Biological Theories of Aging and a Potential Means of Modifying the Aging Process *

In the last 200 years, a substantial increase in human life expectancy has been observed worldwide. This was achieved largely due to environmental changes, along with improved food, water, hygiene and living conditions, the reduced impact of infectious diseases via immunization and antibiotics, and improved medical care at all ages (1-4). Conversely, the abundance of calorie-dense food, together with the low requirements for physical exercise, has resulted in a variety of metabolic diseases that have had a major impact on people of all ages, but particularly contributing to deaths from cardiovascular diseases later in life (5).

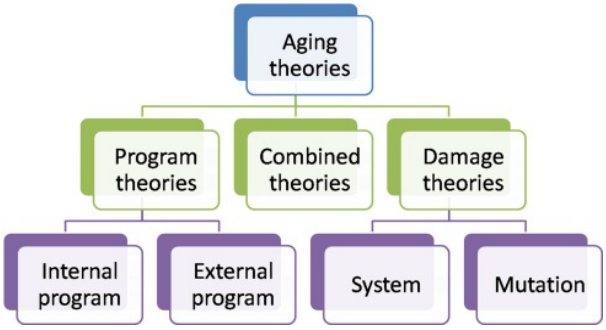
This chapter will briefly summarize the current ideas about the aging process, as well as review mechanisms involved in the acceleration of cellular aging, which consequently age multicellular organisms. Due to the complexity of the subject and the vast amount of material involved, additional information has been moved to the following appendices:

Appendix 1. The Free Radicals Theory of Aging
Appendix 2. The Glycation Theory of Aging
Appendix 3. The Results of the Accumulation of Toxins
Appendix 4. Longevity Genes and Hormonal Influences
Appendix 5. DNA Damage and DNA Repair
Appendix 6. The Cellular Senescence and Apoptosis in Aging
Appendix 7. The Immune System and Aging
Appendix 8. Recommended Herbs to Stay Young

Also, an ETI approach to aging will be described that might help energetically support and maintain the health of cells, organs and the whole organism, and thus enhance longevity.

1. Aging Theories

There are two main categories of theories that have been proposed in science's attempt to explain the process of aging. The first category is comprised of concepts holding that aging is programmed and the second contains those suggesting that aging is caused by the accumulation of damages. The latter category of theories suggests various sources and targets of the damages. It is conceivable that aging could vary across different species, and the hypothesized programmed senescence could accelerate the buildup of damage or decrease the capacity for repair.



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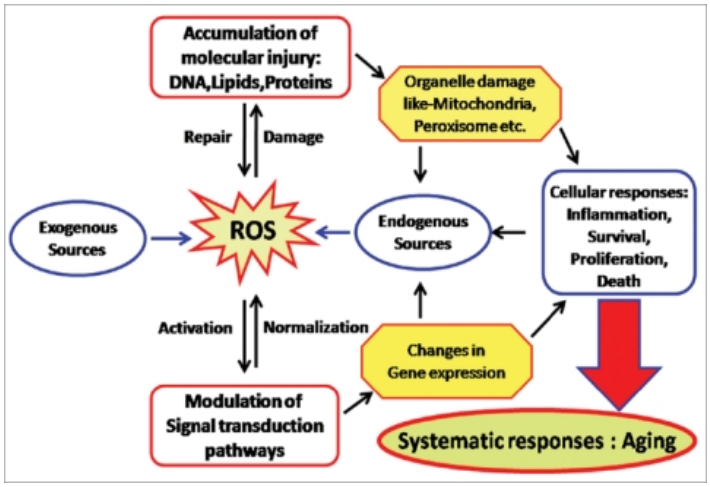
This paper will concentrate primarily on the aging process of those cells where the marks of aging consist of the following attributes: genomic instability, telomere erosion, epigenetic alterations, and loss of proteostasis, leading to deregulated nutrient sensing, altered mitochondrial function and cellular senescence (6).

Metabolism, the life-supporting process, generates toxins, more so in long-living post-mitotic cells, like neurons and cardiac myocytes, and these toxins accumulate in the cells as toxic biological products (7). They are stored in various cellular storage organelles like lysosomes, phagosomes, and proteosomes, with differing degradation mechanisms and kinetics. The toxic and degradation products in the cells weaken the mitochondrial revenue, leading to accumulation of "aged" mitochondria deficient in ATP, but releasing reactive oxygen species, manifesting as "pathology" related to aging (8). These sequences of events appear to compromise cellular adaptability, trigger the apoptotic pathway, and finally lead to cell death.

Mitochondria and reactive oxygen species

The primary function of mitochondria is respiration, which promotes energy production. During respiration, oxygen is reduced in several stages, producing a superoxide radical (O_2^-) and hydrogen peroxide. Most commonly, these molecules, are known as reactive oxygen species (ROS) (8, 9).



From www.researchgate.net/publication/45694952_Markers_of_Oxidative_Stress_in_Erythrocytes_and_Plasma_During_Aging_in_Humans

Short-lived ROS are potent inducers of oxidative damage to any biomolecule. Importantly, the role of ROS in age-associated pathologies has undergone several revisions in past years. Originally, it was believed that damaged mitochondria increase ROS generation and thus accelerate aging (10). However, it was eventually shown that most mitochondrial deficiencies do not end up with an elevated ROS. However, completely inactivated mitochondria pose a threat to the entire organism (11). For additional information, please refer to Appendix 1, *The Free Radicals Theory of Aging*.

Accumulation of by-products of metabolism

Another theory that attempts to explain the process of aging suggests cell senescence arises from the accumulation of biological waste (i.e., by-products) that cannot be completely removed from the organism. According to this theory, each by-product is broken down by an appropriate enzyme or a series of enzymes, which in turn makes the metabolism more complex and increases the array of by-products. The only mechanism by which these agents are diluted in the cells is cell division. The challenge for multicellular organisms, such as a human being, is that many cell types lose replicative capacity or divide slowly, even though they remain active throughout the lifespan. These cells, including cardiomyocytes and brain neurons, accumulate metabolic waste that eventually affects normal cell functioning (12-14).

Amyloid proteins are one class of toxic waste accumulating mainly in the nervous system. The best-described amyloid protein is β-amyloid, which is known to cause Alzheimer's disease (15). Amyloid β-peptide polymerization results in amyloid plaque formation in nerve cells, which brings about Alzheimer's disease (16).

The metabolic waste in the body's blood stream also includes, to a certain degree, spontaneously modified sugar-bound proteins, mainly glucose molecules. The main consequence of spontaneous glycation is impaired elasticity, which is problematic, since elasticity is essential to blood vessels (17). Also, spontaneous glycation affects normal protein functioning. To find out more about glycation, refer to Appendix 2, *The Glycation Theory of Aging*.

