there is no real distinction between soma and germ line (such as hydra and most bacteria) is aging absent.

do about slowing aging and extending the lifespan or, for that matter, protecting ourselves from the diseases of aging? Exactly how does aging lead to the various diseases of aging, and what determines which disease of aging an individual gets? Does someone truly die just from "old age," or do we die of a disease of aging? What are the core biological processes responsible for aging? Are these a few biological processes or many dozens? What are the potential relationships (interactions) among various core processes implicated in aging? What is the relationship between aging in the brain and aging of the body in general? Can the brain be differentially protected from aging and age-related diseases? Would a slowing of aging itself potentially delimit the penetration by the diseases of aging in some or even all individuals? How radically? Is it possible to substantially slow aging, or perhaps even to arrest it? Even more radically, could aging ever be substantially reversed? Many of these questions do not have well-validated scientific answers yet. Most of these questions could be considered central biological questions for all the health-care disciplines and also questions around which there is now a rich and emerging, if still fundamentally young and incomplete, science of aging.

Implications of an aging demographic in Western societies for priorities in health care: prevention versus high-technology medicine

Unfortunately, very little of an emerging science of aging has trickled down into the health-care system and into the awareness of most health-care professionals, where a largely fragmented approach to the diseases of aging predominates theory, clinical research, and treatment. In addition, almost none of it seems to inform the way our health-care system currently works. Substantive prevention in relationship to the diseases of aging (let alone any concerted focus on potentially slowing aging) garners little substantive attention or meaningful share of fiscal resources; instead high-technology intervention, often aimed at an advanced disease of aging (at which little, if any, prevention was typically ever aimed), consumes an enormous fraction of medical resources and costs (Conrad, 2009). Recent estimates are that no more than 5% of health care is spent on prevention, broadly defined, whereas 75–85% is spent on an established illness, typically a disease of aging (Centers for Disease Control and Prevention (CDC), 2010). In 2010, at least $55 billion was spent on the last 2 months of life, and an enormous fraction of total medical costs was spent on end-of-life care (Social Security Advisory Board (SSAB), 2009), often with little evidence that this considerable expenditure improves the quality of life (and may even cause it to deteriorate, in some instances). If one were to extrapolate our current (average)

end-of-life care costs to the baby boomers (a demographic of roughly 60 million people), this could potentially yield a total price tag of about $6 trillion for end-of-life care for the baby boomer generation. Obviously, these trends are unsustainable, but there is little evidence of progress toward addressing, let alone reversing, them.

The emerging and expanding science of the biology of aging, as a vigorous area of scientific inquiry, takes place at a time when the demographics of Western societies are tilting toward an increasingly high percentage of elderly citizens. At the beginning of the twentieth century, when life expectancy was about 47 years in the United States, until today, there has been a roughly 30-year increase in life expectation at birth (Minino et al., 2002). Roughly 25 years of this 30-year gain in lifespan can be attributed to one primary factor: lessening the impact from early mortality due to infectious diseases in children and young adults, in the context of better hygiene and the creation of effective antibiotics and vaccines (CDC, 1999). This has yielded a situation in which many Western societies are now for the first time in human history facing the prospect of having more people over the age of 60 than under the age of 15. Although currently roughly 13% of the United States is over the age of 65, within the next 20 years, this percentage is expected to increase by more than half again, to roughly 20%. By the end of the century, a whole one-third of the world's population will be over the age of 60 (Lutz et al., 2008). These demographic shifts will centrally include a huge increase in the *very old* in the coming four decades. In 2010, more than an estimated 5.5 million Americans were 85 years or older; by the year 2050, that number is expected to almost quadruple to *19 million*. Currently, the number of centenarians in this country (Americans 100 years and older) is estimated at roughly 80,000, but by 2050, there will be more than *500,000* Americans aged 100 years or older. This is unprecedented in human history. However, these significant increases in lifespan have not been accompanied by concomitant increases in "healthspan," or in our ability to substantially prevent (or successfully treat and delimit) the disabling illnesses of later life, the major diseases of aging (centrally including diabetes, cardiovascular disease, stroke, AD, and cancers), which remain largely refractory to amelioration. Some evidence (summarized later in this chapter) argues that these diseases may be largely of Western civilization (primarily due to modern lifestyles) and relatively rare in elders from hunter gatherer (HG) societies, compared to Western societies, even when the younger mortality of HGs is taken into account (Eaton et al., 1988 a,b).

The impact of these large demographic shifts and the associated increased penetration of diseases of aging on health-care economics, combined with the increasing costs of technology-driven health-care interventions, is quietly anticipated to be fiscally catastrophic, involving a steady annual escalation of health-care costs to unsustainable levels (US Government Accountability Office, 2007; Conrad, 2009). The impact on health-care economics of an

aging demographic, combined with an increasing emphasis on high technology, is increasingly penetrant and, frankly, worrisome, particularly in terms of its impact on health-care economics in this country. In 2010, health-care expenditures in the United States were approximately 18% of the gross domestic product (GDP), almost twice as much, in terms of percentage of GDP, as in any other Western society. Even just within the next several years, at a current rate of increase of between 4% and 8% a year (rates of increase moderated more by the recent recession than by changing practice), by 2018–2019, roughly 20% ($1 in every $5) of the US GDP could be spent on health-care expenses, an unprecedented fraction of our national wealth and resources. The health-care expense as a proportion of GDP is projected (without substantive changes in practice trends or chronic illnesses) to rise to 28% in 2030 (more than $1 in every $4) and to 34% by 2040 (more than $1 in every $3; Council of Economic Advisers (CEA), 2009). These are frightening statistics, suggesting that the current rate of escalation in health-care expenditures is totally unsustainable. However, the demographic shifts toward an aging population are only one contributing factor in these accelerating expenditures and are paired with the escalating cost of first-line drugs and high-technology interventions and the high overhead associated with the burgeoning health-care and health-insurance bureaucracy itself (CEA, 2009). *Evidence suggests that as much as three-quarters of the increasing costs are due to factors other than an aging demographic* (CEA, 2009). Despite these enormous and escalating financial outlays in health care, the overall health may be actually declining in the United States, as measured by several indices. Currently, the United States rank around 50th in life expectancy, while other indices, such as infant mortality, are also worrisome and rank 46th, behind all of Western Europe and Canada (CIA Factbook).

Reflecting the major disease of aging with special relevance for this textbook, costs for AD in 2010 were roughly $170 billion in the United States alone (not counting an additional roughly $140 billion in unpaid caretaker costs, suggesting a real cost of over $300 billion in 2010 alone) (Alzheimer's Association, 2010).

These total costs of AD (assuming that current costs continue and no cure or highly effective treatment is found) are expected to potentially reach $2 trillion per year in the United States alone by 2050, with 65 million expected to suffer from the disease in 20 years worldwide, at a cost of many trillions of dollars (Olshansky et al., 2006). As the baby boomers enter the decades of greatest risk for cancers, heart disease, stroke, arthritis, AD, macular degeneration, and other diseases of aging, evidence indicates that the health-care system (as it is currently structured) will eventually undergo a slowly progressive but fundamental collapse in the context of these unsustainable cost escalations. Meaningful strategic options to prevent this fiscal implosion have not yet been developed.

In addition to its financial impact on health-care economics, aging in the Western societies is anticipated to have a more generalized and severely deleterious impact on Western economies, as an increasing percentage of retired elderly severely strain basic social safety net and entitlement programs such as Medicare and Social Security, deteriorate tax and revenue margins, and stretch virtually every societal resource (McKinsey Global Institute, 2008). In this context, scientific work on the biology of aging, particularly if it might reduce or substantially delay penetration by the diseases of aging into an aging population and extend "healthspan" (as distinct from lifespan), appears vitally relevant, if not badly needed. Despite these considerations, the funding of research into *all aspects of aging and age-related disease* garners only 11% of the $31 billion NIH budget (Freudenheim 2010), and research into CR, our only well-replicated lifestyle intervention to slow aging and reduce diseases of aging, garners less than 1/100th of 1% of all biomedical research monies (Guarente, 2003).

Historical and basic evolutionary perspectives on aging

Aging appears somehow woven into the very fabric of life itself; a still controversial question is whether this is accidental (in a sense, evolution did not worry much about aging, as postreproductive deterioration in a complex biological system is inevitable) or whether aging is selected (as nearly immortal organisms would destroy their environment and thus render themselves extinct). These may not be mutually exclusive perspectives. Aging is difficult to define and has no single pathognomonic biomarker, but to paraphrase a famous quote about obscenity, "You'll know it when you see it." Aging can be defined operationally as a progressive and time-dependent "loss of fitness" that begins to manifest itself after the organism attains its maximum reproductive competence (Vijg, 2009) but aging could also be seen as simply the change of the organism over time (Bowen and Atwood, 2004). Although this seems to conflate development with aging, it has other theoretical advantages (see discussion of endocrine dyscrasia). Aging consists of a composite of characteristic and often readily recognizable phenotypic changes and can be defined statistically as a point at which normal or expectable development shows an increasing probability of death from all-cause mortality (excepting traumatic injury, starvation, poisoning, or other accidental death) with increasing chronological age of the organism. Intrinsic to aging is that its characteristic phenotypic changes are progressive and affect virtually every aspect of physiology and every organ of the body, from the skin, to cardiac and muscle tissues, to the brain. Ontologically, aging may reflect "entropy's revenge," as fundamental aspects of life organization become increasingly disorganized,

presumably due to a complex composite of processes (Hayflick, 2007). Modern biological thought holds it axiomatic that purposeful genetic programs drive all biological processes occurring from the beginning of life to reproductive maturity. However, after reproductive competence is attained, current thinking is still divided on the question of whether aging is a continuation of some collection of genetic programs or whether it is the result of the accumulation of random, irreparable losses in cellular organization. Again, these may not be mutually exclusive.

References to aging abound in the earliest human cultures' writings and records, suggesting that humans have been keenly aware of aging for millennia. The Bible refers to aging and death as "the wages of sin," at best, a colorful metaphor and, of course, totally scientifically inadequate. However, a modern biology of aging suggests that the metaphor of aging as a "wage" is both appropriate and heuristic: aging may readily reflect the "wages" of growth, metabolism, and reproduction (excess junk proteins, OS, glycation of proteins, and damage to both mitochondrial and nuclear DNA) and also to the "wages" of organism defense and repair (also known as *inflammation*).

Additionally, one must accept evolutionary principles as fundamental here and grounding any discussion of biological phenomenon, suggesting that aging must, in a direct sense, reflect *a relative absence of selection against aging itself*. However, what this might mean is not clear. Initial evolutionary theories of aging hypothesized that aging was "programmed" to limit the population size (immortal organisms would destroy their environment and render themselves quickly extinct) and/or to accelerate an adaptive turnover of generations, thereby possibly enhancing adaptation to shifting environments. However, this argument has modest evidence for it, at best, as senescence typically contributes minimally to mortality in the wild (Kirkwood and Austad, 2000). Instead, mortality in wild populations (as opposed to that seen in protected populations) is mostly due to extrinsic factors, such as infection, predation, and starvation, and occurs mainly in younger animals (Charlesworth, 1994). As a general rule, many, if not most, wild animals simply do not live long enough to grow old, again due to these extrinsic factors and not to aging. In this sense, *natural selection has a limited opportunity to exert any direct influence over the processes of aging*. Even in species in which aging and senescence do make some contribution to mortality in the wild (for example, in larger mammals and some birds), any hypothetical "aging gene" would be clearly deleterious; thus, it is highly unlikely that it would be selected (Kirkwood and Austad, 2000).

Indeed, the relative rarity of aged animals in the wild is an important clue about how fundamental evolutionary processes relate to aging. With *extrinsic* factors being the primary causes of mortality, there is invariably a progressive weakening in the force of selection with increasing age (Kirkwood and Austad, 2000). By the time an animal in the wild reaches an age at which the percentage of a given population surviving has declined to very low levels, the force of selection is likely far too weakened (if not almost nonexistent, given the low probability of reproductive success in an aged animal) to effectively weed out the accumulation of genes with "late-acting" deleterious (in other words, pro-aging) effects. This constitutes a "selection gap" that allows any alleles with late deleterious *(pro-aging)* effects to accumulate over many generations, with little or no intrinsic "countermechanism" (referred to as the mutation accumulation theory of aging). A prediction emerging from this theory is that because the negative alleles are basically unselected mutations, there might be considerable heterogeneity in their distribution within a population of individuals. There is some evidence both for and against this (Kirkwood and Austad, 2000).

A substantial modification of this basic idea is found in the notion of aging as "antagonistic pleiotropy" (Williams, 1957), that evolution would favor genes that have good effects early in development (for example, genes promoting growth and fecundity) even if these genes had clearly bad effects at later stages of life. A critical and heuristic modification of this basic idea has been provided by Bowen and Atwood (2004), who suggest that alterations in the hypothalamic–pituitary–gonadal (HPG) axis, characterized by increasing gonadotropins and declining sex steroids create aging and by implication its diseases, a process which is "paradoxically" under the control of the very same hormonal systems that regulate growth and reproduction (see Section "Endocrine Dyscrasia"). In this sense, a small but reproductively significant benefit early in life derived from particular genes or alleles would easily outweigh (in terms of selection effect) later deleterious effects, even if those later effects guaranteed eventual senescence and death, especially if those genes promote growth and reproduction. Aging is thus not the "wages of sin" but the wages of growth, reproduction, and metabolism. Of course, this suggests that aging expresses intrinsic trade-offs, a theme also echoed in the widely quoted "disposable soma" theory of aging (Kirkwood, 1977) which suggests a balance of allocation of metabolic resources between somatic maintenance and reproduction. Effective maintenance of the organism is required only for as long as it might typically survive in the wild. For example, because roughly 90% of wild mice die in their first year of life, biological programming for metabolically expensive body maintenance programs beyond this age benefits only 10% of the total population, at most (Phelan and Austad, 1989). Given that a primary cause for early mortality in wild mice is excessive cold (Berry and Bronson, 1992), the disposable soma theory suggests that mice would not benefit from developing body maintenance and repair programs that would slow aging nearly as much as investing metabolic resources into thermogenesis and thermoregulatory mechanisms.

Thus, longevity may be determined in large part by the level of "extrinsic" mortality in the natural environmental niche (Kirkwood and Austad, 2000). If this level is high (life expectancy thus is quite short), there is little chance that the force of selection would create a high level of protracted and successful somatic maintenance; the more critical issue is making sure that organisms either reproduce quickly before extrinsic mortality takes its toll or have high fecundity and reproduction rates to ensure that early mortality for many members of a species does not eliminate reproduction for all members of a species (rendering them extinct). On the other hand, if "extrinsic" mortality is relatively low over long periods of time, selection effects might well direct greater resources toward building and maintaining a more durable organism, by modulating genes that might otherwise contribute to rapid aging. If this set of assumptions is correct, one would predict that, in organisms in relatively safe environments (those with low extrinsic mortality), aging will evolve to be more retarded, while it would be predicted to be more rapid in hazardous environments (slowed aging in these environments would make little difference to procreative success and species survival)—and these predictions are generally well supported (Kirkwood and Austad, 2000). Additionally, evolutionary developments that reduce extrinsic mortality (for example, wings or other adaptations to reduce vulnerability to predation, highly protective armor (such as shells), or large brains (enabling transition from prey species to top predator status) are linked to increased longevity (as seen in birds, turtles, and humans), although mechanisms for this increased longevity are still debated and remain to be conclusively outlined (see Bowen and Atwood, 2004).

