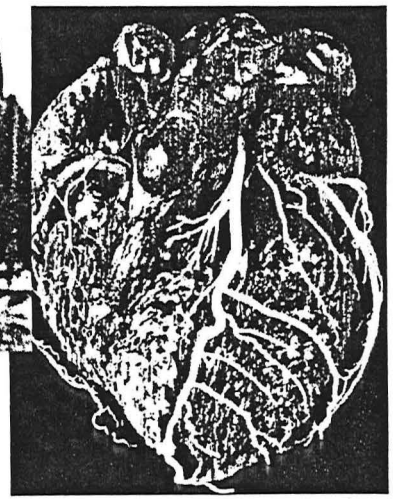
CARDIOVASCULAR AGING: GENES, GENDER AND GRUMPINESS



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Introduction

An Overview

Human aging begins to assume physiological importance in the third decade of life as discrete processes, which are essential either for physiological maintenance or adaptation, begin to fail and, ultimately, impose a finite life-span on the organism. Age is a major risk factor for cardiovascular disease, the leading cause of mortality beginning in the forties. Recent demographic trends and successful treatment of early-onset cardiovascular diseases suggest that an even greater number of individuals with advancing age will require future cardiovascular services. Both politicians and policy-makers recognize there is urgent need to develop research priorities and strategies for coping with the burgeoning number of older Americans in the next millennium.

In spite of its universality, there is an active debate about what is the fundamental mechanism(s) of aging? A substantial literature suggests that genetic factors, related to the finite replicative capacity of living cells or organisms, may be a critical determinant of life-span. Alternatively, several lines of evidence suggest the causal factor is related to the cumulative effects of oxidative damage, which increases exponentially with age in postmitotic tissues such as the brain and heart. One fact is indisputable: increasing chronological age of humans, even in the absence of disease, is accompanied by decreases in a number of age-associated parameters including cardiovascular fitness, attributed to physiologic attrition.

In today's Grand Rounds, I will discuss the evidence and attempt to reconcile how these seemingly disparate, mechanistic theories contribute to the expression of cardiovascular pathologies (cardiac myopathies, ventricular dysfunction, and arrhythmias) in aging individuals. Available evidence will also be reviewed that indicates there is under-utilization of cardiovascular services in the aged, which, if optimized, could retard physiological aging by improving their functional capacity for self-reliance, to their families, and to society.

A case report[1]

J. C. was a French citizen who died recently of apparent natural causes in a nursing home in Arles. At 122, she was the oldest person in recorded history and was born 14 years after the Eiffel Tower was built, and a year after the telephone was patented. Press accounts indicate J.C. enjoyed good health, though almost blind and deaf, until the end. She was married in 1896, had one daughter and became a widow when her husband died after eating a dessert of spoiled cherries, in 1942. After her daughter died in 1934, J. C. raised her grandson who later became a medical doctor and who died in an automobile accident in 1960. She reportedly ate two pounds of chocolate per week, rode a bike until she was 100, and quit smoking five years before she died. Her family history is also significant for longevity; her mother lived to age 86 and her father lived until 93. Asked about her longevity, J. C made this remark about stress "If you can't do anything about it, don't worry about it". She was famous in France for her (bons mots) witty jokes. One favorite of hers: "I've never had but one wrinkle, and I'm sitting on it".

The scope of the problem

The concern for the aging population is of vital importance, and has not escaped the attention of clinicians (i.e. geriatricians), researchers and the nation's policy makers [2]. Estimates from the Bureau of the Census place the number of Americans at approximately 70 million or 20 percent of the population over the age of 65, by the year 2030. Indeed, Americans 65 and older have increased 11 fold in this century alone. Industrial societies such as Japan have reached similar projections. Individuals over 85 "the oldest" is the fastest growing segment of the aging population. Over age 75, most women are widowed, whereas most men live with their wives. Although 5 percent of older individuals live in nursing homes, seventy percent are women. In at least seven Western states-Alaska, Arizona, California, Colorado, Nevada, Utah and Washington-, the elderly population is expected to double by 2020. Seventy percent of older Americans reported

voting in the 1992 presidential election. Four of every five centenarians, like J.C, are women and this number has doubled since 1980 [3, 4].