Until now, no enzyme has been discovered that is capable of metabolizing glycated products. Preventing spontaneous glycation seems to be impossible because all proteins contain lysine residues and glucose is one of the important substances in all living organisms. Glycation and oxidation products contribute to lipofuscin formation in lysosomes, reduced vessel elasticity, and deposition of insoluble aggregates on the walls of blood vessels and the nerve tissue (18). The amount of lipofuscin in a cell strongly correlates to the degree of dysfunctional mitochondria (19). For more information, see Appendix 3, *The Results of the Accumulation of Toxins*.

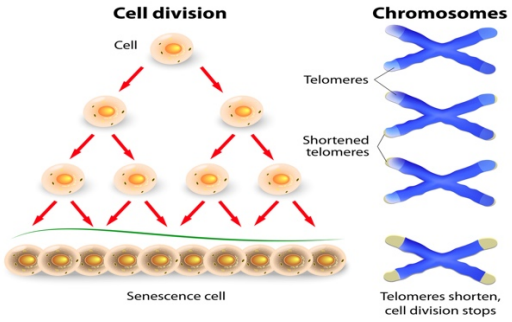
Dysregulation of regulatory pathways

Aging is associated not only with the buildup of metabolic by-products, but also with the dysregulation of regulatory pathways. For example, aging upsets the balance between pro- and anti-inflammatory components, thereby promoting chronic inflammation (20).

Besides the imbalance in pro- and anti-inflammatory responsiveness, aging can also impair other important pathways. The neuroendocrine theory involves the existence of self-regulatory mechanisms of homeostasis – negative feedback pathways (21). One of the essential systems is the hypothalamus-pituitary-adrenal axis. An elevation of the threshold of the hypothalamus to negative feedback signaling has been shown to account for unfavorable age-related changes in human health; in particular, reproductive decline (22). You will find more information in Appendix 4, *Longevity Genes and Hormonal Influences*.

Telomeres are the biological clock of the cell

DNA repetitive sequences (TTAGGG), or telomeres, which are found at the end of a linear chromosome, are lost during repetitive cell division, rendering chromosomes vulnerable to damage. With the shortening of a telomere and thus the chromosomal length, the cell enters irreversible growth arrest, replicative senescence (23). Telomerase, a cellular reverse transcriptase, helps to maintain the telomere length by adding the repetitive sequences (24, 25).



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Also, the oxidative intermediates can travel along the DNA, producing particular damage at triples (the normal version of the genetic code), and the cell's damage repair process is inefficient due to the fundamental biochemical nature of telomere protein (26). The result is the promotion of cellular senescence. These multifactorial events in the cells' lifespan dictate, to a significant degree, the aging process in the whole organism.

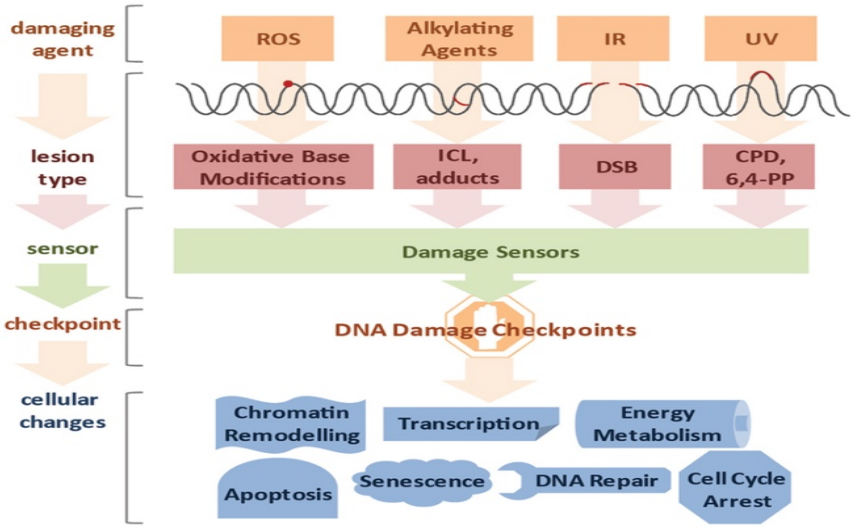
Overall, a thorough analysis of the mutations that cause a lifespan to increase in various model organisms brings us closer to understanding the concept of a biological clock that behaves as a function of time and the metabolic rate. A person's metabolic activity accounts, in large measure, for the formation of by-products that fail to undergo elimination and therefore accumulate. A higher metabolic rate contributes to a faster buildup of toxic metabolic waste and lesions. Conversely, a lowered metabolism, either in the context of calorie restriction or mutations affecting metabolic pathways, triggered by increased food intake, promotes lifespan extension through a decline in the accumulation of toxic by-products.

2. Genomic Instability and Aging

Genomic stability is threatened as soon as a cell is born, due to the intrinsic damage caused by energy generation and errors inflicted by the DNA replication. The damage repair processes are

presumably functioning at their best in new cells, so genomic instability likely does not become an obstacle until much later in life. Over time, equilibrium and cell renewal begin to fail, leading to the reduced replacement of cells lost due to erosion or senescence (27).

There are numerous sources of DNA damage, both endogenous and exogenous, which the cell must deal with. It is thought that a somatic cell may receive as many as 100,000 lesions daily (27, 28). It is not a coincidence that most age-dependent diseases, such as cancer, type II diabetes, and cardiopulmonary and neurodegenerative diseases are associated with increasingly elevated levels of genomic instability that occur over time (29-33). Age-related increase of DNA double-strand breaks is consistently considered as a genetic blueprint of progeroid syndromes because DNA double-strand breaks cause the most deleterious damage to DNA (34-37). For more information, refer to Appendix 3, *DNA Damage and DNA Repair*.



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Also, studies on centenarians have shown that the "oldest-old" population has enhanced DNA repair activity with significantly lower frequency in genomic and cellular damage, when compared to their younger counterparts (38, 39).

3. Aging Neurons

It will help to take a closer look at the normal aging of the nervous system, which is associated with some degree of decline in various cognitive functions. Neurons have significant homeostatic control of essential physiological functions, such as synaptic excitability, gene expression and metabolic regulation (40, 41). Age-related neuronal dysfunction probably involves a host of indirect changes involving the synapses, receptors, neurotransmitters, cytological alterations and electrical transmission, leading to cognitive dysfunction (42, 43), and the "sickness" of neuron is the culmination of a failure of the energy system in mitochondria (44), failure of the protective antioxidant system, and unbalanced calcium homeostasis, leading to exhaustion and neuronal death (45, 46).