However, disposable soma theory has been criticized (Blagosklonny, 2010b) as failing to account for many aspects of aging, most particularly the greater longevity of women and the role of specific genetic pathways (such as mammalian target of rapamycin (mTOR),–see later sections on mTOR) that may heavily modulate aging. Aging is increasingly thought to be not preprogrammed, but more likely the result of a relative absence of selection for "perfect" maintenance of the organism, past the period of reproductive competence. Another way of putting this is that aging is simply the "fading out of adaptation," after achieving the age of reproductive success and moving into the postreproductive age (Rose, 2009). In other words, there is no basis for evolution to have selected against aging and for much better body maintenance, as these issues would escape selection, unless there was a specific selection pressure toward this. An example of a basic selection pressure that could reduce aging significantly might be *progressively delayed reproduction* (procreating at slightly later and later ages), which has been shown in animal models to result in significant enhancement of longevity, in complete concert with basic evolutionary principles

(Teotônio et al., 2009). In animal models of aging, this is referred to as "experimental evolution" (Bennett, 2003). Intriguingly, experimental work with delayed reproduction has successfully developed longer lived species (for example, long-lived *Drosophila*, or fruit flies), but with the cost of depression of early life fecundity, suggesting again intrinsic trade-offs between slowed aging and growth and reproduction (Sgrò and Partridge, 1999). However, there is expert opinion (Johnson, Sinclair, and Guarente, 1999) that there could well be selection to *slow the pace of aging*, as such organisms could potentially have a more protracted period of reproductive fitness, conferring an adaptive advantage. Slower aging also appears intrinsically related to later age of reproductive fitness (Bowen and Atwood, 2004). Additionally, in hominid lines, evolutionary perspectives indicate that the existence of tribal elders, with greater accumulated wisdom and experience, would have improved evolutionary fitness for their tribal groups, despite being largely past a reproductive age, suggesting another potential selection mechanism driving "antiaging" ("group fitness" or "inclusive fitness" in highly social species such as hominids; Carey, 2003).

Basic cellular and molecular theories of aging probably come in two fundamental forms: (1) aging as a genetically modulated process (under the control of discrete genes and molecular pathways—but not "preprogrammed"); (2) aging as an "error" or stochastic or "wear-and-tear" process (the best known of these being the oxidative damage/stress theory). Neither "pure" type of theory is fully able to explain all aspects of aging, suggesting that aging is "quasiprogrammed" (Blagosklonny, 2009) and perhaps related to both growth programs (which are continued past the period of peak reproductive competence, as an example of antagonistic pleiotropy) and stochastic cellular damage/wear and tear aspects (such as emerging from disinhibited inflammation). CR, as the only conserved antiaging physiology yet discovered (see the later sections on CR and CR mimetics) may impact both of these (reducing growth programs and also attenuating factors such as OS and inflammation that may drive stochastic damage). Again, one has to assume that these issues do not contradict or replace a basic evolutionary perspective (in which aging reflects a relative absence of selection against wear and tear, stochastic damage, or failure of inhibition of many genes/pathways that might accelerate or drive age-related decline). Kirkwood and Austad (2000) summarize these considerations for an evolutionary genetics of aging as three basic predictions (p. 236).

1 Specific genes selected to promote ageing are unlikely to exist.

2 Aging is not programmed but results largely from accumulation of somatic damage, owing to limited investments in maintenance and repair. Longevity is thus regulated by genes controlling levels of activities such as DNA repair and antioxidant defense.

3 In addition, there may be adverse gene actions at older ages arising either from purely deleterious genes that escape the force of natural selection or from pleiotropic genes that trade benefit at an early age against harm at older ages.

Thus, aging could reflect the species-variable interactions and intrinsic "tug-of-war" between deleterious and degrading changes (and the declining influence of selection/adaptation in a postreproductive animal), with many of these pro-aging factors intrinsic to growth, reproduction, metabolism, inflammation, and other aspects of physiology ("antagonistic pleiotropy"), versus various (and presumably selected) counterbalanced repair, protection, and maintenance programs. Of course, if aging itself potentially deteriorates those counterbalanced cellular repair and maintenance programs, this suggests that aging is a losing tug-of-war between forces of cellular protection and forces of cellular degradation, and that (as the tug-of-war metaphor suggests), as one side loses, it may lose at an accelerating rate. There is indeed some evidence, although it is hardly conclusive, that aging may actually accelerate (Guarente, 2003). Few elderly would find this possibility surprising.

Cellular and molecular aspects of aging that might map onto these various considerations about the evolutionary basis for aging suggest a dizzying composite of phenotypic changes, including changes in mitochondrial, nuclear, and ribosomal DNA; subsequent genomic and chromatin changes and instability; increasing levels of OS (including pleiotropic and differential expression of OS on membranes and lipids, proteins, and nucleic acids, particularly mitochondrial); increasing systemic inflammation ("inflammaging"), paradoxically concomitant with declining immunocompetence; increasing glycation of proteins (and increasing amounts of advanced glycation end products (AGEs), which potentiate inflammation); increasing cellular senescence and loss of telomeres; dysregulation of apoptosis (programmed cell death is over- or under-recruited); and increasing junk proteins, combined with impaired protein turnover and declining removal of damaged (and glycated) proteins (declining "autophagy"). Last but certainly not least, even our stem cells age and reach senescence, preventing rejuvenation of many organ systems and structures. A clear sense of what are leading versus trailing edges in this process (in other words, clearly distinguished "causes" vs "effects") are still unclear and biology is clearly a place where causes become effects and effects become causes. However, there is evidence for each of these various aspects of cellular change as direct contributors to *all the manifestations of aging, including evidence linking virtually all of these processes ("phenotypes of aging") to <u>all</u> the diseases of aging*. Like many aspects of biological regulation, and indeed life itself, recursive interactions among these various processes may be essential; in other words, the many mechanisms of aging may be highly interactive, suggesting that there cannot be a single pathway into aging (see the discussion of the network of molecular pathways in CR effects), and that instead *aging probably reflects a complex and recursive network of (still incompletely understood) changes.* This is consistent with the severe limitations of all "linear causality" models in biological systems, where causality is intrinsically more recursive, circular, and multifactorial (Freeman, 2000). As critical examples of this principle of reciprocal interaction, inflammation and OS are increasingly linked and seen as mutually reinforcing (Jesmin et al., 2010), OS is thought to drive DNA damage (both mitochondrial and nuclear), glycation promotes inflammation, and declining removal of junk (including glycated) proteins may be related to increased OS (Kurz, Terman, and Brunk, 2007) and mitochondrial decline, while senescence promotes inflammation, as does endocrine decline, as does increasing junk protein while chronic inflammation and OS contribute to senescence. All of these phenotypes may thus be interlinked aspects of declining biological organization and increasing entropy, as basic phenotypes of aging with positive feedback loops between these phenotypes; new interactions seem to be emerging regularly in research into aging and its diseases. Such interaction may explain how processes involved in a modest departure from an ideal youthful physiology gives rise to a process that, over time, *deterministically kills the organism without exception*. Aging in other words may emerge from a deadly 'recursion matrix' of these interactive phenotypes. This is consistent with overwhelming evidence that nothing in biology truly emerges from single factors, but from the concerted crosstalk and feedback between multiple partners. At the same time, several molecular pathways (such as mTOR, and many molecular and cell-signaling pathways with which mTOR interacts) may be particularly critical to aging and the modulation of age-related change. At the end of this chapter, we also summarize evidence that lifestyle factors modulate risk for diseases of aging (and perhaps aging itself), possibly accelerating or retarding it at least to some degree. We also examine the difference between the current Western technological environment and our original evolutionary environment, in terms of the impact that multiple lifestyle variables may have on the cellular mechanisms and the physiology of aging and the diseases of aging.

Basic molecular and cellular perspectives on aging: phenotypes of aging

Although popular conceptions of the molecular basis of aging center around reactive oxygen species (ROS), hard evidence for this as the prime driver of aging is actually very mixed, and increasing evidence argues against it, as least as **the** central process driving aging. However, OS may interact with many of the other phenotypes of aging, particularly inflammation, as well as disinhibited growth factors/programs, suggesting that a softer form of OS theory (that ROS may *contribute to aging*) may still be valid.

Oxidative stress and associated mitochondrial perspectives

A basic assumption about aging is that it must have a fundamental cellular basis, and cellular and molecular perspectives on aging have dominated the scientific landscape of aging research and theory. The oldest and most widely quoted molecular theory about aging was provided by Harman, 1956, who postulated that oxidizing "free radicals" damaged and degraded cells over time, causing aging. Harman's early work on radiation with experimental animals demonstrated that aging had important similarities to the aftereffects of massive exposure to radiation, particularly cancer, inflammation, apoptosis, and other tissue changes not dissimilar to classic phenotypes of aging in older animals and humans. Harman's hypothesis emerged from his familiarity with work on radiation exposure and early findings that large doses of ionizing radiation generated enormous quantities of free radicals. Harman subsequently published what may be the first dietary antioxidant study (1957), studying the effects of dietary 2-mercaptoethylamine, the most potent radioprotective compound known at the time, and demonstrating a modest 20% increase in average lifespan, although the mechanism of action of this compound is still debated. In 1972, Harman published an important extension to the free radical theory, suggesting that the mitochondria were the primary source for OS, as well as the primary site for oxidative damage, and that the mitochondria therefore represented a kind of "biological clock" that he argued determined maximum lifespan. He concluded that his inability to extend maximum lifespan with dietary supplements must derive from the fact that most exogenous antioxidants do not get into the mitochondria. He hypothesized that OS in the mitochondria (vs its endogenous antioxidant defenses) set an outer limit on a given species longevity. Some work has suggested that OS is mostly generated by mitochondrial complex 1 (Mozaffari et al., 2011).

This led to a second "vicious circle hypothesis" about OS in relation to the mitochondria: that OS caused deterioration in mitochondrial antioxidant defense systems and mitochondrial function in general, leading to more OS and, in turn, driving more damage and increasing age-related deterioration. Although this is clearly the most widely quoted and accepted molecular theory of aging, particularly in the popular media and product advertising, the most comprehensive and wide-ranging review of this theory to date (Van Remmen, Lustgarten, and Muller, 2011) concludes that hard support for it is actually quite mixed. Therefore, the authors conclude that this theory remains unproven (but also not clearly falsified either), at least in the original "hard" form of the hypothesis (that OS in the mitochondria was *the* driver of aging. It has also been known for some time that OS markers increase with aging, although debate still rages about how much of this is cause or effect of aging (Sohal and Weindruch, 1996). There are many data points both for and against the oxidative-stress-in-the-mitochondria theory of aging, which might readily lead even the advanced student of aging to a sense of confusion and frustration. On the other hand, a softer form of the hypothesis—*that OS in the mitochondria may significantly contribute to aging—may be better supported, particularly in view of the interaction between ROS and other molecular pathways that clearly have been shown to contribute to aging, and to the diseases of aging, such as inflammatory signaling, and growth signaling* (see Blagosklonny, 2008) (see Section "Mammalian target of rapamycin").

Much experimental work to test the basic hypothesis has focused on genetic manipulations of antioxidant enzyme systems in short-lived species. Support for the hypothesis can be drawn from the results of knockouts of superoxide dismutase (SOD) 2 (Perez et al., 2009) and glutathione peroxidase 4 (Ran et al., 2007), both of which show lethal effects. Other primary data points in favor of the hypothesis emerge from work correlating species longevity with lowered rates of mitochondrial DNA mutation (Sanz et al., 2006) and with other experimental manipulations of OS and mitochondrial function (Hagen et al., 1999). Additionally, longer lived rodents (white-footed mouse (*Peromyscus leucopus*)) exhibit lower levels of ROS (superoxide and hydrogen peroxide), compared to the shorter lived house mouse (*Mus musculus*), and show higher cellular concentrations of some antioxidant enzymes (catalase and glutathione peroxidase) and lowered markers for protein oxidative damage (Sohal et al., 1993). Schriner et al. (2005) generated transgenic mice that overexpressed human catalase localized to peroxisome, nucleus, or mitochondria (MCAT). Median and maximum lifespans were maximally increased (averages of 5 months and 5.5 months, respectively) in the MCAT group. Cardiac pathology and cataract development were both delayed, markers for oxidative damage were reduced, peroxide production was attenuated, and mitochondrial DNA deletions (perhaps the most serious form of mitochondrial damage) were also reduced. These results offer strong support for the free radical theory of aging and also argue that the mitochondria are indeed the most biologically relevant source of these free radicals. In general, there is also broad, although occasionally inconsistent, correlation among OS in the mitochondria, rates of mitochondrial DNA damage, and longevity (Sanz et al., 2006; Barja and Herrero, 2000).

However, there is equally compelling data against this classic hypothesis. The naked mole rat (NMR) demonstrates an unusual phenotype of significantly delayed aging and the longest lifespan of any rodent (about 30 years), five times the expected lifespan based on body size, and exceptional cancer resistance, despite elevated markers for OS and short telomeres (Buffenstein et al., 2011). Additionally, the lack of a significant lifespan decrease or accelerated aging phenotypes in SOD $2^{-/+}$ mice (missing one copy of the gene), despite evidence for increased OS (Mansouri et al., 2006), and increased mitochondrial DNA damage (Osterod et al., 2001) are data points against this classic theory. Further complicating the picture is the evidence that although oxidation

of mitochondrial DNA is elevated in SOD $2^{-/+}$ mice, mitochondrial DNA deletions (thought to reflect the most serious form of mitochondrial DNA damage) are not increased (Lin et al., 2001). This suggests that this particular partial knockout model may not adequately probe the question of the relationship between mitochondrial OS and longevity.

Other animal models demonstrate that increased expression of the major antioxidant enzymes involved in protection from mitochondrial OS, including upregulation of the two isoforms of SOD (MnSOD and Cu/ZnSOD) and catalase, individually or in various combinations, does *not* extend maximum lifespan in mouse models (see Van Remmen, Lustgarten, and Muller, 2011 for detailed review). Mice with genetically reduced individual components of the antioxidant defense system have also been extensively studied, including knockouts of two isoforms of SOD (MnSOD and Cu/ZnSOD), glutathione peroxidases (Gpx-1, Gpx-2, and Gpx-4), catalase, thioredoxin, and peroxiredoxin. Complete ablation of individual components of antioxidant defense can often be embryonically lethal (specifically, homozygous knockout of thioredoxin 2, glutathione peroxidase 4, or MnSOD), but simply a loss of one allele (generating about 50% loss in activity) in heterozygous knockout mouse models ($SOD1^{+/-}$, $SOD2^{+/-}$, and $Gpx4^{+/-}$) does not result in reduced lifespan (Van Remmen, Lustgarten, and Muller, 2011). Lastly, recent work shows that combining a heterozygous knockout of MnSOD and homozygous glutathione peroxidase 1 knockout clearly results in increased OS, indexed through several classic markers (both protein carbonyls and oxidized nucleic acids), but not in a decrease in lifespan (Zhang et al., 2009).

At face value, such negative results might suggest that the "hard" form of the mitochondrial OS hypothesis (OS is *the* primary driver of aging and mortality) is not well supported. However, some very recent work argues that antioxidant defense in the mitochondria involves factors beyond these classic antioxidant enzyme systems and requires activation of one of the seven sirtuins (SIRT3), which promotes acetylation of antioxidant enzymes, significantly enhancing their effectiveness. Hafner et al. (2010) show that $SIRT3^{-/-}$ knockout mice show accelerated aging phenotypes, including classical mitochondrial swelling. Although earlier work on OS and CR emphasized the role of SIRT1 and its homologs (Sinclair, 2005), recent work has demonstrated that SIRT3 appears essential for CR-mediated reduction in OS (Qiu et al., 2010), as homonymous knockout of SIRT3 prevents the expected reduction of OS during CR. SIRT3 reduces OS by increasing activity of SOD2 through deacetylation (Tao et al., 2010; Qiu et al., 2010). In addition to regulating SOD2, SIRT3 reduces OS by modulating the activity of isocitrate dehydrogenase 2 (IDH2), a mitochondrial enzyme generating nicotinamide adenine dinucleotide phosphate (part of antioxidant defense in the MITO; Someya et al., 2010). Thus, there may be many players in the defense against OS in the MITO,

arguing that a comprehensive test of the OS hypothesis of aging may be challenging to design and that single or even combined manipulations of antioxidant enzyme systems may be insufficient to fully probe Harman's original and provocative idea. In general, however, there is increasing skepticism that the OS emerging from mitochondrial respiration is *the* driver of aging or any version of a sole "prime mover" in aging organisms. Additionally, many of the data points supporting a classic OS hypothesis can potentially be reinterpreted in light of evidence that ROS are a secondary driver for mTOR (Blagosklonny, 2008) (see Section "Mammalian target of rapamycin"); antioxidant interventions may therefore reduce overall drive or activation of mTOR (which may slow aging). Additionally, cellular senescence, another fundamental phenotype of aging, may be hinged to DNA damage detection (Chen et al., 2007), damage caused by ROS, suggesting that ROS concepts have to be seen not as operating in etiological isolation, but more as interactive with other phenotypes of aging.