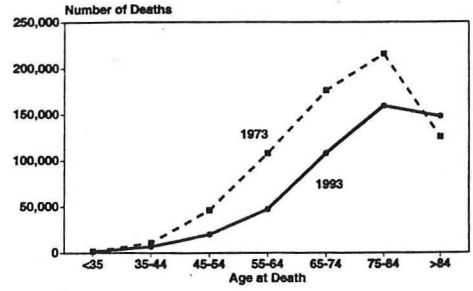
Table 1 shows some terms used commonly in reference to the aging population.

Table 1. Terms	Age group
Middle age	45-65
Aged	65-74
Old	>75
"Oldest old"	>85

Cardiovascular Trends in the Aging Population

Age is a major risk factor for cardiovascular disease. Beginning in the fourth decade of life, death from age-related heart disease disproportionately claims a great number of older men and women. In Americans over 65 years, roughly one-half of all deaths are attributed to cardiovascular diseases. Likewise, the morbidity associated with chronic atrial fibrillation, ventricular arrhythmias, and congestive heart failure, shortens the productive and creative years while increasing the disability from poor quality of life. Formidable progress, from almost 50 years of cardiovascular research and therapeutic interventions, has reduced the early death rate from coronary and other heart diseases. Figure 1 shows the age-adjusted death rates for coronary heart disease in the United States for two periods, 1973 and 1993 [5].

Figure 1



Dr. Claude Lenfant, Director of the National Heart Lung and Blood Institutes, wrote recently: "the fact that we have pushed heart disease in even greater ages is cause for celebration" [6]. Indeed, the absolute death from coronary artery disease alone (684,066 deaths in 1973 vs. 493,063 in 1993) has fallen over the past two decades, despite the striking increases in the aging population. A cause for euphoria! Much will depend on whether there have been shifts in our allocation of resources, and attitudes, towards health-care for older patients with major chronic illnesses including cardiovascular diseases.

Determinants of An Organism's Average and Maximal Life-spans

Aging is an inherent feature of living organisms resulting in physiologic attrition, which occurs even in the absence of overt disease. The pathophysiologic mechanisms of cardiovascular aging are likely related to the biology of the aging process across phylogeny. How long we live, or average life expectancy, is influenced by many factors including prenatal care, occupation, education, public health, and other preventative measures. Table 2 shows the significant increases in average life span, which are mostly derived from improving environmental conditions. For example, the elimination of premature death has had the most dramatic effect from 1900 to 1950 [7].

Why we age is determined by the intrinsic metabolic potential rates of mitochondrial respiration, and levels of oxidative stress/damage which, ultimately, affects maximum life span capacity.

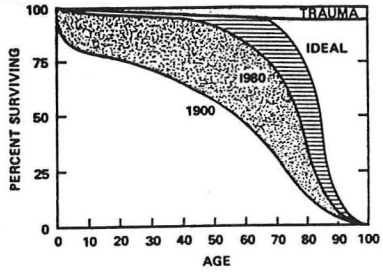
Table 2. Average Life expectancy over the millenniums

PERIODS	Average (years)
Graeco-Roman	20
1000 AD	30
1850 (American)	39
1911	46
1930	55

*Maximum life-span has remained unchanged.

Humans have a finite life span, approximately 100 years, which has not changed perceptibly in recorded history [8]. Unlike average life span, maximal life span is inversely related to the rate of aging [8]. Figure 2 shows the shape of the survival curve is increasingly rectangular [7]. Societies with exceptional longevity have not been satisfactorily documented [9].

Figure 2. The survival curve is increasingly rectangular . About 80 per cent (stippled area) of the difference between the 1900 curve and the ideal curve (stippled area plus hatched area) had been eliminated by 1980. Trauma is now the dominant cause of death in early life [9].