The Ca^{++} ion is a central signaling molecule in various vital cellular functions, including energy production, cell proliferation, gene regulation, membrane excitability, synaptic transmission and apoptosis. Because of its universal nature, vital role in cell signaling and negative effect at high levels, this ion is maintained in the cell at a level that is 10,000 times lower than the concentration in extracellular space (47). Ca^{++} signaling depends essentially on a rapid and transient increase in its intracellular level by influx through ligand-gated glutamate receptors (NMDA-receptor), and voltage-dependent Ca^{++} channels and releases from intracellular stores, such as mitochondria and endoplasmic reticulum (48). A decrease in Ca^{++} buffering or delayed removal results in larger or prolonged calcium responses are characteristic of aged neurons (49). Na^{+} and Ca^{++} exchangers and plasma membrane Ca^{++} ATPase's are the major transport systems capable of rapidly extruding a large amount of Ca^{++} from the cell cytoplasm to extracellular space. An age-related decline in the function of these transport systems disturbing calcium homeostasis has been suggested to contribute to age-related neurodegenerative diseases (50).

4. Anti-Aging Strategies

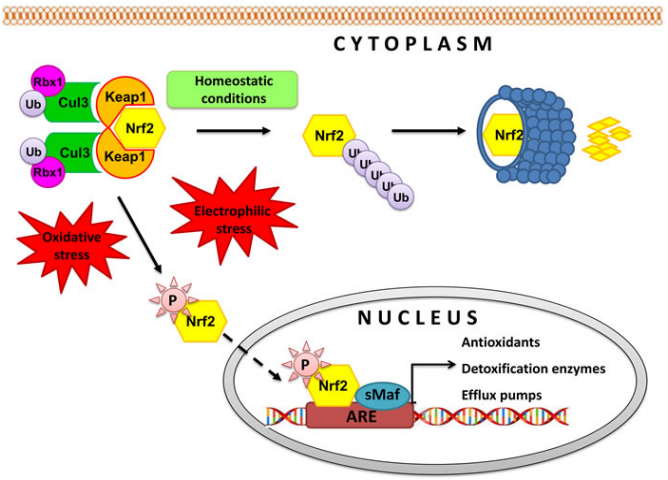
Many of these adaptive pathways are positively influenced by intellectual and physical activity, coupled with caloric restriction and lesser exposure to environmental toxins.

Nrf2 pathways

Under healthy physiological conditions, the steady-state concentrations of reactive oxygen species (ROS) and reactive nitrogen species (RNS) are regulated for proper cellular functions. Reduced surveillance of endogenous antioxidant defenses and/or increased ROS/RNS production leads to oxidative stress, with consequent alteration of physiological processes (51-54).

To avoid the harmful effects of ROS/RNS, cells must promptly respond to their damaging increase. Functionally, the main driver of the cellular antioxidant/detoxifying responses is the nuclear factor erythroid 2-related factor 2 (Nrf2), a transcription factor that induces the expression of a wide range of cytoprotective genes (55-58). The Nrf2 signaling is also involved in other cellular processes, including metabolism and cell proliferation/differentiation (55-59).

Nrf2 controls redox homeostasis, either by regulating basal expression of a range of genes involved in antioxidant defenses or by inducing their expression under stress conditions. In particular, Nrf2 activates the transcription of several antioxidant genes and major phase II detoxifying enzymes (55-57, 60), as well as encoding numerous enzymes of Phase I-III.



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Recent findings based on animal models studies have shown the neuroprotective effects of many compounds, such as the triterpene ginsenoside Rb1(61) and the alkaloid glaucocalyxin B (62), as well as endogenous metabolites like alpha-lipoic acid (63) and estradiol (64), all involved in the activation of the Nrf2/ARE signaling. Consistently, deregulation of the Nrf2 pathways has been linked with aging, as well as with enhanced susceptibility to and/or the accelerated progression of neurodegenerative diseases (65, 66).

Caloric restriction (CR) data

A number of researchers around the world have provided scientific evidence that caloric restriction (CR) is an effective strategy for life extension in several model organisms, including yeasts, fruit flies, fish, and monkeys (67-69). An increase in the lifespan of rats has been observed when nutrient availability drops between 30% and 75% of the species' normal calorie intake. Not only did calorie-restricted rodents live longer, but also a significant part of them (about 30%) died without any apparent pathology, raising the striking possibility that aging is not necessarily tightly linked with pathologies (70-73).

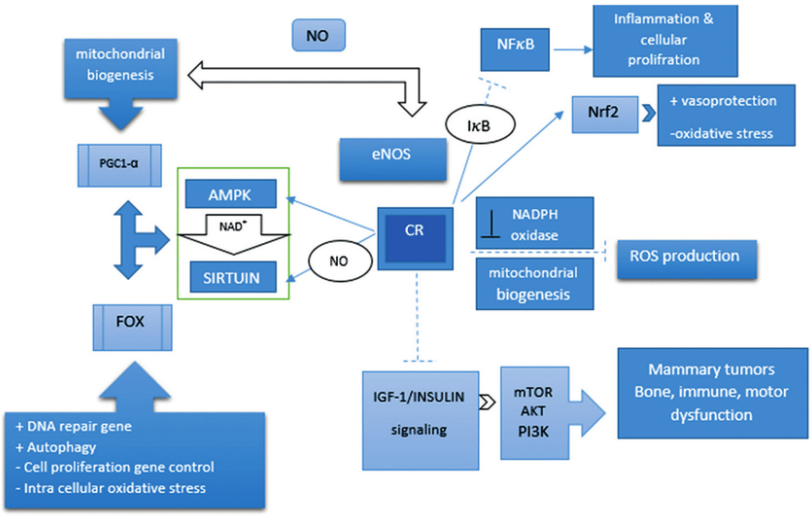
One experiment has shown that a 30% reduction in calorie intake for three months improved memory performance in elderly humans. Also, it has been demonstrated that CR can lead to up-regulation of brain-derived neurotrophic factor (BDNF), which is associated with neuronal plasticity and neurogenesis (74).

A preponderance of evidence suggests that long-term exposure to CR can lead to a reduction of circulating levels of several cytokines, growth factors and hormones, accompanied by a decrease of growth factor signaling, insignificant vascular perturbations and inflammation. Parallel with this data, these changes caused by CR result in decreased cancer risk and its progression (75). Also, findings across the study of human and nonhuman primates have indicated that CR could decrease triglycerides and blood pressure, and increase high-density lipoprotein levels (76). Evidence also exists indicating CR can delay the onset of autoimmune diseases through decreasing the proportion of B cells and preserving high numbers of native T cells and their immune responsiveness (77).

In addition, it is also clear CR feeding can delay the accumulation of oxidative damage markers -- for example, in the case of bases in genomic and mitochondrial DNA. The preservation of membranes and their fluidity by CR feeding is associated with the attenuation of age-related changes in membrane receptor numbers and binding affinity (78).