A major practical challenge to test the basic hypotheses of OS perspectives on aging and also explore therapeutic implications of this idea has been the question of how to deliver antioxidants into the mitochondria (as the primary cellular nexus for OS vs antioxidant protection). Most organic compounds conventionally regarded as antioxidants (particularly the so-called "antioxidant" vitamins A, E, and C) do not get into the mitochondria in meaningful quantities, nor do others common in the diet, such as many polyphenols. Work by Skulachev et al. (2009) however, suggests that one can design molecules that do materially affect OS (SkQs, in this case, comprising plastoquinone, an antioxidant moiety, and a penetrating cation and a decane/pentane link). *In vitro* work indeed confirms that SkQ1 accumulates almost exclusively in mitochondria. In several species of varying phylogenetic complexity (the fungus *Podospora anserina*, the crustacean *Ceriodaphnia affinis*, *Drosophila*, and mice), SkQ1 prolonged lifespan, especially at the early and middle stages of aging. In mammals, SkQs inhibited development of age-related diseases and involutional markers (cataracts, retinopathy, glaucoma, balding, canities, osteoporosis, involution of the thymus, hypothermia, torpor, peroxidation of lipids and proteins). SkQ1 manifested "a strong therapeutic action on some already pronounced retinopathies, in particular, congenital retinal dysplasia." With eye drops containing 250 nM SkQ1, vision was restored to 67 of 89 animals (dogs, cats, and horses) that became blind because of a retinopathy. Moreover, SkQ1 pretreatment of rats significantly decreased hydrogen peroxide or ischemia-induced arrhythmia of the heart, reducing the damaged area in myocardial infarction or stroke and preventing the death of animals from kidney ischemia. In p53 ($-/-$) knockout mice, 5 nmol/kg/day of SkQ1 decreased ROS levels in spleen and inhibited lymphomas. Thus, such "designer antioxidants" show promise

in slowing aging and in both preventing and potentially treating diseases of aging. Intriguingly, of the many common dietary supplements regarded as "antioxidant" (see Section "Polyphenols"), only melatonin has evidence for consistent mitochondrial localization (Srinivasan et al., 2011), with some evidence suggesting that it may function as a significant mitochondrial protectant and regulator of MITO bioenergetic function.

Intriguingly, and underlining the intrinsic connections among the many biological phenotypes of aging, in recent years, the OS theory of aging has forged increasing connections to disinhibited inflammation and inflammatory signaling, with many positive feedback loops between the two processes, such that neatly separating these two processes is difficult (see Section "Inflammation"). Recent work on gene interactions (Jesmin et al., 2010) suggests that OS is perhaps the critical common denominator underpinning the intimate associations between obesity, type II diabetes, and hypertension, and that obesity itself may increase OS (Fernàndez-Sànchez et al., 2011). Evidence also indicates that cancers and AD are hinged to OS, suggesting that the long-term reduction of OS in aging may have significant health benefits and may offer protection against many diseases of aging, even if the hard form of the OS hypothesis (that ROS are *the* driver of aging) is unsupported. Further evidence for critical interactions among these various phenotypes of aging is suggested in the landmark study by Sahin et al. (2011) which shows that telomere dysfunction causes repression of mitochondrial biogenesis regulatory enzymes (PGC-1α/PGC-1β) through activation of p53, leading to increased OS and impaired mitochondrial biogenesis and bioenergetic function. Suggesting another dimension to these dynamic relationships among phenotypes of aging, recent work has suggested that telomere loss may be directly related to lifetime inflammation and OS burden, and that rate of telomere loss in leukocytes predicts cardiovascular mortality in men (Epel et al., 2009).

Inflammation

Increasing evidence argues that aging centrally involves changes in both innate and adaptive immunity (in the direction of declining adaptive immunity and compensatory upregulation of innate immunity), combined with increasing systemic inflammation, recently dubbed "inflammaging" (Franceschi et al., 2007), even in the absence of obvious pathological consequences or lesions. While traditional perspectives on inflammation emphasize acute and local inflammatory processes and the classic cardinal signs of localized inflammation (*rubor et tumor cum calore et dolore*—redness and swelling with heat and pain) involving many "acute phase" proteins, recent work on "inflammaging" emphasizes a different side of inflammation that is more systemic, chronic, and often (at least initially, if not over the long term) asymptomatic.

Of course, inflammation is also a highly adaptive and selected process, central to both organism defense and tissue repair; without it, we could not survive long at all, and it operates at virtually all levels of biological organization, from the small molecular level all the way to the level of behavioral organization (see Chapter 21, "Depression in the Elderly: Interactions with Aging, Stress, Chronic Pain, Inflammation, and Neurodegenerative Disorders"). Yet it is centrally implicated in many, if not virtually all, of the major diseases of aging, particularly atherosclerosis (see Section "Diseases of Aging with Relevance to Neurology"), AD, PD, most cancers, arthritis, and type II diabetes (see Finch, 2011 for a detailed review). This profoundly Janus-faced nature of inflammation may be one of the most striking examples of "antagonistic pleiotropy," suggesting that aging and its acceleration may be at least partially one of the "wages" of successful organism defense and tissue repair. From the perspective of aging and its diseases, the immune system may be simultaneously a best friend and a worst enemy.

Blood levels of proinflammatory cytokines (such as C-reactive protein and interleukin-6) are now widely understood to be primary risk factors for vascular disease and predictors of mortality/morbidity in cardiovascular events. Underlining intimate relationships between proinflammatory and anti-inflammatory signaling, the adaptive up-regulation of IL-6 due to exercise appears critical to the anti-inflammatory production of IL-10 (Walsh et al., 2011) and IL-1ra while inhibiting production of a cardinal proinflammatory cytokine, TNF-$\acute{\alpha}$. IL-6 was suggested to be a "myokine," defined as a cytokine that is produced and released by contracting skeletal muscle fibers; it is responsible for the anti-inflammatory effects of exercise, part of increasing evidence that systemic inflammatory signaling and "tone" are highly plastic and perhaps highly responsive to diet and lifestyle issues (see the last sections on lifestyle and dietary factors.). Indeed, many if not most important lifestyle variables appear to modulate systemic inflammatory tone directly, including classic dietary factors such as fiber consumption (Galland, 2010), omega-3 intake (Mittal et al., 2010), and polyphenol intake (Zhou et al., 2011); sleep quality versus sleep deprivation (Motivala, 2011); aerobic exercise (Walsh et al., 2011); and even social stress (social isolation vs social comfort; Slavich et al., 2010). This suggests that Western lifestyles (sedentary and with typical Western diet patterns) may be, *in toto*, *seriously* proinflammatory and may significantly increase the risk of the diseases of aging most related to chronic and systemic inflammation (many cancers, cardiovascular disease, AD and PD, diabetes, and arthritis).

Glycation, advanced glycation end products, and AGE receptors

Glycation of proteins is a fundamental mechanism in aging and in the deterioration of both organ structure

and function, and is probably neglected in many treatments of aging relative to its importance (Semba et al., 2010; Bengmark, 2007). Glycation appears implicated in almost every disease of aging, and not simply diabetes, with glycation as a primary contributing cause and not simply as a secondary effect. Additionally, AGEs interact with receptors (rAGE) to upregulate inflammation, another primary factor in the biology of aging (see Section "Inflammation"), potentially contributing to another critical dimension of aging. The creation of AGEs involves bonding two or more proteins, a process known as "cross-linking," typically by the creation of sugar–protein bonds. While some AGEs are relatively short lived and fluctuate in response to diet and metabolic state, other AGEs are long lived and virtually impossible for the body to break down. The creation and accumulation of these AGEs, particularly in essential tissues such as coronary arteries and the brain, can have serious effects on function and constitute a major risk factor for a disease of aging in those organs (Semba et al., 2010). For example, areas of arterial glycation are much more likely to eventually become regions of atherosclerosis and plaque accumulation, while glycation of CNS tissue is associated with increasing inflammation and the classic plaque and tangle pathology of AD (Srikanth et al., 2011; Lue et al., 2010), with AGEs a major facilitating cofactor in the creation of both amyloid oligomers and tangles (Gella and Durany, 2009). On the other hand, rAGE activation may also increase autophagy as a protective response, and may reduce apoptosis after oxidative injury (Kang et al., 2011), suggesting yet another layer of interactions between these phenotypes of aging (see Sections "Autophagy" and "Apoptosis").

Glycation of tendons and other connective tissue may form important foundations for loss of flexibility in aging. Obviously, diabetes provides a classic model for the acceleration of glycation and generates a more rapid accumulation of AGEs, with hemoglobin A1C a direct measure of glycation of hemoglobin molecules (an example of a relatively short-lived form of glycation). rAGE receptors are also implicated in AD as a channel for amyloid oligomers to enter cells where the oligomers potentially wreak havoc with multiple cellular compartments, particularly mitochondria and lysosomes (LeFerla, 2008). Glycation can be inhibited by AGE breakers, which includes the amino acid l-carnosine, and also blocked by multiple polyphenols particularly ellagic acid. Green tea extract (Babu et al., 2008), curcumin (Pari and Murugan, 2007), and many flavonoids (Urios et al., 2007) have shown at least some antiglycation functionality, along with alpha lipoic acid (Thirunavukkarasu et al., 2005). This suggests that a diet high in polyphenols and relatively low in free sugars might prevent or reduce long-term glycation of tissues (although this is never been proven in a human clinical assay to our knowledge).

Autophagy

Autophagy is an essential catabolic process through which existing proteins and other cellular components are degraded and recycled, supporting the adaptive function of removal and potential repair of damaged, dysfunctional, or even toxic proteins and cellular organelles. This function is dependent on "autophagosomes" (an intracytoplasmic vacuole containing elements of a cell's own cytoplasm), typically fused with lysosomes to facilitate the digestion of target proteins by lysosomal proteases. Autophagy, like glycation, is perhaps one of the more neglected critical storylines in aging in many popular treatments of the subject, and its importance in aging appears central. Indeed, it appears that aging can be slowed significantly by simply improving this critical process—or, alternatively, perhaps aging itself causes degradation of this process (Madeo et al., 2010). Antiaging effects from improved autophagy are robust (Petrovski and Das, 2010) and include lifespan extension. Severe dysfunction in the various autophagy pathways (typically caused by mutations) can correspondingly generate severe progeroid pathology, affecting multiple organ systems, including muscle, the liver, the immune system, and the brain. Defects in autophagy have shown accelerated aging phenotypes in classic yeast, worm, and fruit fly model organisms (primary models for aging in terms of unraveling its basic cellular and molecular mechanisms). In mammals, autophagy appears essential to life and survival, as genetic knock-out of proteins required for the process is lethal, suggesting a basic role in homeostasis and development. More limited knock-out of genes involved in autophagy in mice results in accelerated aging phenotypes. While the precise underlying mechanisms driving autophagy-related pathology remain obscure, the study of Finkel and colleagues (Wu et al., 2009) suggests that mitochondrial dysfunction is likely a critical factor. Underscoring important reciprocal relationships among the many phenotypes of aging, recent work suggests that disruption of autophagy may manifest itself physiologically in terms of mitochondrial dysfunction and increased OS (Wu et al., 2009).

Growing evidence links declining autophagy to all the neurodegenerative disorders, with their characteristic protein aggregations (often ubiquitinated, suggesting that they are being tagged for removal), although pathological changes can result from excessive or disinhibited as well as deficient autophagy (Cherra and Chu, 2008). Experimental animals genetically defective in autophagy develop neurodegeneration accompanied by ubiquitinated protein aggregates, demonstrating that basic autophagy function is essential for long-term neuronal health. Additionally, both age- and disease-associated (with AD) reductions in the autophagy regulatory protein beclin 1 have been found in patient brain samples (Cherra and Chu, 2008), while treatments that promote autophagy

have been shown to reduce levels of pathological proteins in several *in vivo* and *in vitro* models of neurodegeneration. Rapamycin, lithium, and several polyphenols have been shown to enhance degradation and also possibly reduce synthesis of proteins that may contribute to toxic oligomer formation, as well as larger extracellular aggregates of toxic protein seen in several neurodegenerative diseases. Quercetin, several other polyphenols, and vitamin D all appear to increase autophagy, suggesting important but incompletely mapped roles for diet and lifestyle in modulating this critical aging-related process (Wang et al., 2010b; Wu et al., 2011). These considerations suggest that many neurodegenerative disorders (which are all primary proteinopathies) may have future effective treatments based at least in part on the improvement of autophagy function.

Apoptosis

Apoptosis, originally thought to be a deleterious and primarily negative process, now is appreciated to have a critical role in adaptation and longevity. Apoptosis must balance regulation of the potential benefits of eliminating damaged cells against the pathogenic impact of more maladaptive forms of cell death (such as progressive cell loss in postmitotic tissues, a major mechanism driving atrophy in neurodegenerative disorders and contributing to end-stage organ disease in postmitotic tissues.). Thus, a delicate balance must be struck, and dysfunction in the regulation of programmed cell death can mean that, on one hand, apoptosis potentially contributes to atrophy and a senescent cell phenotype, while, on the other, its failure potentially leads to neoplastic cell proliferation. Apoptosis is thus an important cellular defense for maintaining both genetic stability and physiological function. An intriguing question is whether centenarians may be more or less prone to apoptosis and whether longevity may slightly favor an excessive trimming of still possibly viable cells over allowing an increased percentage of potentially rogue cells to survive–or the reverse (Monti et al., 2000). Additional data points underscoring the importance of a finely tuned apoptosis equation include that cells that avoid apoptosis, particularly proliferating vascular smooth muscle cells, participate centrally in atherosclerosis. Cancer could be thought of as the paradigmatic failure of apoptosis, and several lines of evidence suggest that cellular senescence and apoptosis (both of which contribute to aging) are primary defenses against cancer (Chen et al., 2007). On the other hand, accelerated apoptosis in postmitotic tissues such as the brain clearly contributes to virtually all neurodegenerative disorders. This suggests that adaptive regulation of apoptosis and its tuning and modulation may be highly protective in relation to the diseases of aging and, conversely, that disregulated apoptosis may contribute to both aging and the diseases of aging. Just as future modulators of autophagy may be treatments for neurodegenerative diseases, similar prospects may apply for regulators of apoptosis, although promotion of cancers and perhaps obesity also would be potential concerns. However, promoting apoptosis in senescent cells could be highly desirable and might slow aging significantly (see discussion in later section on Cellular Senescence).

Sarcopenia

Sarcopenia, the loss of both muscle mass and function, is a universal feature of aging that has a major impact on individual health and quality of life, predisposing people to falls and eventual frailty, also often neglected in treatments of aging and its phenotypes. Although the term *sarcopenia* was first coined in 1989, its etiology is still incompletely understood and its precise definition is still debated. It centrally includes losses in muscle fiber quantity and quality, alpha-motor neurons, protein synthesis, and several anabolic and sex hormones (Waters et al., 2010). Other factors may include altered basal metabolic rate, increased protein requirement, and chronic inflammation and OS. These changes lead to decreased overall physical functioning, increased frailty, falls risk, and, ultimately, the loss of independent living.

Sarcopenia is a critical aging phenotype. All elderly show evidence of it, particularly after the seventh decade, with a roughly 40% decline in muscle mass by the age of 80 (Evans, 1995). Mechanisms leading to this are multifactorial and include mitochondrial dysfunction and decline, altered apoptotic and autophagic processes, and even altered trace metal homeostasis (Marzetti et al., 2009). Like virtually every other aspect of aging, CR mitigates this process in a variety of species studied, again via pleiotropic effects of CR, including mitochondrial biogenesis, reduction of OS, and improved apoptotic regulation and autophagic processing. To our knowledge, reduction of sarcopenia has not been demonstrated in humans with CR mimetics.

Cellular senescence

No discussion of aging would be complete and without at least a basic review of cellular senescence, first discovered by Hayflick *in vitro* (Hayflick, 1965). Evidence argues that cellular senescence probably evolved as a defense against cancer and as a response to DNA damage and genomic instability (Chen et al., 2007), and has to be seen as sitting, like apoptosis, as a critical adaptive checkpoint on all cell cycling. In this important sense, the cell cycle, apoptosis, senescence and carcinogenesis have to be all seen as intimately related biological processes. Although cellular senescence is popularly understood mechanistically as driven by a simple loss of telomeres, evidence argues that like all other phenotypes of aging, its true derivation is complex and highly multifactorial, and additionally, that loss of telomeres is not simply due to the total number of replication events, as originally assumed by

Hayflick. Instead, evidence suggest many factors, particularly those related to chronic OS, chronic inflammation and even chronic emotional stress (perhaps as proxy for inflammation but perhaps reflecting other effects in addition to this) determine the rate of telomere loss, suggesting a critical role for lifestyle in protecting against loss of telomeres (Falus et al., 2010). Specifically, recent work has shown that cumulative inflammatory load, as indexed by the combination of high levels of IL-6 and TNF-α, is associated with increased odds for short telomere length in leukocytes (O'Donovan et. al., 2011). Emotional regulation may play an underappreciated role in protection of telomeres, and consistent with this, lifestyle interventions that reduce stress, such as mindfulness meditation, have even been shown to enhance both telomerase (Jacobs et al., 2011) and preserve telomeres (Epel et al., 2009).