Aging in the Cardiovascular System

Table 3. Major Age-associated changes in cardiac structure (reviewed in [10])

- Heart weight increases with advancing ages 30-90 yr. In men 1 gm/year; Women 1.5 gm/yr
- LV mass (adjusted for BMI), which is confined to interventricular septum, is increased in women only.
- By age 75 yr., < 10 percent of the pacemaker cells in the SA node of a young adult remains [11]
- Increases in LV calcification, elastin, collagen and fat tissues infiltrate the conduction system and promote heart block and conduction abnormalities.
- Amyloid deposits are found in roughly 50 percent of older individuals over 70 yr. of age [12, 13].
- Lipofuscin, the end-product of intracellular lipid peroxidation, is ubiquitous finding in tissues including the aging heart [14]

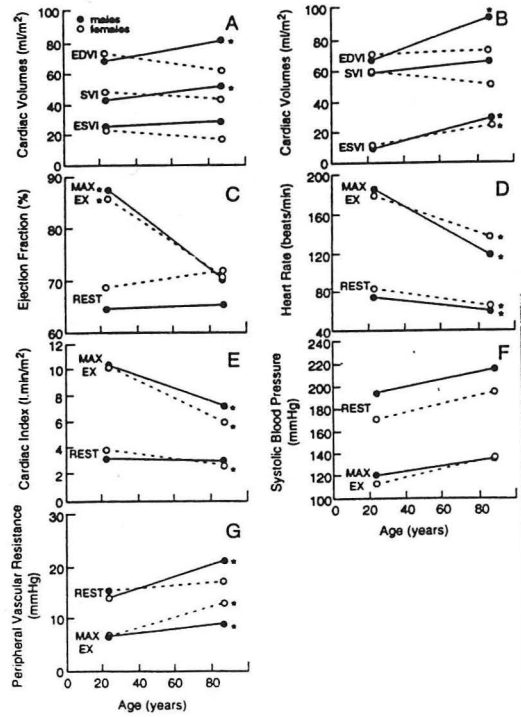
Cardiovascular Structure/Function Relationships in Older Individuals

In an anatomic study of 765 human hearts, without coronary artery disease, Kitzman and colleagues at the Mayo Clinic found that heart mass (adjusted for body mass) was increased in women only with age-related increases in the interventricular septum, and not the left ventricular free wall [15]. Although LV mass has been reported to decrease in the very old (80-100 yr.), whether or not this is because of failure to hypertrophy or just sedentary life-style is unknown. Cardiac muscle-to-collagen ratio stays the same or increases slightly. Lipofuscin is found in the aging heart but most observers can not agree on its physiological significance. Each of the foregoing variables can vary substantially with co-morbid diseases such as hypertension, obesity, coronary artery disease and, in a profound way, lifestyle [13, 16, 17].

Major Age-associated changes in arterial and cardiac responses. Isovolumic myocardial relaxation, a measurement of the time between aortic valve closure and mitral valve opening, is prolonged 40 percent in older individuals without overt CHD [18]. Multiple noninvasive studies (echocardiography, echo-Doppler and radionucleotide) have shown that peak filling rate of the LV in early diastole is reduced in healthy men and women with advancing ages between 20 and 80 yr. [18, 19]

Several indices of cardiovascular reserve function decline with age in otherwise healthy, aging, sedentary individuals. Figure 3 (A to G) shows several age-related differences in cardiac volumes measured at rest (A) and during exercise (B), and ejection fraction (C), heart rate (D), systolic arterial pressure (F), and peripheral vascular resistance (G). The responses were measured at rest and during maximal cycle ergometry in the upright position [10]. The participants in the Baltimore Longitudinal Study of Aging were healthy sedentary male (n=95) and female volunteers (n=50) who were screened for occult coronary artery disease and hypertension [20].

Figure 3



Several age- and gender-related changes in cardiac function have been described during the aging process in humans including reduced maximal heart rate, reduced stroke volume, the ability to achieve maximal ejection fraction and maximal aerobic capacity [21-23]. A higher prevalence in women of a "supernormal" ejection fraction has been described in conditions associated with increased afterload from either aortic stenosis or hypertension [24-27].

One of the most reliable measurements of abnormal cardiovascular function is the maximum oxygen uptake (Vo_2max), or aerobic capacity, which is the largest amount of oxygen an individual can deliver to the tissues of the body [28], as shown in Figure 4. Maximal aerobic capacity is, in turn, determined by the maximum cardiac output and the maximal oxygen difference of the body. The decline in maximum oxygen uptake also appears to be affected by loss in muscle mass [29].