It is also known that IGF-1 plays a key role in the differentiation and proliferation of cells and prevents cell apoptosis of normal and cancer cells. Research has shown CR can protect us against cancer and slow the aging process through decreasing IGF-1 levels by about 40% (79). Together, adaptations that occur due to CR are a down-regulation of the insulin/IGF pathway (PI3K/Akt/mTOR), and upregulation of two energy-sensing pathways (sirtuin [SIRT] and activated protein kinase [AMPK]) which activate forkhead box O (FOXO). FOXO is tailored by upregulation of autophagy genes (known as the DNA repair gene), and down-regulation of genes that control cell proliferation (80-87).

It will be good to discuss further the role of CR-mediated anti-aging benefits to autophagy. Oxidative damage to macromolecules and organelles occurs through normal metabolism. If these damaged molecules are not removed by autophagy, they change into a source for the production of free radicals, which leads to oxidative stress, inflammation and severe diseases. Autophagy functions as a protective mechanism that removes damaged or aged organelles to protect cells from further oxidative stress, dysfunction and cell death. Data suggests that a decrease of amino acids due to CR stimulates autophagy and lysosomal proteolysis activity (88). Moreover, studies carried out on diverse eukaryotic species show that CR is a potent inducer of autophagy and can prevent age-associated diseases (89-90).



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It is also evident that nuclear factor-erythroid 2 (Nrf2) plays a key role in vasoprotection and regulation of the aging process by orchestrating the transcriptional response of cells to oxidative stress. CR restores Nrf2 expression and activity in aged cerebro-microvascular endothelial cells (91). Additionally, CR diminishes telomere erosion associated with aging and decreases cancer incidences through overexpression of telomerase (92). The nuclear factor kappa B (NF κ -B) is

also a redox-sensitive transcription factor that induces the expression of genes involved in cellular proliferation and inflammation. It has been suggested that CR increases cytoplasmic levels of I*kk*B, which prevent NF κ -B translocation into the nucleus (93).

Gut microbiome and aging

It is a known fact that with the growing aging population worldwide, the prevalence of metabolic diseases has radically increased. Dysbiosis in gut microbiome and microbial metabolites are known to be associated with aberrations of gut barrier integrity and enhanced pro-inflammatory cytokines. All these elements can also potentially or partly underlie the pathogenesis and progression of various metabolic diseases that are dominant in elderly people, such as adiposity, insulin resistance, fatty liver, hepatic steatosis, atherosclerosis, cardiovascular diseases and diminished motor activity (94).

Alterations in the aging gut microbiome can also impact the gut-brain axis, thereby hampering neural, endocrine, nutrient and immunological signals between the gut and brain via the enteric nervous system (ENS) and could play a role in diseases of the central nervous system (CNS), including multiple sclerosis, autism, depression and anxiety (95, 96).

Interestingly, the overall target of both probiotics and prebiotics is similar, i.e., restoring and/or maintaining the homeostasis of the gut microbial ecosystem, which can be particularly crucial during old age (97, 98). The fermentation of prebiotics by probiotics, mainly in bifidobacteria and lactobacilli, also increases the intestinal levels of SCFAs, especially butyrate, which has been shown to confer immune-modulating and anti-inflammatory effects (99). These effects could be particularly important for older adults, because they are already going through immune-senescence and are at increased risk of developing miscellaneous infections and illnesses (100, 101).

Other research data that can help to develop an anti-aging strategy

A number of seemingly unrelated studies—ranging from research on the human senses to chewing to aerobic exercise—when reviewed together, may point the way to understanding how to formulate an effective approach for anti-aging.

A continuous study over six years compared whole-brain volumes of older adults with hearing impairment to those without hearing loss, found that those with hearing loss had an accelerated loss of brain tissue (102).

Olfactory dysfunction accompanies or precedes the early symptoms of cognitive disorders, such as Alzheimer's disease, Parkinson's disease and schizophrenia (103).

The results of several animal studies support the idea that the active process of chewing plays a role in cognitive functioning and stress reduction. In humans, a strong correlation was observed between chewing ability (with or without the use of dental prostheses) and cognitive functions (104).

Aerobic exercise can restore some cognitive functioning when problems are encountered in the

aging brain. About 12 months of aerobic exercise was shown to increase the volume of the hippocampus (105, 106), while six months of aerobic training increased the volume of the brain gray and white matter in the experimental groups. In the control groups, it was found that stretching and toning for the same amount of time did not result in brain volume changes (107, 108).

As was mentioned earlier, restraining insulin/IGF-1 signaling has been shown to be an effective and efficient intervention applicable to lifespan extension and the prevention of age-related diseases. Keeping blood sugar in the low normal range can help to prevent the formation of advanced glycation end-products (AGE), one of the constituents of non-degradable cellular garbage (109).

These data (mentioned above), when utilized in the context of a particular person's conditions, can help to develop an effective individual anti-aging program.

Another consideration in developing an anti-aging strategy is to avoid extra iron supplementation, while at the same time increasing one's intake of melatonin and CoQ10. Cells do not have the capacity to rid themselves of intra-lysosomal iron that is bound to lipofuscin (LF). It slowly accumulates over time, even if the uptake of iron is effectively regulated (110, 111). This accumulation is particularly evident in the reticula-endothelial system, in hepatocytes and in long-lived postmitotic cells, such as neurons and cardiac myocytes. It seems most probable that the sensitivity to oxidative stress is enhanced in such cells at the end of their lifespan (112). A study with rats showed that treatment with melatonin or coenzyme Q10 for four weeks reduced the LF content of the hippocampus and carbonyl level (113). In fish, analysis of age-dependent mortality revealed that dietary restriction prevented the accumulation of LF in the liver (114).

5. ETI/VFT Approach to Anti-Aging

Energy is absolutely essential for our body to function in a healthy manner. ETI energy formulas progressively attune the body to more energetic coherence on the physical, emotional and mental levels through the resetting of regulatory points, increasing one's energy level, and potentiating the body's self-recovery process. For the last ten years, VFT has been used to develop several specific formulas that might have anti-aging advantages.

Longevity formula

Description:
This formula was created with the intent to energetically help the body better manage the interaction between our genetic uniqueness and the existing environment, and thereby improve our genetic potential and decrease age-related chronic conditions.

This product works with an energetic template to: a) influence and strengthen the subtle system of whatever organ or system is the most chaotic and disorganized on the energetic level; b) activate the body's anti-aging potential by supporting equilibrium within the hypothalamic-

pituitary-adrenal-thyroid axis; c) create the possibility of energetically mitigating against DNA damage; d) possibly improve the energetic interaction between the human gene matrix and environmentally derived signals, and thus energetically support the human genotype on the level of each individual's needs, as well as energetically maintaining the body's functional reserve.

According to Dr. Jeff Marrongelle and Dr. Stephen Davis, Longevity formula is one of the broadest and most physiological of all ETI energy formulas and might create the global effect on any type of physical conditions. Also, Longevity formula might work in that space of cellular metabolism and respiration and inner cellular function, providing energy to that which creates our life force, and that is one cell at a time. So, whether it is an organ or tissue or organ system, they all need to have a capability to rejuvenate, regenerate and create healthy, normal, vibrant cells. That is what Longevity formula is about.