Additionally, recent work makes a principled distinction between cellular quiescence (cell cycle arrest) and cellular senescence (Blagosklonny, 2011), with the former reversible, and paradoxically, with activation of the progrowth mTOR pathways increasing the likelihood of senescence, while inhibition of TOR saves cells from this biological "dead-end" and shifts them into quiescence. Thus, cell signaling pathways involved in aging also have a critical role as well, suggesting that conjoined activation of DNA-damage sensing systems such as p53 and p21 (which orchestrate blocks on cell cycling) and growth pathways simultaneously helps to select senescence. Additionally, and perhaps critically important in many clinical situations, senescent cells develop a large cell morphology and become hypersecretory in a proinflammatory direction. This is part of the evidence that aging is a kind of a dysregulated "hyper-functional" state, driven in part by disinhibited growth signals (mTOR acting as a central integrator of those signals). As Blagosklonny states, "cellular functions are tissue-specific: contraction for smooth muscle cells, secretion of lipoproteins for hepatocytes, aggregation for platelets, oxidative burst for neutrophils, bone resorption for osteoclasts and so on. These hyperfunctions lead to age-related diseases, such as atherosclerosis, hypertension, macular degeneration, increasing the probability of organismal death" (Blagosklonny, 2011. p 95). Thus, as Blagosklonny notes, senescence reflects a biological version of cells responding simultaneously to "pressing the gas pedal" (growth drive) and "getting on the brakes" at the same time (cell cycle blocks driven by DNA-damage sensing systems). Additionally, senescence both promotes inflammation and is promoted by it, further underscoring recursive relationships between these phenotypes of aging, and offering further evidence of the Janus-faced nature of inflammation, as an example of antagonistic pleiotropy (Blagosklonny, 2011; Figure 1.1).That removing senescent cells slows aging in a progeroid mouse model demonstrates that senescence is not simply an aging phenotype

(an effect or component of aging), but a driver of aging itself (Baker et al., 2011). This is consistent with much other evidence that *most if not all the phenotypes of aging reciprocally reinforce one another, consistent with a circular/recursive causality model of biological causation.*

Endocrine dyscrasia

It has been only in the last 10 years or so (since the seminal paper of Bowen and Atwood, 2004) that evidence has accumulated for a primary role in aging for changes in the hormonal-reproductive (HPG) axis potentially characterized as an "endocrine dyscrasia". Although many are aware of the more famous components of this dyscrasia (age-related declines in classic sex steroids with the decline in male testosterone more gradual but starting earlier than the steep menopausal decline of estrogen and progesterone in females), Bowen and Atwood have argued persuasively that the less appreciated upregulation of luteinizing hormone and follicle stimulating hormone from the pituitary and the associated increase in gonadotropin-releasing hormone (GnRH) from the hypothalamus to the pituitary (along with associated down regulation of inhibins and upregulation of activins—as peripheral modulators of HPG axis function) may play a central role in aging and its phenotypes. As Atwood and Bowen (2011) summarize, this theory is a clear extension of basic antagonistic pleiotropy concepts of aging: "hormones that regulate reproduction act in an antagonistic pleiotropic manner to control aging via cell cycle signaling—promoting growth and development early in life in order to achieve reproduction, but later in life, in a futile attempt to maintain reproduction, become dysregulated and drive senescence. Since reproduction is the most important function of an organism from the perspective of the survival of the species, if reproductive-cell cycle signaling factors determine the rate of growth, determine the rate of development, determine the rate of reproduction, and determine the rate of senescence, then by definition they determine the rate of aging and thus lifespan." (p.100). As support for the theory, HPG axis dysregulation may be a primary factor in AD, with elevation of luteinizing hormone and FSH, and decline of sex steroids as etiological, and as contributing to an exaggerated mitogenic signal that promotes beta-amyloid pathways, hyperphosphorylation of tau, synaptic retraction, and drives dysfunctional neurons into the cell cycle and from there into programmed cell death (Atwood et al., 2005; Casadesus et al., 2006). Challenges to this novel and heuristic theory of aging include relatively its undeveloped linkages to classic mTOR and insulin signaling pathways, as well as links to other classic aging phenotypes, such as mitochondrial decline, OS, and "inflammaging". However, recent updates (Atwood and Bowen, 2011) summarize data linking evidence for endocrine dyscrasia with multiple diseases of aging, suggesting that an endocrine dyscrasia
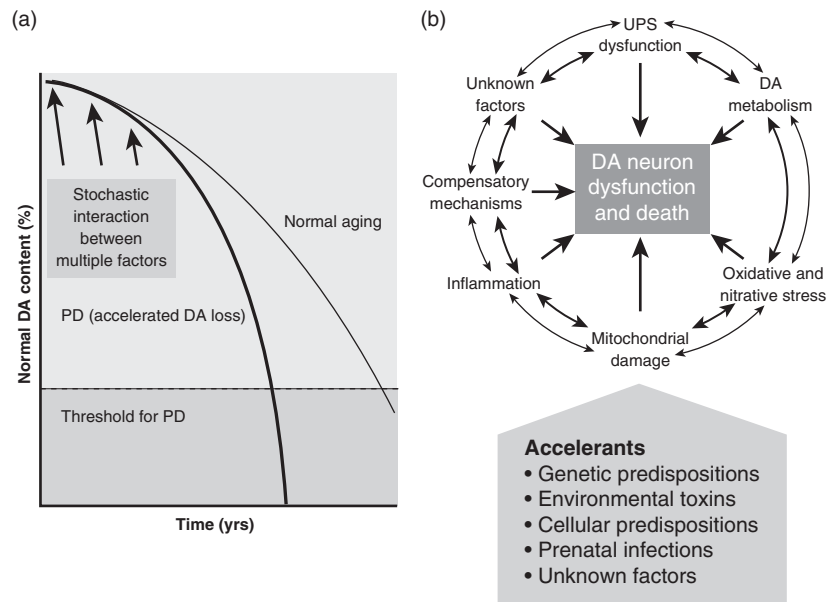
Figure 1.1 Cell cycle factors related to aging based on the stochastic acceleration hypothesis of Collier, Kanaan & Kordower (2011). A revised hypothesis of the relationship between aging and Parkinson's disease (PD) as they affect the biology of midbrain dopamine (DA) neurons. The hypothesis incorporates evidence that supports the involvement of common cellular mechanisms in dopamine neuron dysfunction in ageing and degeneration in Parkinson's disease. (a) The effects of these altered cellular mechanisms as they accumulate during normal ageing result in Parkinsonian dopamine neuron dysfunction, either very late in life or not at all (shown by the light gray line). However, when these same cellular mechanisms are accelerated by specific, individually determined factors, Parkinsonism emerges earlier in the lifespan (shown by the dark gray line). (b) The hypothesis contends that the cellular mechanisms that threaten dopamine neuron function are identical, but are not linked in an orderly cascade of cause and effect; instead, they can contribute to varying degrees and combine in patient-specific patterns, thus fulfilling the definition of a stochastic interaction: incorporating elements of randomness with directionality toward dopamine neuron dysfunction. Light gray double-ended arrows show cellular events in normal ageing. Thicker, dark gray double-ended arrows show accelerated cellular events in PD. UPS, ubiquitin-proteasome system. Similar mechanisms are implicated in cancer pathogenesis also. *Source:* Blagosklonny (2011). Reproduced with permission from US Administration on Aging.

may interdigitate with and generate reciprocal synergies with many other core phenotypes of aging mentioned in this chapter, particularly disinhibited particularly inflammation via promotion of TNF-α (Clark and Atwood, 2011). Novel approaches to antiaging therapies from this theory would centrally include efforts to normalize HPG axis function, not just through classic supplementation of sex steroids, but also intercepting other aspects of altered cell signaling, particularly overactivation of activins and an undersupply of inhibins, although these two latter manipulations are currently unavailable and represent highly appealing targets for future technologies.

The slowing of aging: dietary or calorie restriction and lifestyle interventions

Calorie restriction: evolutionary and animal models

Although the effects of CR on longevity were described more than 115 years ago (Jones, 1884), and its protection against the diseases of aging has been appreciated for almost a century (Rous, 1914), only more recently have we begin to unravel the molecular mechanisms by which CR extends lifespan and protects the organism from age-related change. CR functions as a kind of global metabolic reprogramming for virtually all organisms, extends lifespan, and reduces penetration of the diseases of aging significantly, if not dramatically, in most species in which it has been studied. Although the precise molecular pathways and cellular effects of CR are still being studied and debated, in general, it is viewed as a selected and phylogenetically conserved trade-off between reproductive fecundity and physiological conservation/preservation, and consistent with ideas in the previous section, results in a downregulation of the gonadotropic axis (Bowen and Atwood, 2004). A basic speculation has been that some version of a basic CR mechanism arose relatively early in evolution, during common periods of nutrient shortfalls, to allow organisms to trade off reproduction for conservation (when major energy shortages would have made reproductive efforts too metabolically costly), allowing an adaptive shift back to growth and reproduction at a time when nutritional supplies were more abundant.

Recent work has confirmed that CR effects are conserved virtually throughout the entire animal kingdom, starting with organisms as primitive as yeast and extending into insects and other invertebrates, lower vertebrates such as fish, mammals (Fernandes et al., 1976), primates (Lane et al., 2001; Roth et al., 2001), and even humans (Rochon et al., 2011), although long-term studies on CR effects in humans are still lacking. (Short-term studies clearly demonstrate that the basic physiology of CR is well conserved in humans, but life extension—confirming that aging is indeed slowed—has not yet been empirically confirmed. Most researchers, however, anticipate that this will be eventually demonstrated.)

CR/DR lacks a precise quantitative definition but might be considered to reflect a roughly 30% reduction in calories from eating freely until satiation (Richardson, 1985). CR effects for many species might begin at around a 25% to 30% reduction and extend to a 50% to 65% reduction, at which point CR transitions into starvation, a process that does not demonstrate any of the protective effects of CR and actively destroys global health. CR also requires that basic macro- and micronutrients be obtained (vitamins, minerals, fatty acids, and at least some protein). CR/DR is probably not a simple "homogeneous" issue, and can include differential restriction of proteins, carbohydrates, and fats, with these different forms of DR probably activating different cellular pathways involved in nutrient sensing and, therefore, having somewhat different physiological effects. However, protein and amino acid restriction clearly appears to be the more critical component, as protein restriction without CR elicits a significantly more robust profile of CR effects (Simpson and Raubenheimer, 2009) than the reverse (CR but without protein restriction; Kim et al., 2010a). Reasons for this may hinge on the importance of protein restriction for downregulation of mTOR, which is required for maximal CR benefits (see Section "Mammalian target of rapamycin").

Protein restriction may cause downregulation of growth factors and growth hormones (particularly GH, but also IGF), as well as provide downstream inhibition of TOR pathways (Figures 1.2, 1.3 and 1.4), improving autophagy and decreasing protein synthesis, among other effects, and may be particularly protective in relation to carcinogenesis (Anisimov et al., 2010); CR without protein restriction may not be nearly as protective in relation to cancers (Baur et al., 2006). Carbohydrate and glucose restriction, on the other hand, may more directly modulate insulin pathways and their several downstream targets. Intriguingly, evidence indicates that single amino acid restriction (specifically limiting dietary methionine or tryptophan) can yield CR effects (Caro et al., 2009), with subsequent reduced ROS in the mitochondria, lowered insulin and blood sugar levels, improved insulin sensitivity, and more (in other words, a CR physiology). This suggests an intriguing and perhaps less burdensome
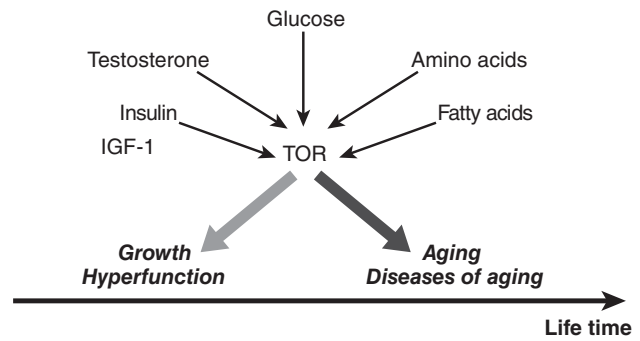

Figure 1.2 A simple schematic for the molecular pathway of mTOR as "antagonistic pleiotropy"–that, in some sense, aging is simply the flip side of a protracted growth process that is not sufficiently turned off after a peak reproductive period. *Source:* Blagosklonny (2009). Reproduced with permission from US Administration on Aging.

option to classic CR approaches, without at least some of the aversive effects of classic CR diets (foods high in methionine include eggs, fish, soy, and many seeds, especially sesame seeds). CR *without* protein restriction, on the other hand, may not produce lifespan extension, probably because of a blunting of the CR protective effects against carcinogenesis, as well as perhaps a more limited downregulation of IGF (and other growth factors) and lessened overall inhibition of mTOR (Anisimov et al., 2010; see the next sections on mTOR).
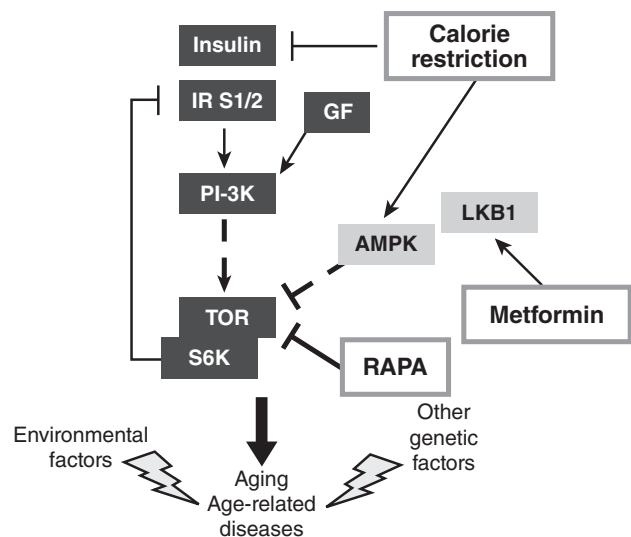

Figure 1.3 A simple schematic of some of the cellular pathways implicated in calorie restriction, aging, and the slowing of aging. Nutrients, growth factors (GF), and insulin activate the TOR pathway, which is involved in aging and age-related diseases. Other genetic factors and environmental factors (such as smoking, sedentary lifestyles, and obesity) contribute to age-related diseases. Several potential antiaging modalities (metformin, calorie restriction, and rapamycin and several polyphenols particularly resveratrol) all directly or indirectly (via impact on AMP kinase) inhibit the TOR pathway. *Source:* Blagosklonny (2009, 2010a). Reproduced with permission from US Administration on Aging.
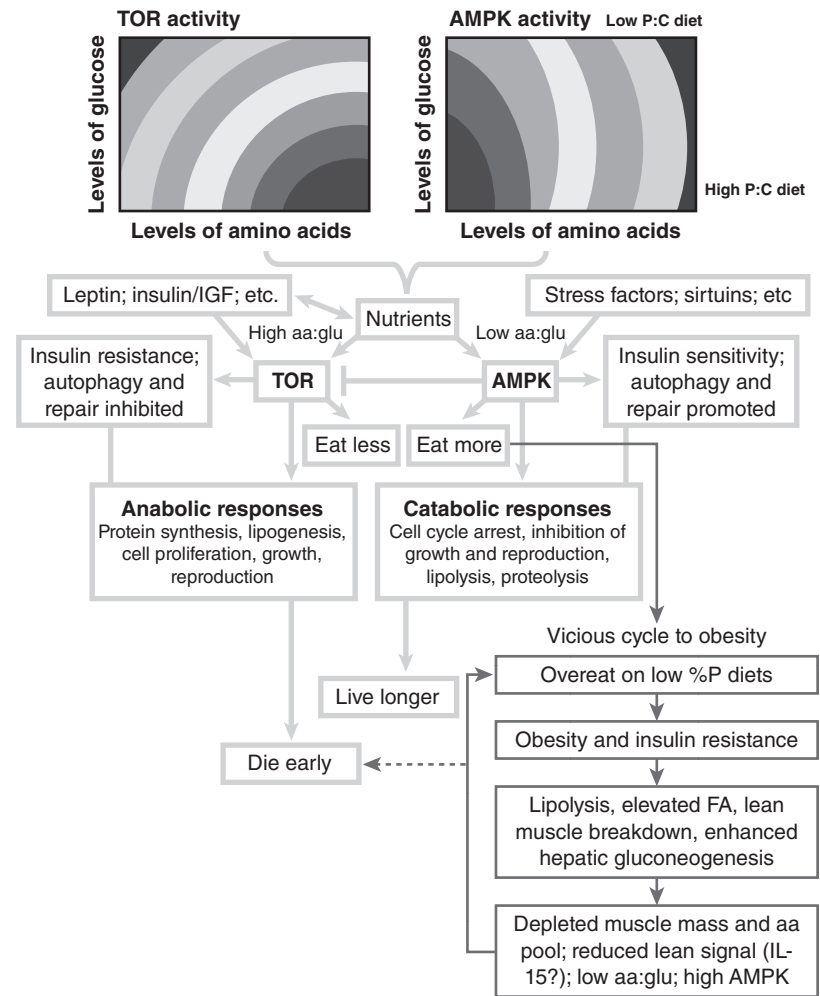
Figure 1.4 A schematic summarizing the hypothesis for how diet balance might affect lifespan via the TOR and AMPK signaling pathways. *Source:* Simpson and Raubenheimer (2009). Reproduced with permission from US Administration on Aging.