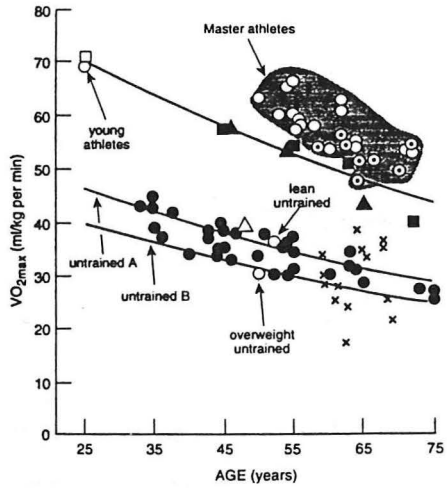


Figure 4

Arterial stiffness is thought to be secondary to a diffuse process involving the vessel wall rather than the increased prevalence of atherosclerosis in older persons [30].Total muco-polysaccharide content, or the ground substance of the interstitial matrix, are unaltered whereas chondroitin sulfate and heparin sulfate increase, and chondroitin and hyaluronate decrease with aging [31]. Finally, differences in life-styles among participants such as diet, body weight and smoking are likely confounding variables in cross-sectional and longitudinal studies. Despite these limitations, a pattern of cardiovascular regulatory mechanisms has emerged at the molecular, cellular and organismic levels.

Table 5. Studies of Cardiovascular Aging in Humans: Special Considerations.

- Aging is a continuous variable, beginning at the fetal, post-partum, neonatal, adolescent, adult and senescent periods.
- Cardiovascular aging is influenced by co-morbid diseases, life-styles and gene-environment interactions.
- Occult heart disease is a problem; Framingham data: 60% males over 65 year have 75-100 percent occlusion of at least one major coronary vessel, but only 15-20 percent are symptomatic [32].
- Age-related physiological changes differ among individuals.
- Cellular mechanisms may differ at varying periods of the life cycle.
- A paucity of studies have been performed on individuals who are 80+yr.
- Human studies of cardiovascular aging are correlative and descriptive, and not mechanistic; humans are often inappropriate.
- Animal studies, rodents and non-human primates, are meritorious to understand the aging mechanism.
- There is no "ideal model" for studies of fundamental mechanisms.

Cardiovascular Aging is a multiphasic process

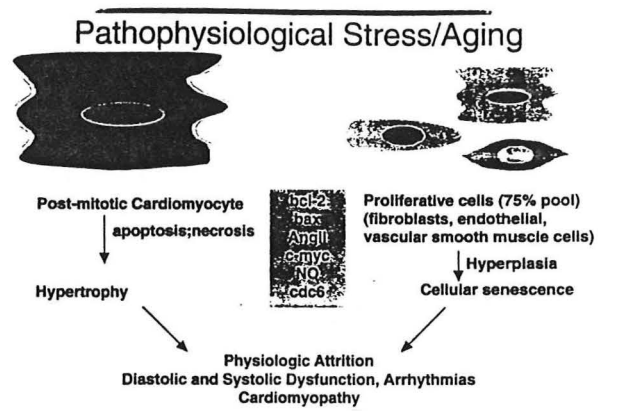
The evidence that cardiovascular aging involves multiple cellular changes can be summarized temporally as follows: (1) the initiation of event "hits" which accrue with time; (2) susceptible targets are injured; (3) reduced efficiency of cellular reparative capacity, and (4) irreversible damage, a harbinger of the end-stage phenotype. In physiological terms, the expression of cardiovascular aging is represented by diverse diseases such as acute myocardial infarctions, congestive heart failure, and systolic and diastolic dysfunction, which frequently accompany hypertensive heart disease.

Composition of the cellular pool in the adult heart

Although the heart is a highly efficient muscle pump, it is not a homogenous collection of muscle cells. It is not widely appreciated that seventy-five (75%) of the heart's volume comes from cardiomyocytes that comprise only ~25-30% of the myocardial cell pool. As shown in Figure 5, the predominant fraction of myocardial cells (75 %) consists of distinct cell types: vascular smooth muscle, endothelial, fibroblasts, and interstitial cells of varying proliferative capacities in both health and disease. Therefore any unifying hypotheses of aging must account for these distinct structure-functions relationships that likely contribute to the diverse pathologies exhibited in the aging heart of humans. The two major points of view: namely, replicative senescence and oxidative damage, which shape current thinking of the casual factors, seem to be divided more on the basis of specific systems from which they have evolved rather from the integration of diverse processes that contribute to human pathology. Thus, these seemingly divergent paradigms must be reconciled in the aging heart, which is a syncytium of myocardial

cells with distinct post-mitotic and proliferative capacities . First, I will review the replicative senescence hypothesis before discussing the oxidative damage theory, for which there is more accumulative evidence in cardiovascular aging.