Dose: 10-15 drops per dose diluted in 2-4 oz. of water 2-3 times a day.

Adverse Effects: No side effects expected within the recommended dosage.

Cells Longevity formula

Description:

The in-vitro experiment with Cells Longevity formula was done at the Riga Stradins University in Latvia. It was focused on subtle energy's ability to influence cell's viability and the mitochondrial membrane potential under "food deprivation" conditions. A human embryonic kidney cell line (HEK-293) was used for the experiment.

The experimental results showed an extraordinary ability of the Cells Longevity formula to provide high viability and proliferation of cells in food deprivation conditions, actually increasing the cell viability for 46% higher than the control group. In addition, the researchers studied subtle energy's effects on cells when strong mitochondrial toxins are present, and found that in the infused media, the viability of cells was better—not only in comparison with the control containing mitochondrial poison, but even in comparison with the non-poisoned control.

Dose: 10-15 drops per dose diluted in 2-4 oz. of water 2-3 times a day.

Adverse Effects: No side effects expected within the recommended dosage.

Anti-aging Formula

Anti-aging formula, from the energetic point of view, might progressively attune the body to more energetic coherence on the physical, emotional and mental levels through the resetting of regulatory points, increasing one's energy level, and potentiating the body's self-recovery process via:

- supporting **equilibrium within the hypothalamic-pituitary-adrenal-thyroid axis** by using the energetic blueprint of the following herbs: astragalus, Korean ginseng, reishi, kava, as well as ETI's Total Adaptogen, Longevity, Perfect Vitality, and Stress Relief formulas

- maintaining **the body's functional reserve** via ETI's Perfect Vitality and Kidney Support formulas

- improving **the immune system profile and status** via ETI's Chronic immune and Thymus formulas

- supporting **optimal production of the Nrf2 factor** and thus improving the transcription of several antioxidant genes and major detoxifying enzymes**

- enhancing **the mitochondria biogenesis** via ETI's Mitochondria support formula

- possibly improving **the interaction** between our gene matrix and environmentally derived signals and thus supporting our genotype on the level of the individual's needs via the ETI's Cells Longevity and Longevity formulas

- supporting **efficient cell cycle control** via Formula AC, Cells Longevity, and the energy pattern of the Protocel.

- supporting the **balancing expression** of telomerase, therefore diminishing its erosion associated with aging and reducing the probability of cancer incidences through overexpression of telomerase via ETI's Telomerase formula;

Dose: 10-15 drops per dose diluted in 2-4 oz. of water two times a day for at least 2 months

Adverse Effects: No side effects expected within the recommended dosage.

***NOTE:** These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease.

**We used the energetic blueprint of a specific herbal combinations that supports the Nrf2 factors activation like ashwagandha, bacopa, green tea, milk thistle, and turmeric.

Appendix 1. *The Free Radicals Theory of Aging*

Free radicals are highly reactive molecules or atoms that have an unpaired electron in an outer orbital that is not contributing to molecular bonding. Normal molecular oxygen has two unpaired electrons in outer orbitals with two pi–bonds formed from two p–orbitals, each containing one electron. If an electron is added to normal triplet oxygen, the new electron completes one orbital, leaving the other orbital with an unpaired electron, resulting in a superoxide $O2^-$, which is a conventional free radical.

Other oxygen free radicals in biological systems are nitric oxide (NO) and the hydroxyl radical (OH), which can damage nucleic acids, proteins or lipids. About 0.3% of superoxide exists in the form of (HO2), which is more reactive than superoxide itself. Because this form is uncharged, it can penetrate cell membranes more effectively than superoxide. Nitric oxide is a relatively unreactive free radical, which has a half-life of a few seconds, normally reacting quickly with oxygen (O2). But if nitric oxide comes across a superoxide ($O2^-$), it forms peroxynitrite ($ONOO^-$), which can form a hydroxyl radical (OH). Peroxynitrite can react directly with proteins and other macromolecules to produce aldehydes and ketones, cross-linking and lipid peroxidation.

A cell's superoxide ions tend to be concentrated in the mitochondria, because they are too reactive to travel very far in an unchanged state and are much less frequently found in the nucleus than in the cytoplasm. Similarly, hydroxyl radicals (which have a billionth-of-a-second half-life) do not drift far from their site of formation. But hydrogen peroxide molecules are more stable and can drift across the nuclear membrane into the nucleus or near cell membranes, where hydroxyl radicals can be generated when heavy metal ions are come across. These radicals can then react directly with oxygen forming lipid peroxides (lipid peroxyl radicals, i.e., lipid molecules containing paired-oxygen groups -OO-).

In living cells, peroxidized membranes lose their permeability, becoming inflexible, reactive and nonfunctional. Lipid peroxidation can produce singlet oxygen, hydroperoxides and lipid epoxides. In addition, many damaging aldehydes are formed during lipid peroxidation. Unlike free radicals, the aldehydes MDA, 4–HNE and other aldehydes are long lived and can drift far from membranes, damaging a wide variety of proteins, lipids and nucleic acids.

Animal cells contain three important enzymes to deal with superoxide and hydrogen peroxide: Superoxide dismutase, glutathione peroxidase and catalase.

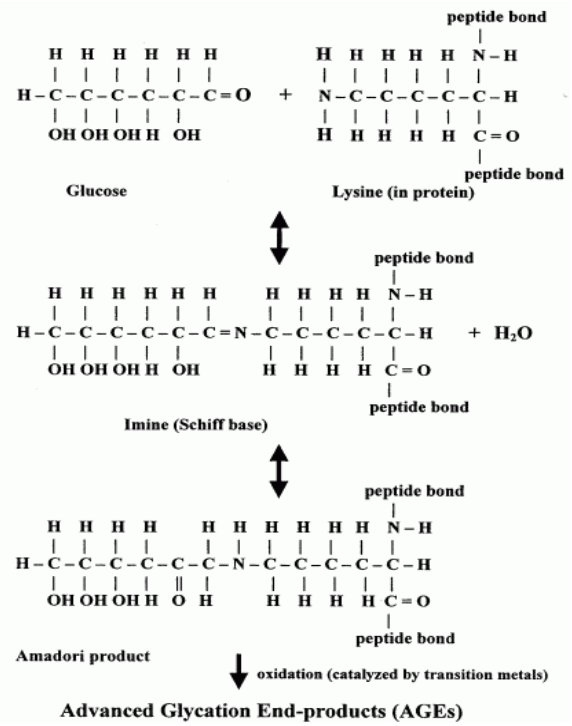
Superoxide dismutase (SOD) is an enzyme that catalyzes the reaction of two identical molecules to produce molecules in different oxidative states. In the absence of SOD, two superoxide ions can spontaneously dismutase to produce hydrogen peroxide and singlet oxygen. SOD catalyzes a reaction between two superoxide ions to produce hydrogen peroxide and triplet oxygen. The liver, in particular, is very high in SOD. Catalase (CAT) catalyzes the formation of water and free oxygen from hydrogen peroxide.