Calorie restriction: genes and pathways

Many genes and molecular pathways are implicated in CR effects, consistent with the previous discussion. Indeed, many researchers and theorists at this point believe that CR involves a whole family or network of interacting molecular pathways. These would include insulin signaling 1/2, IGF and other growth factors, PI3 kinase, AKT (protein kinase B), forkhead transcription factors, PGC1-ά, AMP kinase, sirtuins, and mTOR (Figures 1.3 and 1.4). This network of pathways argues against any version of a single primary pathway being responsible for CR effects, and suggests a highly pleiotropic phenotype, consistent with other evidence that adaptive growth processes must be, by necessity, sensitive to a host of signals (see Section Mammalian Target of Rapamycin). Thus CR as a protective and antiaging intervention, probably operates through a network of linked molecular pathways, where recursive interactions and relationships may be incompletely understood at present. Although a class of transcription factors called sirtuins, particularly SIRT1, were initially conceptualized as *the* critical regulators of CR effects (Sinclair, 2005),

recent work suggests that SIRT1 may operate on and influence some, but not all, of the CR network, while SIRT3 may also be critical as well. However, research suggests that CR (if it includes significant protein restriction) downregulates mTOR while also upregulating AMPK (Baur, 2006), up-regulates several sirtuins (Sinclair 2005), promotes mitochondrial biogenesis, and significantly reduces inflammation (Figures 1.3 and 1.4). Effects from inhibition of TOR are increasingly thought to be critical to mediating lifespan extension and slowing the aging process with DR. As a result, this TOR pathway has supplanted the sirtuins as the most studied and most intriguing cell-signaling group of pathways in aging (and antiaging) science. As such, it merits a detailed overview.

Mammalian Target of Rapamycin

Target of rapamycin (TOR) belongs to a highly conserved group of kinases from the PIKK (phosphatidylinositol) family, increasingly conceptualized as core and essential integrators of growth signaling. Knockout of mTOR is consistently embryonically lethal across several species,

suggesting a strong antagonistic pleiotropy affect for this particular gene (Blagosklonny, 2010a). Rapamycin, an immunosuppressive macrolide, was first discovered as the product of a soil bacteria from Easter Island. It directly and potently inhibits the activity of TOR (TOR complex 1 (TORC1), but not until recently did we understand that it also impacts TOR complex 2 (TORC2)). TOR was first identified in yeast but subsequently has been found to exist in all eukaryotic organisms. TORC1 (rapamycin sensitive) is thought to be the central element of the TOR signaling network, monitoring and integrating a large set of intra- and extracellular processes and controlling growth, proliferation, and lifespan with a host of complex downstream effects (Kapahi et al., 2010). TORC2 is also rapamycin sensitive, but contributes to the full activation of AKT, an upstream and critical signaler of TORC1; it also mediates spatial control of cell growth by regulating the actin cytoskeleton (Hall, 2008) and disruption of TORC2 by rapalogs appears to drive the "paradoxical" insulin resistance seen in chronic administration (Lamming et al., 2012). TOR plays a highly conserved and central role in coupling nutrient sensing to growth signals, integrating signals from wnt-β-catenin signaling pathway (growth factors involved in stem cell differentiation and regulation), glucose and lipid availability (signaled by AMP kinase), protein and amino acids deficiency or availability (growth resources), signals from multiple other growth factors and hormones, and even oxygen availability and hypoxia signals *to dynamically determine the envelope of growth versus conservation signaling in the cell*. TORC1 is thus thought to act as a growth "checkpoint" and signal integrator, determining whether the extra- and intra cellular milieu is favorable to growth and, if not, producing effects consistent with a CR phenotype. TORC1 has many output targets, altered in either CR or CR mimetic effects from rapamycin, including messenger RNA translation (inhibited in CR), autophagy (increased in CR), transcription and ribosome biogenesis (inhibited in CR), proliferation and growth (inhibited in CR), and several other key cellular processes, including stress resistance (increased by CR); for a fine technical review of TOR research, see Kapahi et al. (2010). Inhibition of mTOR by rapamycin has been shown experimentally to increase lifespan, even when given to mice in late middle age (Harrison et al., 2009). This finding suggests that rapamycin is a more powerful CR mimetic than resveratrol, which has failed to extend lifespan outside of obese animals (Baur et al., 2006; Miller et al., 2011). On the basis of age at 90% mortality, rapamycin led to increased lifespan of 14% for females and 9% for males. Intriguingly, patterns of mortality and disease in rapamycin-treated mice did not differ from those of control mice, suggesting that treatment with rapamycin globally delays aging and age-related disease in a nonspecific and fairly "even" fashion (Harrison et al., 2009),

arguing for at least some involvement of mTOR in virtually all age-related disease that might cause or contribute to mortality (at least in mice). Inhibition of TOR's major downstream targets, such as S6K, a kinase involved in ribosome biogenesis, appears to be important to the protective (antiaging) effects of TOR inhibition, and a knockout of this gene (S6K) also increases lifespan in mice and, intriguingly, generates activation of AMP kinase; this suggests dynamic relationships between mTOR and AMP kinase (Selman et al., 2009) that are probably incompletely mapped at this time (as two core primary mediators of CR/DR effects).

Figure 1.4 (from Simpson and Raubenheimer, 2009) schematically summarizes relationships between AMP kinase and mTOR. These two kinases are increasingly viewed as possibly integrating much of CR physiology, with an upregulation of AMP kinase and a downregulation of mTOR potentially orchestrating the entire range of CR effects through their conjoint activity. These two kinases are differentially involved in nutrient sensing, with TOR activated by high amino acid/glucose ratios (in other words, plenty of amino acids and proteins to build new tissue, thus releasing a "go" signal to anabolic processes and growth) and AMP kinase activated by low amino acid/glucose ratios. Thus, protein/carbohydrate dietary ratio may influence differential activation/inhibition of TOR and of AMP kinase (and these two integrators of CR physiology are also interactive, with AMP kinase inhibiting mTOR). These differential nutrient-sensing systems may help explain why CR without at least some protein restriction may not be as effective as a general antiaging strategy (Blagosklonny, 2010a, 2010b), particularly in relation to the prevention of cancers, because such a diet does not maximally downregulate mTOR. Additionally, Figure 1.4 may help explain why resveratrol by itself (a primary activator of AMP kinase, but not a primary or direct inhibitor of mTOR) does not produce a lifespan extension in animal models (outside of obesity) because it does not inhibit mTOR sufficiently.

Calorie-restriction mimetics

Given the intrinsically stressful and unpleasant nature of basic CR approaches (for example, CR animals typically cannot be housed together because they are too irritable and will fight), most believe that CR is simply not a viable health-maintenance strategy for most people. If anything, the recent pandemic of obesity has underlined that most individuals, when given ready access to tasty and addicting high-calorie-density foods, are simply not going to restrict their calorie intake voluntarily, regardless of the well-known and widely appreciated negative consequences. This has led to increasing interest in CR mimetics, defined as any substance that potentially mimics the molecular effects and physiology of CR (without the stress of making a person hungry much of the time). There are probably many substances that cause

mild nausea, visceral upset, or other GI distress and that subsequently inhibit food intake, but although these can show CR effects in sustained administration in animal models, they cannot be considered CR mimetics. Additionally, drugs that may directly modulate appetite (such as Rimonabant, an endocannabinoid-1 receptor blocker) might also show CR effects in sustained administration by modulating consumption and hunger drive at central levels, but they also cannot be considered true CR mimetics. One emerging prediction might be that CR mimetics will occupy an increasingly central role in primary prevention in relation to the diseases of aging in the coming decades, but an enormous amount of basic research remains to be done before widespread implementation of CR mimetics would be advisable or feasible; long-term data in both preclinical and clinical populations also is lacking (although data collection and trials of CR mimetics are underway in relation to many diseases of aging).

There are actually a number of CR mimetics with accumulating research supporting CR effects, but the most famous of these is clearly resveratrol, a molecule that has received enormous research attention in the last 15 years. In addition, metformin is a true CR mimetic (a drug commonly used to treat type II diabetes and rarely categorized in conventional medical literature as a CR mimetic) and 2-deoxyglucose are CR mimetics (2-deoxyglucose was actually the first described CR mimetic and interferes with glycolysis, preventing glucose utilization by cells even when abundant glucose is available, but it is cardiotoxic in chronic administration). Fisetin, derived from Fustet shrubs, is a flavonoid polyphenol that has also demonstrated CR mimetic effects. Rapamycin (as a primary inhibitor of TOR) is also a potent CR mimetic; to date, only rapamycin has demonstrated lifespan extension when given to older mammals (many CR mimetics have demonstrated lifespan extension in other target species, such as yeast, fruit flies, fish, and worms). Other polyphenols (a very large group of compounds found in fruits and vegetables, totaling perhaps as many as 6000 substances) may have mild CR effects, particularly quercetin, resveratrol, and its first cousin, pterostilbene (Belinha et al., 2007). However, single-polyphenol regimens, particularly resveratrol, have not shown lifespan extension (Pearson et al., 2008)[2], except in obese animals (protecting mice from premature mortality and the undesirable physiology of obesity (Baur et al., 2006) or cases in which resveratrol was combined with every-other-day dieting (EOD) as a

mild CR alternative (also demonstrated in a mouse model in Pearson et al., 2008). Although resveratrol was initially assumed to have its protective effects through SIRT1 activation, recent work has clarified that AMP kinase is probably the necessary and sufficient target for the protective effects of resveratrol (Um et al., 2010). Recent work has suggested that pterostilbene may be a more effective CR mimetic, with better bioavailability than resveratrol, and also a better activator of PPAR-α (Rimando et al., 2005), with more beneficial effects on lipid profiles, while still showing extraordinarily low toxicity (Ruiz et al., 2009).

Evidence suggest that resveratrol and its analogs, like pterostilbene (along with metformin and quercetin, two other CR mimetics), are probably only *partial CR mimetics*; even moderately high-dose resveratrol (20–30 mg per kilogram) does not appear to protect mice against late-life cancers (particularly a form of virally induced lymphoma, a very common cause of death in aged laboratory mice; Pearson et al., 2008) and does not extend lifespan outside of obese animals. Intriguingly, a nutraceutical combination of resveratrol and quercetin appeared to provide better mimicking of CR physiology than resveratrol alone (Barger et al., 2008; although longevity was not indexed specifically). This suggests that combinations of partial CR agents may get us closer to mimicking a full CR physiology than a single compound particularly a combination of rapamycin and an AMPK modulator such as resveratrol or metformin -- a logical combination that has yet to be tested, and where AMPK modulation might help reduce the insulin resistance seen on chronic administration of rapalogs (associated with its TORC2 disruption).

These considerations (Figure 1.4, by Simpson and Raubenheimer 2009) suggest that a complete or ideal CR mimetic might both activate AMP kinase and directly inhibit mTOR (not simply indirectly through increased AMPK activity), without toxicities or major side effects, a design target that no single known compound at this time yet achieves. Inhibition of mTOR (via rapamycin) has shown promising protection against diseases of aging in mammalian animal models (Stanfel et al., 2009). Perhaps a combination of low-dose rapamycin and resveratrol or pterostilbene might achieve the desirable targets of mTOR inhibition and AMPK activation, and thus function as a full CR/DR mimetic. To prove this in a mouse model, one would have to show further protective benefits from those achieved with rapamycin alone if resveratrol or pterostilbene were added in late middle age. This intriguing hypothesis has never been probed or tested even in a mammalian animal model. Full testing of these ideas in humans appears even further away, underlining an enormous gap between research promise and clinical reality in this vital area of biological science. Given the potential impact that a full, robust, and safe CR mimetic could have on aging and the diseases of aging (particularly the potential extension of "healthspan"), there is remarkably little

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[2] Given that AMP kinase inhibits mTOR, resveratrol might have some modest indirect effects on this critical pathway. Studies on resveratrol reviewed in later sections (see the section on CR mimetics) suggest that mTOR inhibition is likely to be modest, given the absence of lifespan extension in mammalian animal models, outside of obese animals, where its AMPK promotion may be protective and promote similar aging trajectories to non-obese animals.

research into this area, relative to its potential biological promise. Indeed, conventional medicine still sees CR/DR and CR mimetics largely as biological "fringe" subjects, instead of appreciating their potentially enormous protective functions and central and paradigmatic insights. Large pharmaceutical firms have just recently begun to pay more attention to this area of CR and its mimetics (see the recent GSK acquisition of Sirtris, www.gsk.com/ media/pressreleases/2008/2008_us_pressrelease_10038 .htm).

Calorie-restriction variants and mutants

There are many ways to generate CR effects, beyond classic CR approaches. One of the most basic of these is simply intermittent fasting (which may not result in nearly as much weight loss as full CR but still activates a CR physiology), along with methionine restriction (as noted earlier). In addition, there is manipulation of growth hormone (such as growth hormone knockout) and IGF-1 and insulin signaling manipulations (consistent with overwhelming evidence that insulin-signaling pathways are primary targets for CR effects; Figures 1.3 and 1.4). A dwarf mouse implementing a growth hormone knockout shows a roughly 60% life extension (and won a recent Methuselah prize; Bartke and Brown-Borg, 2004). This animal showed reduced hepatic synthesis of IGF-1, reduced secretion of insulin, increased sensitivity to insulin actions, reduced plasma glucose, reduced generation of ROS markers, upregulated antioxidant defenses, increased resistance to OS, and reduced oxidative damage, all quite consistent with CR physiology. Probably many dozens of genes can be modified to yield some variation of a CR physiology and at least some increase in longevity (and therefore slowing of aging), consistent with the evidence that CR/DR activates a complex and highly interactive network of cell signaling and regulatory pathways (Yuan et al., 2011; Lorenz et al., 2009).

Lifestyle and dietary factors

There is increasing, if not collectively convincing, evidence that core lifestyle factors such as exercise and diet (as well as sleep quality and social stress vs social comfort) potentially influence many aspects of aging, thus constituting a complex collection of negative and positive risk factors for all the diseases of aging. This collection of lifestyle variables are also presumably interactive with a small group of known polymorphisms and a likely much larger group of unmapped polymorphisms that collectively may have a large effect on longevity (Yashin et al., 2010) and risk for specific diseases of aging. Future mapping of those polymorphisms (and their likely complex interactions with lifestyle variables) may allow much better prediction of risk, and eventually allow for more effective and tailored early interventions, to reduce specific

risk for a particular disease of aging. As but a small example of these issues, IL-10 endowment may affect risk for AD. Although a good night's sleep, a healthy and more balanced diet, regular aerobic exercise, and social support are generally regarded as having nothing to do with each other biologically, recent work in relationship to all of these lifestyle factors suggests that they impact a broad but fundamentally shared set of cellular and molecular pathways. These shared effect pathways include multiple if not most aspects of cell signaling (internal cellular regulation): regulation of cell cycling, regulation of inflammatory, stress, defense and growth pathways, including mTOR. Although our understanding of diet, exercise versus sedentary lifestyle, sleep, and stress versus social comfort is still evolving, evidence suggests that basic lifestyle factors either promote or inhibit inflammation, protect insulin sensitivity versus generating insulin resistance, and create more OS versus protect against it, while promoting (or inhibiting) autophagy, cellular senescence, and apoptosis in aging, thus modulating virtually every known phenotype of aging. Additionally and rarely appreciated within traditional medicine, *all the individual components of so-called healthy lifestyle practices appear to be part of our ancient evolutionary environment and reflect HG lifestyle characteristics*. This suggests the possibility of a version of a "unified field theory" in relationship to long-term health versus chronic disease, and that healthy living may reduce complex and still poorly understood "mismatches" between our genome and our current biological environment in Western societies. In general, such ideas have little current visibility within conventional medical circles (although a reprioritizing of prevention is now being widely emphasized), but a nascent awareness of these more global biological perspectives on health versus chronic disease is slowly emerging, energized by increasing research into lifestyle and its complex biological impact.