Figure 5



The senescence hypothesis of aging

Telomeres, telomerases and loss of replicative capacity

Model systems ranging from cultured human fibroblasts (except germ-line or immortalized tumor cells) to yeast to the primitive worm to humans exhibit a finite replicative capacity [33-38]. What is the sensing, mechanism used by somatic cells to cease cell division? Recent interest has been focused on the telomere-telomerase hypothesis almost exclusively on cancer and aging [39-41]. However, the potential relevance in cardiovascular aging is more related to the predominant pool of proliferative myocardial cells than the post-mitotic and terminally differentiated cardiomyocytes. Telomeres are stretches of repetitive DNA sequences at the ends of chromosomes, and telomeric length is maintained by a balance between processes that lengthen and shorten telomeres.

Progressive telomere shortening is observed normally as somatic cells divide. Greatly shortened telomeres eventually lead to limited replicative capacity. The enzyme, telomerase, is a ribonucleoprotein which stabilizes telomere length by adding back hexameric (TTAGGG) repeats to the telomeric ends of the chromosomes, thus compensating for the continued erosion of telomere length, estimated ~ 25-200 DNA base pairs off the telomere ends that occurs during each cell division. Cellular senescence (or aging), or a cessation of cell division, occurs once the number of these trimming events exceed 100 times [the end-replication problem]. Thus, telomeres are often referred to as biological "clocks", which count the number of cell divisions, serving as a buffer and that determines when cellular senescence occurs.

From a teleological standpoint, senescence is thought to evolve as a defense mechanism against uncontrolled proliferation. While cell type and donor age are dependent factors, replicative senescence is thought to involve proliferative control by dominant-acting genes, loss of function mutations, cellular transformation by oncogenes and/or the inactivation of tumor suppresser genes [38, 42]. Immortalization may therefore occur through mutation(s) of the gene(s) responsible for telomerase repression pathway allowing the expression of telomerase in cancer cells [43]. Conceivably, telomerase activity may be un-regulated or reactivated during escape from replicative

senescence. Aging cells also exhibit resistance to apoptosis or programmed cell death, cellular differentiation, and shortening of the telomere after oxidative stress [44].

OXIDATIVE STRESS/DAMAGE THEORY OF AGING

There is increasing evidence suggesting that oxidative stress/damage may be a major causal factor in the aging process [45, 46]. The level of oxidative stress and the susceptibility of tissues to experimentally-induced oxidative stress seem to increase during the aging process [47]. The hypothesis that free radicals can promote, and antioxidants or free radical scavengers can ameliorate oxidative damage in the heart has been pursued by clinicians and researchers [48, 49]. Physiological attrition is thought to be the consequence of a dual between prooxidant and antioxidant pathways. Table 5 lists the accumulative evidence, which is mostly correlative supporting the hypothesis that acceleration of oxidative molecular damage increases exponentially with aging, becomes irreversible, and leads to functional impairment.

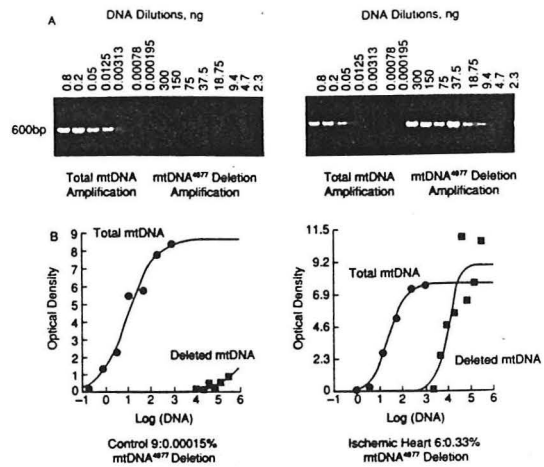
Table 5. Major features of the Oxidative Damage Hypothesis

- Mitochondria are the sources and targets of oxidative damage;
- Rates of reactive oxygen species increase during aging;
- Oxidative stress/damage increases during aging;
- Antioxidant defenses decrease during aging;
- Overexpression of antioxidative enzymes for Cu, Zn, superoxide dismutase and Catalase increases life span; and
- Synthetic/degradative reparative pathways are impaired during aging.