Glutathione is a peptide composed of the amino acids cysteine, glycine and glutamic acid. It is also the major antioxidant in the non-lipid portion of cells (most of the cytoplasm). Glutathione peroxidase/glutathione destroys fat peroxides in the same way it eliminates hydrogen peroxide.

Appendix 2. *The Glycation Theory of Aging*

Proteins are long chains of amino acids (amino acid polymers, or polypeptides). Amino acids are all organic compounds with an amino group [$-NH_3^+$] and an ionized carboxyl group [$-COO^-$] attached to the same (alpha-position) carbon atom.

Proteins can be damaged both by free-radicals and by glycation. Glycation is a reaction by which reducing sugars become attached to proteins without the assistance of an enzyme.



Advanced glycation end products (AGEs) in tissues increase the rate of free radical production to 50-times the rate of free radical production by unglycated proteins. AGEs attached to LDL cholesterol accelerates oxidation and subsequent atherosclerosis. The irreversible cross-linked protein of AGEs in vessel collagen also contributes to atherosclerosis, as well as to kidney failure. AGEs aggravate protein cross-linking in the plaques, thus accelerating neuron death.

AGEs can be formed in the body from glycation and oxidation or can be ingested directly from foods (such as fried poultry skin) or tobacco smoke. Approximately one third of absorbed dietary AGEs are excreted in urine, but the rest is incorporated into body tissues. AGEs are universal symptoms of aging affecting skin, lungs, muscles, blood vessels and general organ function.

Lipids, as well as proteins, are subject to glycation. Lipid glycation of LDL cholesterol increases the LDL oxidation associated with atherosclerosis. Another form of protein damage is racemization, although this kind of protein damage is less serious than glycation. Cells can only make proteins from L–isomer ("left-handed") amino acids. Only L–isomer proteins are functional. Some D–isomer ("right-handed") proteins are not only non-functional, but harmful.

Thermal energy causes a small percentage of proteins to spontaneously change from the L–form to the D–form, and this form of molecular deterioration is known as racemization.

Also, carbonyl (aldehyde or ketone) content of protein is used as a measure of protein oxidation. Carbonyl formation is irreversible, so oxidized proteins must be removed by degradation. Carbonyl content of protein in an animal cell increases exponentially with age.

Cellular proteins are continually being degraded (hydrolyzed) within cells by proteolytic enzymes, both for regulation of cellular processes and for "quality control" of proteins. The four major classes of cellular proteolytic enzymes are: (1) caspases, (2) calpains, (3) cathepsins and (4) proteasomes. Caspases are mainly active in apoptosis and are therefore in the category of regulatory proteases. Calpains are Ca^{2+} dependent, ATP independent proteases that mainly degrade membrane and cytoskeletal proteins (as well as certain transcription factors).

Proteins can also be brought into the lysosome for degradation by chaperone-mediated autophagy (CMA), in which members of the hsp70 heat shock protein family (the chaperones) attach to a target protein and then bind to a lysosome receptor protein. With aging, lysosomes of postmitotic cells increasingly become bloated with aggregates of oxidized, glycated, cross-linked proteins that are resistant to enzymatic degradation.

The "error catastrophe" theory of aging proposes that accumulating damage to synthesized proteins results in damage to the machinery of synthesis itself, leading to an escalating circle of malfunctioning cellular components. But the rate of both protein synthesis and protein degradation declines with age, and the inability to eliminate damaged macromolecules may be more catastrophic than the synthesis of new defective ones.

Appendix 3. *Accumulation of Toxins*

Many chemicals accumulate in the cells with age, including toxic and inactive substances from the environment and similar substances arising as byproducts of cellular metabolism. Fat-soluble substances (such as DDT and PCBs) are particularly slow to be eliminated. Iron tends to accumulate in cell nuclei with aging, as does aluminum. Aluminum transforms metabolically active DNA into an inert state. Lead also accumulates in cells, and is neurotoxic. Cytochrome P–450 detoxification enzymes of the liver decline with age.

For non-dividing cells that cannot be replaced, the accumulation of cellular debris may be a very significant factor in cellular aging. A species' survival may therefore be dependent on the creation of new organisms, once the old ones have accumulated too much chemical debris to be functional.

Of particular note is lipofuscin (age pigment), which can accumulate in large quantities in non-dividing cells. Lipofuscin is regarded as a product of lysosomes' organelles that contain hydrolytic enzymes to degrade proteins, lipids and damaged organelles. As production of lysosome enzymes decline with age and as lysosomes overcome increasingly cross-linked proteins and lipids that are resistant to enzyme degradation, dysfunctional lysosomes accumulate in cells as lipofuscin granules. Lipofuscin granules are characterized by a single membrane

envelope, enclosing yellowish-brown material. Inhibitors of proteases (enzymes that degrade protein) and vitamin E deficiency result in lipofuscin-like cellular residues lipofuscin. There is evidence that lipofuscin formation inhibits protein degradation, thereby creating a vicious cycle that promotes its own. Lipofuscin accumulation in the non-dividing cells of the brain and heart is very prominent and is, in fact, regarded as a biomarker of aging. Lipofuscin accumulation in retinal pigment epithelial cells may lead to age-related macular degeneration, the leading cause of blindness in the developed world. The fact that lipofuscin accumulates at a higher than normal rate in those with Alzheimer's disease and the fact that the disease is also characterized by abnormal tau-protein and amyloid-protein suggests that creation of defective protein and/or problems with removal of defective protein could be the underlying cause of Alzheimer's disease.

Lysosomes are normally responsible for degradation of aging mitochondria. But as lysosomes become increasingly dysfunctional due to ingestion of indigestible lipofuscin, cells become increasingly populated with aging, swollen mitochondria that produce less energy and more superoxide. Reactive oxygen species produce more aldehydes and more aldehyde-bridges between proteins, resulting in more lipofuscin. Thus, there is a positive feedback loop of lipofuscin production, impaired lysosomes, dysfunctional mitochondria and aldehyde formation.

Appendix 4. ***Longevity Genes and Hormonal Influences***

Insulin-like growth factor–1 (IGF–1) is a mitogen and an important mediator of the growth hormone (GH) effect. High GH and IGF–1 increase tissue development, metabolism and glucose utilization at the cost of higher oxidative stress, more protein glycation and higher proliferation.