Exercise

Regular aerobic exercise is widely recognized as an essential component of a healthy lifestyle, yet fewer than 15% of individuals living in the United States engage in adequate amounts of aerobic exercise; a majority of people in the United States are almost completely sedentary (Roberts and Barnard, 2005). Sedentary lifestyles are thought to contribute to risk for all diseases of aging, particularly cardiovascular disease, metabolic syndrome, and type II diabetes, especially when combined with a Western diet. Exercise has an extremely complex biological footprint, but among its many effects, exercise offers protection against all-cause mortality, particularly against atherosclerosis, DMII, and several but perhaps not all cancers, notably colon and breast cancer. It also significantly reduces frailty and sarcopenia. Regular exercise appears specifically protective against diseases associated

with chronic low-grade systemic inflammation (Peterson and Peterson, 2005), perhaps due the anti-inflammatory response elicited by an acute bout of exercise, largely mediated by muscle-derived IL-6. IL-6 stimulates production of anti-inflammatory cytokines (such as IL-1ra and IL-10) and inhibits subsequent (postexercise) production of the key proinflammatory cytokine TNF-α. In addition, IL-6 stimulates lipolysis and fat oxidation and metabolism (see Peterson and Peterson, 2005 for a detailed review). These anti-inflammatory effects also inhibit insulin resistance, which is partly modulated by TNF-α and by NFκ-B/AP-1, transcription factors centrally involved in inflammatory signaling.

Exercise may also upregulate antioxidant defenses (Kaliman et al., 2011), while OS actually initially increases during a bout of exercise, with subsequent upregulation of endogenous defenses (referred to as mitochondrial hormesis or "mitohormesis"). Some work on the effects of exercise calls into question the conventional wisdom of blocking OS, as evidence suggests that this actually impairs exercise benefit and even may prevent beneficial effects of CR (Ristow and Schmeisser, 2011). Exercise may also increase neurotrophins, improve stress resistance, improve mood, increase emotional and stress resilience, and enhance cognitive function and learning (Ratey, 2009), and consistent with these effects, at least some preventative/protective effects against most neurodegenerative disorders, particularly AD, have also been demonstrated.

Obesity

One of the most worrisome public health trends over the last 20 years has been a steady and dramatic increase in the prevalence of overweight and obese individuals. Current statistics suggest that roughly one-third of the United States is obese (with a body mass index (BMI), greater than 30), with another one-third of the population overweight (BMI over 25 but less than 30; Wang et al., 2007). Additionally some evidence suggests that the rate of obesity is still increasing despite much attention to this public health issue, and may reach 50% penetration in the United States by 2025. Equally worrisome is the emerging evidence that the rates of obesity in the United States are actually higher in children than in adults, perhaps due to a highly undesirable combination of increasingly sedentary gameplay (in which video games have largely supplanted more physical activity), increasing fast food consumption, and overconsumption of sugary beverages. Obesity is increasingly appreciated as a risk factor for virtually every disease of aging, beyond its popular link to risk for cardiovascular disease. Obesity contributes significantly to risk for hypertension, dyslipidemia, insulin resistance and type II diabetes, multiple cancers, and even AD. Evidence suggests that increased abdominal fat (vs subcutaneous fat) is a more significant risk factor than generalized obesity, and this relationship is potentiated,

curiously enough, in otherwise leaner subjects (Pischon et al., 2008), as abdominal fat may have a particularly potent effect on dysregulation of inflammation (Fontana et al., 2007) via promotion of proinflammatory cytokines. Aging itself decreases subcutaneous fat while increasing abdominal fat, and simply reducing abdominal fat surgically has a prolongevity effect in animal models. Increased visceral fat is independently associated with all-cause mortality, insulin resistance and diabetes, cardiovascular disease, cerebrovascular disease, AD, and disability in the elderly (Florido et al., 2011). Additionally, there is evidence for intrinsic relationships between obesity and upregulated inflammation (in part as compensatory and a way of using more energy) and, on the other hand, CR and reduced inflammation (Ye and Keller, 2010).

Polyphenols

Although conventionally regarded as "antioxidants", polyphenols are an enormous class of substances (constituting perhaps as many as 6000 distinct compounds) found in plants, principally fruits and vegetables, that have enormously pleiotropic effects on human and mammalian physiology. Some of these effects may be more biologically significant than any direct "free radical scavenging" done by any polyphenol; they include many effects on cell signaling, the regulation of growth factors and apoptosis, the regulation of cell cycling, the regulation of inflammation, the modulation of many (if not most) cellular stress pathways, an impact on multiple transcription factors (including those involved in energy homeostasis), and (consistent with their conventional designation) the management of OS (Virgili and Marino, 2008). Many of these effects on aspects of cell signaling require much lower levels of polyphenol than any direct free radical scavenging in serum or tissues. Indeed, from this perspective, polyphenols look less like "antioxidants" and more like complex cell physiology and cell signaling modulators. However, it seems unlikely that such a designation will replace the catchy title of "antioxidant," even in the context of increasing evidence that such a title may be fundamentally if not profoundly misleading. Many, if not most, of the phenotypes of aging (OS, mitochondrial dysfunction, inflammation, and declining autophagy, among others) appear to be partially modulated by various polyphenols. From this perspective, if our ancestors consumed more plants than we do and did so over tens of thousands of years (if not much longer), the relative removal of polyphenols from the human diet (in those eating minimal fruits and vegetables) would be predicted to have complex but potentially profound effects on physiology and on the biological trajectories of aging. Conversely, those eating a rich variety of plants may be more protected against accelerated aging and the diseases

of aging. Of these two predictions, the second has been better studied, and is generally supported, while the first has some evidence for it as well, but is not well elucidated.

Polyphenols consist of several classes of chemical substances, including nonflavonoid compounds (such as resveratrol, other stilbenes, and curcuminoids), and classic flavonoids (consisting of two large classes, anthocyanins, which are colorful and pigmented, and anthoxanthins, which are colorless). Resveratrol and its first cousin, pterostilbene, are both naturally occurring phytoalexins produced by plants in response to fungal infection (phytoalexins are all "plant defense" compounds). Of the anthoxanthin family, quercetin is one of the best-known and best-studied members, along with EGCG (a member of the catechins family, with catechins constituting a large group of polyphenols in tea and wine). Dietary sources for polyphenols include many foods that have been ancient components of the human diet for many hundreds and even thousands of years: fruits and their juices (typically containing both anthocyanins and anthoxanthins), tea (catechins), coffee (chlorogenic, caffeic and ferulic acids), red wine (anthocyanins, resveratrol, and quercetin), vegetables (many anthoxanthins and anthocyanins), some cereals, chocolate (multiple flavonoids, including catechins and proanthocyanidins), and various legumes, particularly soy (isoflavones) and peanuts.

In this context, there are multiple challenges to any emerging science that might explain the roles polyphenols could play in health maintenance and the slowing of at least some aspects of aging and/or age-related disease. First, there are many thousands of different bioflavonoids *in toto*, but only a handful with much *in vivo* research (resveratrol, curcumin, green tea extract, and quercetin are perhaps best studied). Most of the studies of polyphenols use *in vitro* approaches; although there are increasing numbers of *in vivo* studies in animal models, very few clinical studies have taken place in humans. As an additional major challenge to potential therapeutic use, virtually all bioflavonoids have relatively poor bioavailability, which may be part of their extraordinarily nontoxic biological footprint. Most polyphenols are rapidly conjugated (typically sulfated and glucuronided), and variably metabolized, often with an uncertain biological status of their multiple metabolites. The proper study of any polyphenol in potentially slowing or preventing any disease of aging is methodologically challenging and also expensive (long time frames are needed and it is difficult to control for many other positive and negative lifestyle risk factors). With all these scientific and methodological challenges, there is little financial incentive to study polyphenols in humans in relation to the diseases of aging or aging itself, given the poor return on investment with inexpensive agents that cannot be patented. This collection of factors has generated the current situation, where one finds much promising animal-model data for

multiple polyphenols in relation to a disease of aging, but a dearth of good human clinical studies. This is changing slowly, and several polyphenols are in clinical trails related to several diseases of aging.

One of the few completed studies of a polyphenol in a human clinical population demonstrated that resveratrol is effective at higher doses in treating diabetes (Patel et al., 2011). Clinical studies are underway related to cancer, AD, and heart disease. Curcumin is also being increasingly studied for its anti-inflammatory, antiproliferative, and antiaging effects. Curcuminoids are thought to affect many dozens of cellular pathways and, like many polyphenols, block NF kappa-B, a transcription factor involved in the regulation and activation of inflammatory responses (Aggarwal, 2010). Curcumin is also one of several polyphenolic inhibitors of mTOR, a critical nutrient-sensing and growth factor integrative pathway that is increasingly implicated as a molecular target of CR; if inhibited, it may slow aging and also inhibit or delay diseases of aging (Beevers et al., 2009), but curcumin has notoriously poor bioavailability and rapid metabolism (Bengmark, 2006).

Diseases of aging (with particular relevance to neurology)

This list of diseases is truncated due to space considerations, and does not include many important illnesses, including motor neuron diseases, frontotemporal lobar degenerative disorders, and various brain cancers.

Cardiovascular disease

Although "cardiovascular disease" technically refers to any disease that affects the heart or blood vessels, the term has become increasingly synonymous over the last 20 years with atherosclerosis. This disease of aging is directly responsible for more deaths than any other in Western societies, killing twice as many individuals as all cancers combined and probably more than all the other diseases of aging put together (Minino et al., 2006). Thus, it clearly merits a summary review. Evidence argues that lifestyle and cultural factors have to be considered as primary etiological issues here. As Kones pointedly states "Americans are under assault by a fierce epidemic of obesity, diabetes, and cardiovascular disease, of their own doing. Latest data indicate that 32% of children are overweight or obese, and fewer than 17% exercise sufficiently. Over 68% of adults are overweight, 35% are obese, nearly 40% fulfill criteria for metabolic syndrome, 8–13% have diabetes, 34% have hypertension, 36% have prehypertension, 29% have prediabetes, 15% of the population with either diabetes, hypertension, or dyslipidemia are undiagnosed, 59% engage in no vigorous activity, and fewer than 5% of the US population qualifies for the American Heart Association (AHA) definition of ideal cardiovascular health. Health, nutrition, and exercise illiteracy is prevalent, while misinformation and unrealistic expectations are the norm. Half of American

adults have at least one cardiovascular risk factor. Up to 65% do not have their conventional risk biomarkers under control. Of those patients with multiple risk factors, fewer than 10% have all of them adequately controlled. Even when patients are treated according to evidence-based protocols, about 70% of cardiac events remain unaddressed. Undertreatment is also common. Poor patient adherence, probably well below 50%, adds further difficulty in reducing cardiovascular risk. Available data indicate that only a modest fraction of the total cardiovascular risk burden in the population is actually now being eliminated. A fresh view of these issues, a change in current philosophy, leading to new and different, multimechanistic methods of prevention may be needed. Adherence to published guidelines will improve substantially outcomes in both primary and secondary prevention. Primordial prevention, which does not allow risk values to appear in a population, affords more complete protection than subsequent partial reversal of elevated risk factors or biomarkers" (Kones, 2011, p. 61).

Consistent with these statements, recent research demonstrates that the underlying process of atherogenesis is a complex and long-term process involving many players, including endothelial cells, cytokines and immunoglobulins, immune cells, growth factors, extracellular matrix molecules, and lipids, but with a primary role for OS and inflammation. Atherogenesis requires a cascade of processes, starting with a maladaptive, sustained proinflammatory reaction to oxidized lipid deposition in the arterial wall. The initiating event appears to be the deposition of apoB-containing lipids, typically oxidized low-density lipoproteins. Oxidation of these lipids dramatically increases the likelihood that the deposition process will irritate the vessel, promoting increased proinflammatory cytokine release; this suggests that plasma redox balance may be a critical variable (Maharjan et al., 2008). Hyperlipidemia is also associated with declining endothelial nitric oxide synthase (eNOS) and increasing nitroxidative stress in the endothelium (Heeba et al., 2009). These inflammatory cascades lead to accumulation and swelling in arterial structures, mostly from macrophage cells combined with lipids (principally, oxidized low-density lipoprotein (LDL), VLDL, and other fatty acids), calcium (particularly in advanced lesions), and a certain amount of fibrous connective tissue. Glycation of proteins (an intrinsic component or phenotype of aging), as well as foreign antigens, can also promote these fundamental inflammatory changes (Milioti et al., 2008), with regions of more glycated tissue and AGEs promoting and accelerating the formation of these plaque structures (Kim et al., 2010b).

These slowly developing structures (atheromatous plaques) are found at least to some degree in most individuals in Western societies, and early asymptomatic stages of this process are found in many young adults; however, they are rare in HGs (Eaton et al., 1988 a,b). LDL is the most common ApoB plasma lipoprotein, but ApoB-containing VLDL, remnant VLDL (depleted of triglycerides), intermediate-density lipoprotein (IDL), and LP(a) have also been shown to be atherogenic, along with ApoB from chylomicron remnants; this suggests that many forms of lipid contribute to risk. These lesions actually begin in childhood and develop slowly over many, many decades. The early stages of deposition are called "fatty streaks," but they are not composed of adipose cells; instead, they consist of white cells, especially macrophages, that have taken up oxidized LDL. After these cells accumulate large amounts of cytoplasmic membranes (and high cholesterol content), they become "foam cells." When foam cells undergo apoptosis, the contents are deposited into the surrounding tissue, attracting more macrophages and inflammation, and causing a positive and self-sustaining feedback loop. Upon activation by proinflammatory stimuli, macrophages and lymphocytes release proinflammatory cytokines that stimulate the migration of smooth muscle cells (SMCs) from the medium of the vessel wall. SMCs then contribute to more foam cell and fibrous cap formation, also under the influence of proinflammatory cytokines (for example, IFN-γ and TNF-α secreted by T helper cells, and IL-12 secreted by macrophages and foam cells; Milioti et al., 2008). Eventually, foam cells die via apoptosis, dumping nondegradable cholesterol crystals that form the lipid core of the plaque structure. Plaque structures can be either stable or unstable, with vulnerable plaque tending to be faster growing and with higher macrophage content, suggesting that autoinflammatory processes not only contribute to the early, more silent stage of the process, but also drive the deadly late stages of the process well. Recent work by Wang et al., 2011 suggests a potentially pivotal role by immunoglobulins (IgE) as a critical player in the activation of macrophages, and with high correlations between IgE levels and degree of plaque instability.

Although popularly viewed as a disease of cholesterol (a perspective that dominated the earlier conceptualizations of vascular disease in the 1960s and 1970s), increasing scientific opinion favors atherosclerotic vascular disease as a disease of inflammation and OS. Consistent with this, increasing evidence shows that statins actually impact both inflammatory and OS issues (Heeba et al., 2009), while promoting upregulation of heme oxygenase (an important antioxidant defense enzyme). Statins appear to inhibit vascular disease through pleiotropic mechanisms, including decreased synthesis of LDL, increased removal of LDL (through hepatic LDL receptors), upregulation of eNOS, increased tissue-type plasminogen activator, and also inhibited endothelin 1, a potent vasoconstrictor and mitogen. All of these promote improved endothelial function. Statins also reduce free radical release, thus inhibiting LDL-C oxidation (Liao and Laufs, 2005), while increasing endothelial progenitor cells and reducing both the number and activity of inflammatory cells and cytokines. They also may help stabilize atherosclerotic plaques, reduce production of

metalloproteinases, and inhibit platelet adhesion/aggregation (Liao and Laufs, 2005).