Mutations of mitochondrial DNA increase in human ischemic hearts

The accumulative evidence in support of the oxidative damage theory has important clinical implications in ischemic heart disease. One hypothesis proposes that the production of oxygen radicals damages mitochondrial DNA, reduces ATP generation, and evokes compensatory mechanisms of OXPHOS gene expression [50, 51]. Figure 6 shows a study by Wallace and colleagues at Emory reporting that a 4977 base pair mitochondrial DNA deletion (mt DNA), which appears in control hearts only after age 40 years, was present in greater amounts (between ~8-2000 fold increase) in ischemic hearts of patients with significant triple vessel coronary artery disease compared to age-matched controls [51]. Figure 6. Polymerase chain reaction quantitation of the 4977 base pair DNA (mtDNA) deletion. The template was genomic DNA which was serially diluted and amplified in control (left) and ischemic (right) hearts. Panel B shows the results of a PCR amplification. The appearance of the major band is related to the primers that can now amplify across a region in close proximity because of the putative deletion in the ischemic heart.

Figure 6



In addition, transcripts for OXPHOS enzymes were significantly elevated in the diseased hearts supporting the hypothesis that free radicals, generated during chronic ischemia, may contribute to the pathology and alter gene expression. However a correlation between OXPHOS induction and mtDNA damage does not establish causality since the primary mechanism(s) for the deletion in the mitochondrial genome are unknown, and remain speculative. Furthermore, no prior studies have rigorously examined and established that specific mitochondrial deletions directly affect cardiac metabolism, function and, ultimately, cardiac pathology. Recently, Sohal and colleagues at Southern Methodist University in Dallas have demonstrated that mitochondrial aconitase, an enzyme in the citric acid cycle, is a specific target of random oxidative damage in flight muscles of the housefly [52]. This intriguing finding awaits testing whether or not this specific 'biomarker' of mitochondrial oxidative produces cardiac dysfunction in aging mammals.

Mitochondrial respiration and the aging process

Mitochondria are essential for cell survival as the primary source of adenosine triphosphate (ATP) synthesis which is particularly high in oxidative tissues such as the brain, kidney, skeletal muscle and the heart. Paradoxically, mitochondria are the principal sites for the generation of reactive oxygen species (ROS) which are proposed to imperil the organism and promote several degenerative diseases of aging [53, 54]. The process of mitochondrial respiration is also referred to as oxidative phosphorylation (OXPHOS)

Antioxidative defenses decrease during aging

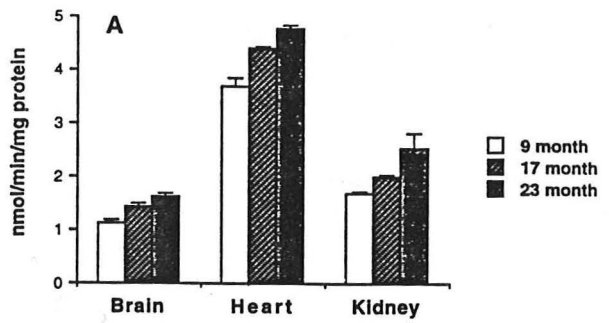
Oxidative stress denotes a redox state where the balance between pro-oxidants and antioxidants is tilted in favor of the oxidants. Theoretically, shifts in the level of oxidative stress in cells may occur by variations in the rates of oxidant generation and/or alterations in the efficiency of antioxidative defenses. Activities of superoxide dismutase (KCN-sensitive and KCN-insensitive, believed to be Cu Zn- and Mn-SOD, respectively), catalase, glutathione peroxidase, and glutathione reductase, which collectively represent the first line of enzymatic antioxidative defenses, provide an indication of whether or not antioxidative potential is affected. However, measurements of individual antioxidative enzymes have not provided consistent or conclusive

results of the direction of age-related activities in antioxidant defenses [54]. Presently, there is no single or simple measure of the level of oxidative stress. Consequently, a battery of tests is deemed necessary to infer the direction and the magnitude of the shift in the redox state of the tissues. Several biomarkers of oxidative stress/damage are used to provide an indication of the relative level of oxidative stress, namely (i) rates of ROS generation, (ii) activities of antioxidative enzymes, (iii) oxidative molecular damage (i.e. DNA damage and lipid peroxidation).