According to the glucocorticoid cascade hypothesis, glucocorticoid steroid hormones show rising blood levels with age, which increasingly damage feedback inhibition neurons in the hippocampus, resulting in even greater increases of blood glucocorticoid and a destructive feedback loop. Glucocorticoid hormone (cortisol in humans) is a normal response to stress. Cortisol mobilizes blood glucose and depresses the immune/inflammatory response, among other effects. Although useful in emergencies, chronic stress can be catabolic. High blood levels of glucocorticoids are sensed by neurons in the hippocampus, which signal the brain to release less vasopressin. The involvement of hippocampal neurons makes sense, because stressful situations are often associated with intense and detailed memories. Cortisol can reduce neuron uptake of glucose by 15-25%, which can contribute to neuron death. Also, glucocorticoids reduce cellular SOD and glutathione peroxidase activity in all brain areas.

Humans and other primates are the only species that produce and secrete the hormone dehydroepiandosterone (DHEA) in quantities greater than any other steroid. DHEA levels peak in the late 20s and decline to 10% of the peak by age 80. DHEA may protect against the harmful effects of cortisol, while contributing to androgen and estrogen synthesis in peripheral tissues, promoting lean body mass, reducing depression and improving immune function.

Growth hormone (GH) also declines with age (about 14% per decade after age 25), and this decline has been blamed for increased fat deposition, loss of muscle mass and bone demineralization. There is evidence that GH replacement can improve cardiovascular health,

boost immune function and improve cognitive function in older adults, but there is also the danger that GH replacement can increase insulin resistance and cancer risk.

Current data demonstrate the decline in receptor sensitivity, rather than declining hormone release, is associated with age. Therefore, hormone replacement will not get to the root of the problem and can be harmful, whether or not it is shown to cause cancer.

Appendix 5. *DNA Damage and DNA Repair*

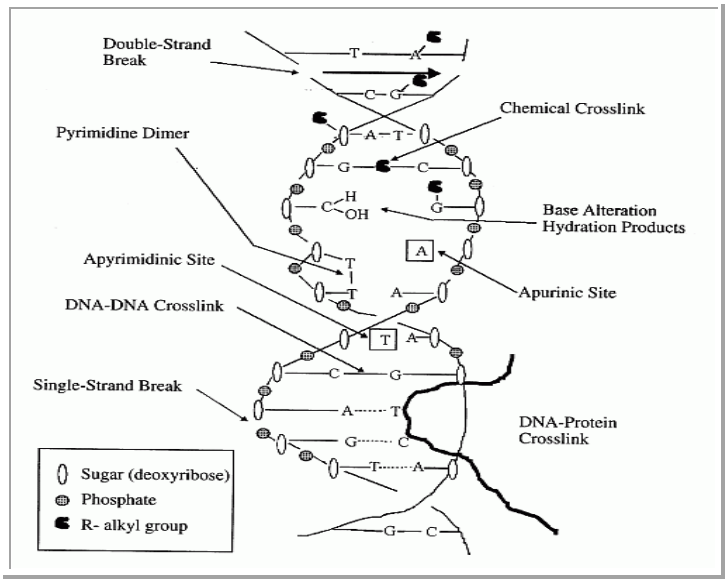
Cell structure and metabolism operates under the direction of genes, which are located in the DNA (deoxyribonucleic acid) of the chromosomes of all animal cell nuclei.

Animal genetic material in the cell nucleus exists as a complex known as chromatin, which consists of DNA, five histone proteins and some non-histone proteins. With age, the compacting of chromatin increases, probably due to increasing covalent linking between DNA and the chromosomal proteins. Because compacting helps determine which genes are expressed and which genes are not, the increased compacting of aging probably means a decline in gene expression.

Aging theories associated with DNA include programmed aging (or programmed aging resistance) and theories that link aging with DNA damage/mutation or DNA repair capability.

Environmental influence on DNA can take two forms: mutation and DNA damage. DNA damage tends to interfere with gene expression by preventing transcription of RNA from DNA, whereas mutation usually results in transcription, which typically produces proteins with diminished or altered functionality. Mutations that are not lethal to a cell are more likely to be perpetuated in dividing cells. DNA damage, rather than DNA mutation, is posited as a cause of aging. There are more than 200,000 DNA damage events per mammalian cell per day due to oxidation, hydrolysis, alkylation, radiation or toxic chemicals. Types and frequency of DNA damage are roughly illustrated in the following picture:

Summary of Types of DNA Damage



Mitochondrial DNA, rather than nuclear DNA, is the primary site of damage. The most active DNA repair enzymes, excision repair enzymes, all operate on the basis of damage or mutilation occurring to only one of the two strands of the DNA double-helix, such that the undamaged strand can be used as a template to repair the damaged strand. The damaged area of the injured strand is cut-away by a nuclease enzyme, and a new strand (or a single nucleotide) is constructed.

The amount of oxidative DNA damage in neurons is likely to be many times greater than in most other cells. The human brain accounts for only 2% of total body weight, but 20% of resting oxygen consumption due to the high metabolic demand required to maintain membrane ion potentials. Neurons transcribe about 2–4 times as much DNA as do cells from the kidneys, liver or spleen.

Because of the rapid revenue of mitochondria in cells, oxidative damage to mitochondrial lipids (membranes) and proteins is normally less of a concern than oxidative damage to mtDNA. But with age, lysosomes become less efficient at removing defective mitochondria. Oxidative damage to cardiolipin in the inner mitochondrial membrane reduces oxidative, which is probably an important factor in the declining ATP production by mitochondria associated with aging. Additionally, the enzymes responsible for importing DNA repair proteins into the mitochondria become increasingly defective with age (possibly due to oxidative damage).

Apoptosis (cell suicide) is the most effective defense against DNA damage and mutation when DNA repair enzymes are inadequate to fix the damage. The resultant decline of DNA repair associated with decreased apoptosis for DNA damage can contribute to cancer, and to aging.

Appendix 6. *Cellular Senescence and Apoptosis in Aging*

The relationship between cellular aging and aging of the whole organism is complex. Cellular immortality is essential for stem cells, but an immortal somatic cell is cancerous. As has been

alluded to, apoptosis is a programmed cell suicide that causes cells to shrink and be eliminated without the tissue traumas associated with the inflammation that goes with uncontrolled cell death (necrosis).

A youthful, healthy organism has efficient cell cycle control and can thereby resist undesirable apoptosis, while efficiently using apoptosis when needed. Aged cells with less effective cell cycle control will less readily undergo normal apoptosis when defective, but will more often result in dysfunctional apoptosis. High levels of apoptosis in aged tissues result in tissue degeneration.

Senescent cells (cells that no longer proliferate or divide in response to growth factors or mitogens) can function like normal cells, but display a number of distinctive characteristics. Some of these characteristics, such as increased free radical production, increased oxidative damage, increased glycation damage and reduced heat shock protein expression may simply be due to the fact that senescent cells are "old." Senescent cells are not only more sensitive to cell injury, they have larger nuclei and less regular shape. The accumulation of numbers of senescent cells within tissues could contribute to the aging of tissues and organs. So-called premature cellular senescence can be provoked by various sublethal cellular stresses, such as hydrogen peroxide, ultraviolet irradiation and similarly damaging agents, which either accelerate the number of telomeres lost per division or directly induce DNA damage or both.