Although it is extremely common in Western societies (at least in some stage, even if clinically silent), extensive vascular disease is virtually nonexistent in HG groups (Eaton and Eaton, 2002). This suggests a primary role for etiology in the Western lifestyle and diet (see later sections on diet and lifestyle variables), in which multiple, if not virtually all, components of the Western diet and lifestyle appear proinflammatory relative to HG lifestyles (sedentary vs highly aerobically active, altered omega-6/omega-3 ratios, poorer sleep, greater social isolation, lower consumption of fiber, lower consumption of protective polyphenol phytochemicals, and high BMI vs low BMI in HG groups).

In addition to atherosclerosis (which is clearly the largest problem in pathological vascular aging in Western cultures), there is also vascular aging independent of atherogenesis. Increasing evidence implicates angiotensin II (Ang II) signaling as central to this process (Wang, Khazan, and Lakatta 2010). Arterial remodeling and decline in aging (even without atherosclerosis) is increasingly thought to be linked to Ang II signaling (Wang, Khazan, and Lakatta, 2010a). Components of Ang II signaling (including several reactive oxygen species, multiple growth factors, matrix metalloproteinases, chemokines, and nicotinamide adenine dinucleotide phosphate-oxidase) are upregulated within arterial walls in many species including humans, during aging. *In vivo* studies suggest that elevation of Ang II signaling drives accumulation of AGE (advanced glycated end products, which are themselves proinflammatory), increased collagen, disruption of elastin, and invasive hypertrophy of both smooth muscle and endothelial cells (Wang, Khazan, and Lakatta 2010a). Obvious clinical implications are that attenuating Ang II signaling may significantly retard this age-associated arterial remodeling, suggesting important protective effects for ACE inhibitors and ARB compounds. Intriguingly, multiple polyphenols, including those in pomegranate juice (rich in tannins and anthocyanins), appear to inhibit angiotensin signaling (perhaps in part from nonspecific antioxidant effects, but also from inhibition of angiotensin-converting enzyme activity) and may also reduce blood pressure (Stowe, 2011). Ang II also enhances ROS production by activating NAD(P)H oxidase and uncoupling eNOS. Systemic inhibition of Ang II thus may potentially have CR mimetic (antiaging) effects, due to its central role in coordination of vascular aging, OS, and impact on the mitochondria (de Cavanagh, et al., 2011).

These processes driving vascular aging and disease are of obvious primary relevance to vascular dementias, as well as to common findings of white matter erosion (typically referred to as white matter hyperintensities or white matter ischemic change on MRI and CT scans), sometimes appearing as a highly comorbid pathology with AD (Brickman et al., 2009). Indeed, separating amyloid angiopathy (a frequent vascular concomitant to AD) from other forms of atherosclerosis is almost impossible within clinical settings.

Alzheimer's disease

As the disease of aging with perhaps the greatest relevance to this textbook, there has been a paradigm shift over the last 20 years away from the original assumption that AD has nothing to do with aging. Of course, this could not possibly have been true, given the simple fact that AD roughly doubles in incidence every 5 years after the age of 60–65 and that aging remains the greatest risk factor for nonfamilial sporadic AD. Recent research suggests that markers for OS and mitochondrial decline (Pratico, 2010; Aliev et al., 2010; Mancuso et al., 2007) are elevated even prior to the appearance of extracellular amyloid deposition, which takes place in the preclinical stages of the disease. Indeed, multiple lines of evidence link AD to many, if not virtually all, of the phenotypes of aging, including inflammation (Masters and O'Neill, 2011), OS, accumulation and/or clearance failure of characteristic pathogenic proteins (Barnett and Brewer, 2011), and increasing deleterious synaptic effects from those proteins and from associated inflammation (De Strooper, 2010; Mondragon-Rodriguez et al., 2010; Palop and Mucke, 2010). Recent work has suggested that pathogenic proteins (such as oligomeric amyloid) are not being cleared out (Mawuenyega et al., 2010), underlining an important role for declining autophagy in the etiology.

These considerations suggest that AD is indeed a highly pleiotropic and complex disease with several stages in which we may still not understand fully all the critical factors, or exactly how they interact to create a cascade with distinct stages, and with different processes and interactions presumably critical at different stages. What were originally adaptive mechanisms (such as inflammation, recruitment of amyloid pathways by various stresses and neuroplasticity challenge, phosphorylation, apoptosis, cell cycling, and so on) may become pathogenic in the context of chronic synergistic recruitment, biological stress, and neuroplasticity challenge. This suggests an image of AD in which a host of individually adaptive and compensatory mechanisms jointly "conspire" to drive the brain into a neurodegenerative process (Mondragon-Rodriguez et al., 2010). Given that these interactions among a host of individually adaptive processes occur well past a reproductive period, they would escape virtually any conceivable selection pressure or modification. In this sense, the vulnerability to AD may reflect a "fault line" in the human genome consistent with the evolutionary perspectives outlined earlier. Thus, AD itself may be an expression of antagonistic pleiotropy in which genes and molecular pathways that were adaptive during periods of youth and fecundity potentially backfire in aging, particularly when synergistically recruited. Table 1.1 summarizes some, but not all, of the complex interactions

Table 1.1 Factors contributing to a neurodegenerative mratrix in AD

Biomarker	Produced by	Producing	Clinical/Other correlates
Beta amyloid plaque (extracellular Aβ)	Aging, ↓ clearance, oxidative stress/inflammation APOE4, altered BBB function? ↑ gonadotropins (LH/FSH) and declining sex steroids?	Inflammation (glial activation), oxidative stress, more oligomers?	Subtle regional atrophic changes. Second biomarker appearing after OS/MITO decline
Small aggregate amyloid (oligomeric Aβ)	β/γ secretases, inflammation, oxidative stress, ↓ clearance, endocrine dyscrasia? Plaque?	Synaptic loss and dysfunction, OS, inflammation	Synaptic loss (NMDA, AMPA), loss of LTP, increased LTD
Inflammation (INFLAM) (↑ innate immunity)	Amyloid fibrils and oligomers, ↓ ACh, ↑ rAGE signaling, aging, OS, endocrine dyscrasia?	Synaptic dysfunction, apoptosis, declining neurotrophins, OS, ↑Aβ?	Contributes directly to cognitive dysfunction via multiple effects
Central insulin resistance (in CNS)	Inflammation (↑ NFk-b, AP-1, TNF-α, other proinflammatory cytokines), chronic stress?	↓ Energy, HC damage, ↑ kinases (→ neurofibrillary tangles?), declining autophagy?	Promotes synaptic dysfunction and loss; promotes amyloidosis
Oxidative stress (OS), MITO decline	Declining control over OS in aging, Aβ oligomers in MITO, metal ions, INFLAM, advanced glycation end products, junk protein	Synaptic and neural loss, INFLAM, Aβ, increased tangling? aberrant cell cycling → apoptosis	Appears *before* plaques/tangling; membrane OS increases with disease, but DNA OS markers do not
Excitotoxicity and Ca++ dysfunction	Oligomers (Aβ) in MITO, and at Ca++ channels, ↑ kynurenine (from increased cytokines)	Synaptic dysfunction, apoptosis, esp. in HC/EC regions	Synaptic dysfunction, eventually SL/NL
Neurotrophin and neuro-transmitter depletion	Oligomers (Aβ) → receptor internalization, tau pathology → microtubule dysfunction, inflammation	ACh loss → ↑ Aβ, BDNF/ NGF declines, aberrant cell cycling and apoptosis	Synaptic dysfunction, promotion of both SL and apoptosis
Neurofibrillary tangling and tau aggregates	Oxidative stress (OS) → ↑ kinases (w/ ↓ phosphatases), insulin resistance? Downstream effect of Aβ oligomers? Aberrant cell cycling?	Basal forebrain (ACh) loss, SL, apoptosis	Tracks atrophic change (SL/NL) and declining cognitive function closely. Precursors (PHF) appear long before beta amyloid deposition
Atrophy HC/EC → lateral temporal → frontal/parietal	Multifactorial, with many factors listed contributing to synaptic loss and apoptosis	Proceeds functional declines (slightly)	Major biomarker for degenerative changes in clinical stages of AD
Cognitive loss, especially STM, then language and executive function	Synaptic loss early, SL plus NL later (apoptosis)	Declining fxn, compensatory neuroplasticity effort?	Primary functional measure, necessary for diagnosis

Source: Adapted from Watt et al. (2012) with permission from Springer.

SL: synaptic loss, NL: neural loss (neuronal cell death), Aβ: beta-amyloid, BBB fx: Blood Brain Barrier Function, MITO: mitochondria, ACh: acetylcholine, NGF: nerve growth factor, BDNF: Brain Derived Neurotrophic Factor, rAGE: receptors for advanced glycation end products (which promote inflammation), HC: hippocampus, EC: entorhinal cortex, apoptosis: programmed cell death, NFk-b: Nuclear Factor Kappa B (transcription factor involved in inflammatory signaling), AP-1: activator protein 1 (transcription factor involved in inflammatory signaling), Oligomers: several molecules of beta amyloid stuck together, Kinases: enzymes promoting phosphorylation and tangling, NMDA/AMPA: subtypes of glutamate receptor, LTP: long-term potentiation, LTD: long-term depression, lateral temporal: lateral temporal lobe, frontal/parietal: frontal and parietal convexity.

between putative etiological factors in AD, emphasizing an image of the disease as highly multifactorial, but one in which many primary phenotypes of aging (OS, disordered cell cycling, inflammation, glycation, apoptosis, mitochondrial decline, accumulation of junk proteins, and declining autophagy) appear not only contributory, but also highly interactive, arguing against any version of a single factor etiology.

Parkinson's disease

PD and its more aggressive and malignant close relative, diffuse Lewy body disease (DLBD), are idiopathic neurodegenerative diseases characterized by intraneuronal accumulation of Lewy bodies (aggregates of alpha-synuclein), particularly in substantia nigra (midbrain dopamine-producing regions) in classical PD (and much more widely in DLBD). It is marked by progressive loss of DA cell bodies, deafferentation of basal ganglia, and dysfunction in direct and indirect corticostriatal pathways. Subsequent primary symptoms include resting tremor, slowing of movement, rigidity and gait difficulties, and eventual postural instability. There is evidence of differential vulnerability to degeneration in nigral regions, with "ventral tier" neurons more vulnerable than "dorsal tier," and with VTA neurons least effected (Collier et al., 2011), despite the fact that these fields form a continuous sheet of DA neurons. This differential vulnerability is viewed in recent work as multifactorial. In animal models, it appears linked to several markers, including the appearance of alpha-synuclein, ubiquitin (as a marker of proteasome activation), lipofuscin (as a marker of lysosome

activation), 3-nitrotyrosine (as a marker for nitroxidative stress), dopamine transporter activation, and markers of astrocyte and microglial activation (inflammation markers). Dysfunctional mitochondria and activated microglial cells are thought to be the primary intracellular source of reactive oxygen species, and lysosome-mediated autophagy is the primary cellular mechanism for removing defective mitochondria. The progressive accumulation of lipofuscin (conventionally regarded as "age pigment") is thought to reflect an index of mitochondrial damage and subsequent lysosomal degradation of defective mitochondria (Terman et al., 2006). Collier et al. (2011) argue that the etiology of PD, while still uncertain, may reflect stochastic interactions among inflammation, OS, declining autophagy, and accumulations of pathogenic junk proteins, producing a "stochastic acceleration hypothesis". These kinds of basic models (although omitting inclusion of many other aging phenotypes such as glycation, endocrine change, and telomere loss) (Figure 1.1) may provide a template for unraveling the etiology of other neurodegenerative disorders, particularly AD, but also the FTD family and some types of cancer, where the connections to aging and aging phenotypes are less clearly established. The high percentage of AD pathology in patients with DLBD argues also for a fundamental relationship between AD and PD that is still incompletely mapped.

Aging processes and the brain: cognitive changes in aging

Although enormous evidence suggests that aging in the brain cannot be neatly separated from aging of the whole organism, at the same time, one has to consider that aging may be differentially expressed across different organ systems, and that the brain might be exposed differentially to aging processes (and perhaps differentially protected as well), including effects on the brain of pathological forms of aging, as described in the discussions of AD and PD. Much work suggests that a variety of neurocognitive functions decline with aging, even in those without demonstrable neurological disease (although the enormous difficulty in removing preclinical AD completely from one's aging cohort/control group, plus the ubiquitous penetration of vascular disease in Western societies raises serious questions about how many studies purporting to show age-related cognitive change may be measuring at least in part prodromal stages of neurological decline from a major disease of aging). In any case, robust evidence suggests that a host of neurocognitive processes decline in aging, including episodic memory, working memory, spatial memory, processing speed, and even implicit (skill) learning, along with various motor functions, particularly motor speed and fine motor control (see Yeoman et al., 2012 for overview). The precise neural bases for these declines are still open to debate, and although initial

assumptions heavily emphasized age-related neuronal loss, increasing evidence argues that these neurocognitive declines are probably pleiotropic in origin, with synaptic loss possibly more important than actual neuronal loss. This itself also appears multifactorial, with roles for aminergic and neurotrophin decline, and where increased CNS inflammation might also play a role, but this has until recently been minimally probed, both in clinical and preclinical approaches (Cribbs et al., 2012). Loss of the smaller and highly plastic thin dendritic spines (more than the "fat" mushroom spines which appear more resistant to aging) appears to be one of the best candidates for an ultrastructural basis to age-related cognitive change, at least in relevant animal models (and thin spines which are more NMDA receptor-dense also appear more sensitive to deprivation of classic sex steroid hormones) (Dumitriu et al., 2010). A physiological correlate to cognitive declines in aging related to declarative (episodic) memory appears to be the phenomenon of prolonged hyperpolarization in aged hippocampal neurons, associated with changes in NMDA, AMPA, calcium channels, and other ion channels (Yeoman et al., 2012).

Departure from ancient evolutionary environment: impact on aging processes and promotion of diseases of aging

Enormous evidence indicates that Western societies involve diets and lifestyles that are radically different from HG lifestyles and diets and, indeed, radically different from the original evolutionary environment in which the entire hominid line evolved. This may produce an "evolutionary discordance" (Konner, 2001) that may have profound effects on human health and a major influence on the biological trajectories of human aging. This notion of a radical departure from an evolutionary environment and a subsequent mismatch between our genes and our environment may provide a unifying context for connecting all increased risk factors for all the diseases of aging: Humans in modern technological societies are now living much longer (primarily due to our successful control over predation, starvation, and infection as primary causes of early mortality for children and younger adults). Put differently, *all of the so-called healthy lifestyle practices that have been discovered piecemeal through many empirical studies (such as a diet high in fruits and vegetables, healthy omega-3/omega-6 ratios, high intake of fiber, and regular exercise) all have as a unifying context that they are components of our original long-term biological environment as HG*s (Eaton and Eaton, 2002). This suggests that healthy lifestyle practices reduce or perhaps even virtually eliminate chronic mismatches between a genome carved in a more ancient HG environment and our current technological environment. Unfortunately, adoption of these healthy lifestyle practices is far from widespread in the United States or in other Western societies, and it may be relatively restricted

to those better educated and those belonging to more fortunate socioeconomic groups (Johannson et al., 1999).

The fundamental hominid diet for probably more than two million years (preagriculture) was lean protein sources (game and fish), supplemented by significant quantities of fruits and vegetables (Cordain et al., 2005). Modern technological diets are higher in fat (particularly omega-6 fats) and carbohydrates (largely from grains and other agricultural products) and now contain significant transfats (which did not exist in our original biological environment); they also are frequently deficient in fiber and multiple protective phytochemicals (polyphenols) and possibly low in other several critical micronutrients, including choline and phospolipids, multiple B vitamins, and several minerals (Eaton et al., 2007). In addition, vitamin D deficiency is now quite common (Holick, 2007), while this was probably very rare, if not nonexistent, in ancient HG societies, in which skin color seems to have evolved to match latitudes and to balance vitamin D production with skin protection, given that both modern sunscreens and indoor living were nonexistent.