Rates of ROS generation increase during aging

Figure 7 shows the age-related increase in the rates of superoxide anion radical (O_2^-) generation (below) and H_2O_2 (not shown) from the brain, heart and kidney of 9-, 17- and 23-month-old mice [55].

Figure 7



Superoxide anion radical (O_2^-) is produced at several intracellular sites, but the main source of generation is the mitochondrion [56]. O_2^- is converted by SOD into H_2O_2, which, being freely diffusible through membranes, is released into the cytosol [55, 57]. The rate of H_2O_2 release is usually measured in state 4 respiratory conditions in coupled mitochondria or in conjunction with respiratory inhibitors to identify the site of generation [55, 57, 58].

Oxidative stress/damage increases during aging

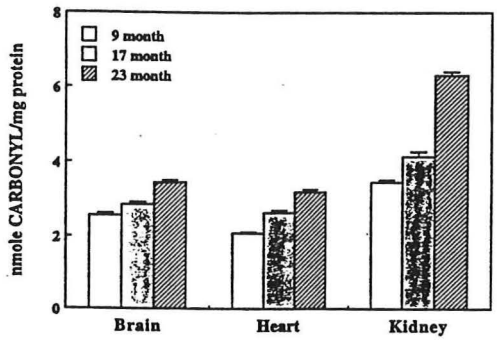
The steady state level of oxidatively modified macromolecules is arguably the key indicator of oxidative stress because it represents the net amount of oxidative damage accrued by the cells after taking into account the degradation or repair of the oxidatively damaged molecules. An increase in such damage has been observed during aging and under a variety of experimental conditions that affect the balance between pro-oxidants and antioxidants [46, 59]. Different approaches and methodologies have been used to measure the level of oxidative damage [60]. Oxidative modifications of DNA, proteins, and lipids are commonly used as indicators of molecular oxidative damage.

DNA damage. A variety of studies have indicated that DNA oxidative damage under normal physiological conditions is relatively extensive and the oxidized nucleoside, 8-hydroxydeoxyguanosine (OHdG) is a specific product of such oxidative damage [61-63]. The concentration of OHdG also increases with age in mouse tissues from the brain, heart skeletal muscle, kidney and the liver [64]. Similar findings have been reported in flies [65, 66].

Protein damage. Accumulation of protein carbonyl derivatives, and loss of -SH groups in membrane proteins are used as indicators of protein oxidative damage. Studies from Stadtman's laboratory in the last decade have convincingly demonstrated that proteins undergo extensive oxidative damage under normal physiological conditions and that carbonyl content is an indicator of such damage. Protein carbonyl content has been shown to increase in response to experimental

oxidative stress as well as during aging in different model systems [67]. Sohal and colleagues have also reported that protein carbonyl content is associated with the life expectancy of flies [68], and in aging rodents [64]. Figure 8 shows protein carbonyl content in the homogenates of brain, heart, skeletal muscle, kidney and the liver [55]

Figure 8



Lipid peroxidation. Cellular membranes contain a considerable amount of polyunsaturated fatty acid side-chains which are prone to undergo oxidation. Indeed, one of the earliest types of molecular oxidative damage ascribed to reactive oxygen species was the peroxidation of lipids [60]. Measurement of malondialdehyde (MDA) concentration can be used as an indicator of lipid peroxidation in the aging heart.

Overexpression of antioxidative enzymes retards aging

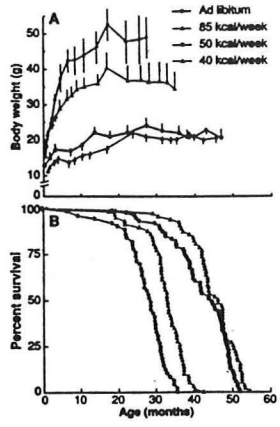
In model systems ranging from transgenic *Drosophila* to mice, overexpression of either catalase or superoxide dismutase or glutathione peroxidase tends to be protective against oxidative stress [Sohal, 1995 #3793; Chan, 1995 #3794; Sohal, 1996 #3795; Kondo, 1997 #3796]. Oxidative stress, from pathological stress such as ischemia/reperfusion, also plays a central role in cell mediated injury of vital organs such as the brain, kidney, and heart [69-72].