Appendix 7. *The Immune System and Aging*

Many aging effects are due to the declining ability of the immune system to differentiate "foreign" from "self" proteins. Not only does the immune system become less capable of resisting infection and cancer, but declining cell function could be due to attacks by the immune system against native tissues. Arthritis, psoriasis and other autoimmune diseases are known to increase with age.

The thymus gland of the immune system reaches its greatest weight during puberty, and shrinks thereafter, with lymphoid tissue being replaced by fat. The shrinking of the thymus gland proceeds far more rapidly than the progress of aging and at age 50, the thymus of humans is typically only 5–10% of its original mass. Nonetheless, T–cells remain fairly constant over most of one's adult life due to peripheral proliferation (although proliferation declines in the elderly). Because the thymus is the organ in which T–cells "mature," once maturation occurs most of the work of the thymus is done. In the maturing T–lymphocyte system, the thymus creates a broad diversity of T–cells, each of which is programmed to recognize and combat a different antigen.

The immune system uses proliferation and apoptosis to create and refine T–cells. The immune system uses clonal expansion (rapid multiplication of lymphocytes of a single "clone" against a single antigen) and apoptosis to control the numbers of T–cells available to fight specific antigen threats.

There are two types of helper T–cells, designated TH1 (type 1) and TH2 (type 2). The TH1 cells promote the growth of T–lymphocytes with the cytokine interleukin–2 (IL–2), whereas the TH2 cells promote growth of B–lympocytes with the cytokine interleukin–4 (IL–4). TH1 cells are

more prominent in autoimmune conditions, whereas TH2 cells are more prominent in viral infections. In youth and maturity, the TH1 cells predominate, but in the elderly the TH2 cells predominate. Moreover, aging is accompanied by a significant loss of IL–2, as well as of IL–2 receptors, a phenomenon thought to be responsible for the significant decline of proliferation (clonal expansion) in response to antigens seen with aging. The decline of T–cell activation due to reduced IL–2 production is at least partially due to oxidation-damaged proteasomes being less capable of inducing the gene transcription factor NFκB.

Proliferation of T–cells in response to antigenic or mitogenic (cell-division stimulating) signals also declines with aging, especially due to a decline in the activity of the mitogen activating protein kinase (MAPK) cascade, which causes cell surface signals to alter gene expression. Caloric restriction significantly reduces the decline of MAPK activity associated with aging.

Natural killer (NK) cells differ from cytotoxic T–cells by their ability to destroy pathogenic cells without the need of antigens. NK cells decline in activity with age, but this decline is compensated for by an increase in NK cell numbers. B–cells from older animals produce fewer antibodies and express less of the surface CD40 protein, which causes B–cell activation and differentiation. The decline in T–cell activity with age is responsible for most of the decline in B–cell numbers and activity.

Macrophages are immune system cells that "eat" foreign particles (including bacteria) and digest the particles in lysosomes. Monocytes are the small blood stream cells that swell to become macrophages after migrating into tissues. Monocytes from elderly humans have a greatly reduced capacity to produce the cytokine interleukin–1 (IL–1) and the toxic free radicals that macrophages use to kill foreign or cancerous cells. Nonetheless, the superoxide, hydrogen peroxide, hydroxyl ions and nitric oxide produced by neutrophils and macrophages to kill bacteria can attack native tissues in age-associated chronic inflammation.

Appendix 8. *Recommended Herbs to Stay Young*

Since ancient times, people have tried to find medications to cure different illnesses. The healing properties of certain medicinal plants were identified, noted and used. The recommendations made below are based on this herbal tradition of medicinal healing. To learn more about the specific herbs and herbal supplements, go to the NIH Medline Plus database of herbs and supplements: https://medlineplus.gov/druginfo/herb_All.html.

1. **Bilberry** (*Vaccinium myrtillus)* contains a special antioxidant called anthocyanosides that helps prevent many age-related problems. This herb is particularly good for preserving vision and preventing degenerative eye diseases.
2. **Celery seeds** (*Apium graveolens)* help decrease blood pressure, preventing gout and other types of arthritis, contain dosage of anti-inflammatory ingredients, including apigenin, a COX-2 –inhibiting compound.
3. **Echinacea** (*Echinacea, spp.)* is one of the best herbal immune-system enhancers.
4. **Evening Primrose** *(Genothera biennis)*. For premenopausal woman, this oil prevents PMS and menstrual cramps. It is also useful in combination with **Saw Palmetto** to protect from prostate enlargement.

5. **Garlic** *(Allium sativum)* is a potent antimicrobial agent that helps defeat a wide range of viruses, bacteria and fungi, lowers blood pressure and cholesterol, and can be used to inhibit the formation of internal blood clots.
6. **Ginkgo (***Ginkgo biloba)*. Among ginkgo's many benefits, it improves blood flow through your body, especially in your brain. Additionally, it helps protect against heart attacks, muscular degeneration and the kind of impotence caused by reduced blood flow into the penis.
7. **Hawthorn** *(Crataegus monogyna)*. It gently normalizes one's heart function in both congestive heart failure and coronary artery disease.
8. **Horse Chestnut** *(Aesculus hippocastanum)* helps protect from varicose veins.
9. **Kava** *(Piper methysticum)* helps to relieve and slow the aging process itself.
10. **Milk Thistle** *(Silybum marianum)* is the best herb to support the liver against cirrhosis and hepatitis.
11. **St. John's Wort** *(Hypericum perforatum)* is an effective treatment for mild to moderate depression.
12. **Saw Palmetto** *(Serenoa repens)* prevents prostate enlargement.
13. **Tumeric** (*Curcuma longa*) has remarkable anti-inflammatory activity, helps with pain, aches, inflammation of joints, tendons and muscle problems.
14. **Ashwagandh**a *(Withania somnifera*) is an adaptogenic, anxiety reducer, anti-inflammatory, sexual enhancement and anti-spasmolytic, and has blood pressure-reducing properties.
15. **Astragalus** *(Astragalus membranaceus*) helps to decrease fatigue, is a tonic and stimulates the immune system.
16. **Asian Ginseng (***Panax ginseng)* helps to increase vitality, especially after illness, is a tonic for the elderly, improves appetite, tone of skin and muscles, and restores sexual energy.
17. **Licorice (***Glycyrrhiza glabra)* treats ulcers, has potent anti-inflammatory properties, protects liver, stimulates immune system, and is an antioxidant.

*__Disclaimer__: *These statements have not been evaluated by the Food and Drug Administration (FDA). The provided information is not intended to diagnose, treat, cure or prevent any diseases or medical problems*.

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