The following tables summarize some of these fundamental differences between an ancient biological environment for humans and the current environment, including work on biomarkers from studies of HG societies (Eaton and Eaton, 1999; Eaton et al., 2007; Eaton et al., 1998a,b; Cordain et al., 2005). This evidence for huge biological environment shifts during a period of minimal genetic change for humans (the last 10,000 years) suggests a potential "unified field theory" for the diseases of aging (and that diseases of aging are largely "diseases of civilization"; Melnik et al., 2011). Ironically, humans have never lived longer than they are living in modern technological societies: The average life expectancy at birth within preindustrial HG societies was probably roughly 30–35 years (Konner and Eaton, 2010). However, this significantly extended lifespan in technological cultures is one in which penetration by a major disease of aging (excepting osteoarthritis, which is common in HG groups) appears more likely, relative to the few elders who existed in HG societies (Dunn, 1968; Konner and Eaton, 2010). Conclusive data on this question is lacking, however, and reconstruction of more ancient (Paleolithic) HG lifestyles and biological state involves extrapolating from the relatively few HG societies that survived into the twentieth century (columns adapted from Eaton and Eaton, 1999; Eaton et al., 2007; Eaton et al., 1998a,b; Cordain et al., 2005).

Original Evolutionary Environment	Modern Technological Environment
1 Regular aerobic exercise (2-3+ hours per day)	1 Minimal to no aerobic exercise (< 15 min/d)
2 9+ hours sleep (see #1)	2 7 hours or less of sleep (see #1)
3 Calorie limitations (intermittent CR)	3 Unlimited calories
4 High-phytochemical/polyphenol diets	4 Low phytochemical/polyphenol diets
5 Omega-6/omega-3 ratio 1:1 to 3:1 with modest intake of overall fats	5 Omega-6/Omega-3 ratio 10:1 to 20:1 with typically higher intake of fats
6 High intake of fiber (about 50-100 g per day)	6 Low intake of fiber (≤ 15 gm/d)
7 Low sugar/carbs, except fruits/veggies	7 High sugar/carbs, not from fruits/veggies
8 Intake of K+ > Na+ (K+ > 4 gm/d)	8 Intake of Na+ > K+ (Na+ > 4 gm/d)
9 Pro-alkaline diet	9 Pro-acidic diet
10 Minimal to no glycated proteins	10 Common glycated protein (especially milk products)
11 Intimate social groups/tribes	11 Social isolation common
12 Early mortality: infection, starvation, predation, and intraspecies violence: life expectancy 35-45 years	12 Death from an advanced disease of aging: life expectancy 75-85 years

Biomarkers

Hunter Gatherers	Current Technological Societies
1 BMI 21–24	1 About 30% BMI >30, about 30% BMI 25–30
2 Total cholesterol under 125	2 Total cholesterol about 200 or higher
3 Blood pressure 100–110/70–75	3 120/80 (normative), with hypertension common
4 VO2 max good to superior	4 VO2 max fair to poor (sedentary lifestyles)
5 Homocysteine low	5 Homocysteine significantly higher
6 Vitamin D about 50–100 ng/mL	6 Vitamin D deficiency common (10–30 ng/mL)
7 Higher B vitamin/folate levels	7 Common B12 and folate deficiencies
8 High insulin sensitivity	8 Variable degrees of insulin resistance
9 Fasting plasma leptin 2–4ng/mL	9 Fasting plasma leptin 4–8ng/mL
10 Waist/height ratio <45	10 Waist/height ratio 52–56
11 Physical activity >1000 kcal/d	11 Physical activity about 150–490 kcal/d for most

Although conclusive data is still lacking, preliminary evidence suggests that HG societies did not appear to have nearly the incidence of cancer and heart disease (Eaton and Eaton, 2002), diabetes (Eaton et al., 2002), or AD (Eaton and Eaton, 1999) suffered by modern societies, even when the relative rarity of elder members is taken into account (Konner and Eaton, 2010). Consistent with these findings and hypotheses, a paleolithic diet improved diabetic bio-markers more than the highly touted Mediterranean diet (Lindeberg et al., 2007) and improved BP and glucose tolerance, decreased insulin secretion, increased insulin sensitivity, and improved lipid profiles, all without weight loss in healthy sedentary humans (Frassetto et al., 2009). Additional evidence (summarized in Spreadbury, 2012) suggests the provocative hypothesis that virtually all processed or "acellular" carbohydrates—which tend to be high-density carbohydrate foods—(ancient sources of carbohydrates in fruits and vegetables were low density) contribute directly to an inflammatory gastrointestinal microbiota which leads directly to leptin resistance, disordering of fundamental energy homeostasis through effects on multiple satiety peptides, and promotion of obesity. Spreadbury further argues that modern diets are truly distinct from ancient diets not in relationship to either nutrient density or glycemic index but only around *carbohydrate density* (Spreadbury, 2012) due to acellular grain-based foods.

It is difficult to know precisely what the sum total or composite effect of such global and pervasive shifts in our basic biological environment might be, or what each factor may contribute to the overall increasing burden of diseases of aging in Western societies. However, the evidence favors the hypothesis that these shifts are first of all individually deleterious. Therefore, collectively, they are likely to be highly undesirable and potentially profound. Indeed, there may be poorly mapped synergisms among these various factors in promoting diseases of aging, as virtually every one of these factors—the complex multifactorial dietary shifts, sedentary versus aerobic lifestyles, common obesity generated by these two factors, vitamin D deficiency, low-grade sleep deprivation, and increased social isolation and stress (vs the intimate social groups of our ancestors)—*all impact the regulation and management of inflammation* (as even psychosocial isolation and social stress is a proinflammatory event). This suggests that, collectively, Western lifestyles (when compared to the lifestyles of our HG ancestors) may be *hugely proinflammatory*. There is evidence that autoinflammation involves increased OS (Finch, 2011), drives insulin resistance, and is potentiated by glycation (Semba et al., 2010), and increases cellular senescence. Such a global view of the biological environment also suggests strongly that single-component "fad diet" approaches, such as the elimination of all fructose, sugar, or carbohydrates, are not likely to be successful unless combined with a larger group of dietary and lifestyle changes (although refined carbohydrate reduction as noted may help with reducing obesity, inflammation,

and pulsatile insulin over-production all of which may be critical in the Western society burden of diseases of aging). In any case, this analysis, which suggests a complex and highly interactive composite of environmental shifts relative to ancient HG environments that collectively are probably biologically profound.

Many, if not most, of these lifestyle and dietary factors may also deteriorate the endogenous management of OS (Kaliman et al., 2011). Given that autoinflammation creates OS for "bystander" tissues (Finch, 2011), these lifestyle variables may impose a double burden: increasing OS while depriving us of several protective factors (found in our ancient evolutionary diet and lifestyle) that might ameliorate or protect against OS. OS, modulated by both diet and exercise, is also believed to be a primary factor in genetic damage and genomic instability (Prado et al., 2010), leading potentially into cancers and the acceleration of cellular senescence, as a primary defense against cancer (Ogrunc and Fagagna 2011). Cellular senescence in turn appears to be proinflammatory, creating a so-called "senescence-associated secretory phenotype" (SASP) (Blagosklonny, 2011). Many of these dietary and lifestyle factors also modulate the glycation of proteins and the formation of AGEs (particularly diets low in fiber and polyphenols and high in refined sugars/carbs), with AGE products a primary regulator and inducer of inflammation. Inflammation itself may promote insulin resistance and, thus, glycation, suggesting many positive feedback loops between these classic metabolic and age-related processes. Common vitamin D, B12 and folate deficiencies may contribute to declining autophagy, and also increasing inflammation (Holick, 2007), promoting cognitive decline in aging, increased homocysteine (as a marker and proxy for OS and inflammation), and possibly increased AD (Tangney et al., 2011). Many lifestyle factors also impinge on the cell signaling related to endogenous defenses against OS, particularly exercise, polyphenol intake, inflammatory state, obesity and excessive energy, and insulin resistance. Indeed, the typical alterations in energy homeostasis in Western diets and lifestyles, leading to an excess of energy (in turn, leading to obesity), are a primary activator of mTOR (mTOR, as a pathway that integrates nutrient signaling and growth factors), increasingly implicated as a central factor in the regulation and induction of aging (Blagosklonny, 2009, 2010a). In addition, multiple polyphenols (modestly) and DR, particularly protein restriction, inhibit mTOR. Collectively, these considerations suggest that Western lifestyles may directly impact the biology of the diseases of aging (and aging itself) directly and powerfully in a multitude of undesirable ways. Thus, although the central prolongevity triumph of Western civilization and medicine, the prevention and treatment of bacterial infection, has had a very positive impact on median survival to old age, Western lifestyles may accelerate aging and the diseases of aging in a multitude of other ways. Preventing the diseases of aging therefore has to begin with an appreciation for the central importance of lifestyle change,

back toward at least some approximation of our evolutionary environment.

What constitutes optimal prevention of the diseases of aging?

In sum, this large constellation of globally altered lifestyle variables impacts the fundamental biology of aging and also modulates the underlying mechanisms directly driving all the diseases of aging. Jointly, these lifestyle factors, interacting with our genome (containing many currently unmapped polymorphisms that presumably directly modulate aging processes and the vulnerability to diseases of aging variably across individuals), in concert with multiple lifestyle behaviors, determine what aging trajectories our systems enter as we get older. These basic interactions between lifestyle (which we can map out) and many polymorphisms in our genetic endowment (which we can now map only minimally) determine how much our fundamental cellular repair mechanisms and defenses against cellular damage and aging are supported and enhanced as much as possible, versus overtaxed and overwhelmed. The primary and multifactorial mechanisms of aging reviewed in this chapter appear to lead invariably into the diseases of aging, if given enough time and enough room to work. *Indeed, the sum total of presence or absence of all the diseases of aging in an individual may be one of the best ways to globally index aging itself* (Blagosklonny, 2009). Challenges remain in operationalizing such a definition, of course, given that practical, cost-effective (and nonintrusive) metrics in relation to many of the diseases of aging are not yet clinically available. Unfortunately, the conventional medical perspective on diseases of aging in this country is still largely unaware of evidence that they may reflect common mechanisms operating in different tissues and systems; instead, conventional medicine mostly approaches each major disease of aging in a piecemeal and fragmented fashion. This chapter argues strongly against that traditional approach.

Western lifestyles (consisting of a typical Western diet pattern and a sedentary lifestyle with poor sleep and increased social isolation) appear quite undesirable in terms of aging of the brain and body, deteriorate capacities to deal with various biological and social stresses, and remove us from our proper and ancient evolutionary environment. We have changed remarkably little genetically since our days as HGs, but our lifestyles have changed dramatically. This suggests that much of our current difficulties with health are not due to some exotic collection of esoteric biological derailments that can only be interpreted and treated by a "medical–industrial complex" and understood by someone with a doctoral degree; instead, they are due to a fundamental, if not profound, mismatch between our genes and our environment (Stipp, 2011). This suggests that basic health considerations should focus on approximating that ancient

biological environment as much as possible: regular aerobic exercise, large amounts of fruits and vegetables, not too many calories, minimal processed food and other products of "food technology" (particularly our highly addicting fast food), a better omega-6/omega-3 ratio (typically very high in most Western diets with significant omega-3 deficiency), reduced social isolation, and improved sleep quality and quantity. As noted earlier, all these common recommendations place us closer to our ancient evolutionary environment and reduce this fundamental and destructive discordance between genes and environment in Western lifestyles.

At this point, there is no cure for virtually any disease of aging (perhaps excepting some cancers), so meaningful prevention needs to a genuine priority instead of an afterthought in our health-care system. We must be willing to spend money on prevention and to make lifestyle changes a genuine cultural priority. It is also quite sobering to realize that, even in the context of the best possible preventative efforts, all one can do is *delay the onset of a major disease of aging: Eventually, we will all succumb to one of these manifestations of aging*. However, such delay in onset of a major disease of aging can potentially increase healthspan (even if major lifespan extension remains elusive) and substantially decrease the burden of diseases of aging in old age, along with their often punitive impact on quality of life and personal and societal economics (see Chapter 21).

Prevention, in this context of the many considerations reviewed in this chapter, thus has to mean much more than "statins and beta-blockers" (controlling multiple conventional risk biomarkers that clearly have some prognostic value but may only minimally index our deceptive yet radical physiological departure from our ancestors). *Instead, real prevention must mean, for the large majority of individuals in a culture and not simply for a fortunate few, reapproaching our original evolutionary environment*. In simplest terms, as a culture, these major lifestyle changes must mean that we exercise and sleep significantly more, eat significantly less, and eat more wisely (consuming more of the "paleolithic" foods of our ancestors and less the questionable and addictive products of food technology). In addition, we need to aim more for quality of social connection than quantity of material consumption, as quality and depth of social attachment is emerging as one of the better predictors of long-term health (Seeman and Crimmins, 2001; see Chapter 10).

Making these critical changes in priorities and approach, both individually and in terms of the embedded high-tech priorities of our health-care systems, is likely to be painful in many ways, as well as profoundly politically contentious. However, one cannot envision any viable long-term prescription or big-picture view of biological health that does not place these simple principles first. Additionally, this view of health (that it emerges from the basic fit between genes and environment) places health back into a proper evolutionary perspective that is badly lacking in many treatments of diseases of aging. There seems to be little

sense in the current health-care environment that Darwin's central insights (about the match between genetic endowment and environment determining adaptive success) has any relevance to discussions of basic health or illness. Has modern medicine abandoned Darwin? A central implicit myth of the "medical–industrial complex" (implicit in the sense that it is largely embedded in relentless advertising and is never explicitly stated) may be that high-tech medicine and first-line drugs are our best defense against the chronic diseases of aging, a supposition for which there is very little substantive evidence, and much counterevidence.

An additional option for the future may be the possibility of a highly effective CR mimetic: perhaps a future version of resveratrol or rapamycin, some combination of our current (partial) CR mimetics, or perhaps even a completely new and different compound yet to be discovered. It seems an easy prediction that a truly safe and effective CR mimetic (which, by definition, would give the physiology of CR without the pain of chronic hunger) that could both slow aging and substantively delay onset of all the diseases of aging would be a compound that almost everyone would readily consider taking and many if not virtually everyone would find highly attractive. Indeed, if a patentable agent were proven highly effective and safe, one could easily predict that it would eventually become the best-selling prescription medicine of all time. However, such considerations (potential widespread use of CR mimetics) embed a major conundrum, similar to that posed by the potential creation of an "exercise pill." Would individuals with the option to take a safe and effective CR mimetic still be adequately motivated to modify problematic lifestyle habits and move closer toward the original evolutionary environment of humans, which we believe promotes long-term health and healthy (or at least *healthier*) aging? One can readily appreciate the temptation to continue eating problematic but tasty foods and remaining overweight and sedentary, if one's anxiety about any potential disease of aging could be significantly ameliorated by simply taking a pill.

Such a dilemma in many ways goes to the heart of difficult choices confronting modern technological *Homo sapiens* in relation to both health care and, more fundamentally, long-term health. *Do we trust in our high technology first and foremost? Do we place exclusive faith in our technological competencies, to the exclusion of trusting in biological relationships that are (at least, in some sense) pretechnological? Or must we place equal or even greater trust in our basic evolutionary heritage and our embeddedness in a complex biological matrix and ecology, the environment that carved our genome? Put in simplest terms, do we think that health promotion is primarily a technological or a lifestyle matter?* Answers to these questions may determine a great many things about our long-term health in the coming century and our health-care system. Additionally, these choices mirror much larger and even more difficult choices about our basic relationship to a complex biological matrix (the extended environment), which is clearly showing

the negative impact of human technologies. A tempting hypothesis is that our disregard of the environment may be intrinsically hinged to the overvaluation of technology and the undervaluation of our biological "embeddedness" and our fundamental evolutionary context; these considerations were summarized in the previous sections regarding the basic notion of an evolutionary discordance between our genes and our current technological environment, diet, and lifestyles. In simplest terms, overvaluing high-tech medicine over "low-tech" lifestyle change may be a mistake we are culturally primed to make in how we view health and how we construct and finance our health-care systems.

Whatever answers we might construct to such questions, there seems little question that Western societies face enormous challenges in a tsunami of age-related disease, in an aging population, at a time when fundamentally unhealthy lifestyles promoting those very same diseases of aging are widespread within the United States and in other Western societies. Health-care professionals of virtually all disciplinary persuasions need to take responsibility for educating both patients and the general public about these issues, as a critical part of reprioritizing genuinely proactive and early prevention efforts and health maintenance via lifestyle change over much later high-technology interventions that are proving to be prohibitively costly while at the same time yielding very uncertain if not minimal benefits in relation to quality of life.

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