Caloric intake, maximal life-span and aging

Beginning in the 1930's results of numerous studies consistently and reproducibly indicate that caloric restriction, a maneuver that reduces caloric intake by 33 percent, can achieve a 40 percent increase in the maximal life-span of experimental rodents ([73], see review [74]). Figure 9 shows the effect of caloric intake on (A) body weight, (B) percent survival, and (C) life-span in female C3B10F1 mice [75])

Besides an increase of life-span, caloric restriction delays or substantially reduces several age-associated chronic conditions including diabetes, cancer and renal disease with simultaneous increases in the immune responses of experimental animals [76]. Interestingly, these effects are independent of caloric composition (fat, carbohydrate, mineral, vitamin and protein content) suggesting than the critical determinant was the amount of energy intake and not the caloric source. Dietary restriction of adult monkeys at constant body weight for 15+ years prevented the age-associated onset of glucose intolerance, and NIDDM [77]. Whether such maneuvers that reduce the complications of atherosclerosis and, ultimately, increase the life span of these non-human primates is presently unknown.

Figure 9



Caloric restriction may reduce the incidence of chronic illnesses

The underlying mechanisms for increased longevity by caloric restriction remain a mystery. Current investigations are focused on the potential alterations in glucose utilization, oxidative stress/damage, reduced glycation of macromolecules, stress hormones and gene expression. Similarly, lower blood pressure, lower triglycerides, reduced lipoprotein adhesion to blood vessels, and higher HDL cholesterol are potential factors likely to yield reduced cardiovascular morbidity and mortality. However, direct evidence that lean humans have reduced mortality is lacking. Critics of caloric restriction hypothesis argue that the primary reason for reduced mortality is an unrestricted diet, and not increased longevity from caloric restriction [78]. However, this is a circular argument that seemingly discounts the intuitive and substantial body of clinical evidence of obesity on cardiovascular morbidity and mortality.

Potential cardiovascular benefits of caloric restriction in humans

Biosphere 2 is considered an 'experiment of nature' in which eight persons (4 men and 4 women) were sealed off and maintained in a closed environment for 2 years near Tucson, Arizona [79]. Adaptive changes on a low calorie but nutritionally adequate diet (1800-2200 kcal per day/person) included substantial weight loss (18 % men, 10 % women), lower blood pressure, lower cholesterol, fasting blood sugar, and fall in white blood cell counts [79]; precisely mimicking caloric restricted rodents [80]. However, it remains a testable hypothesis whether or not caloric restriction can increase life-span and reduce chronic illnesses in humans. Adequate, controlled trials have not been conducted, although guidelines for human trials have recently been formulated [81]. Even a small beneficial change from caloric restriction on cardiovascular morbidity and mortality can have dramatic effects on outcomes since the number of affected individuals is so large. Strategies which reduce oxidative stress/damage are likely to improve cardiac structure and function and, thereby, extend disease-free intervals. Whereas the public desire for caloric restriction is questionable, George Roth at the NIA and his industry partners see the overwhelming potential of a lucrative market for 'safe pills', which mimic the caloric restricted state [78].

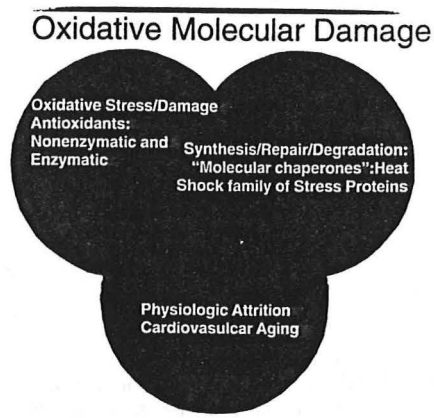
REPAIR/DEGRADATION OF OXIDATIVE MOLECULAR DAMAGE

Stress proteins and antioxidants can protect against oxidative stress/damage.

A potentially effective strategy may be to physiologically minimize the **possibility** of the production of •OH. Indeed, overexpression of members of the heat shock family of

stress proteins may provide one such avenue. The main distinguishing features of the stress protein pathway are: (i) a regulatory mechanism that facilitates rapid synthesis of the highly inducible members of the heat shock family of stress proteins [82]; (ii) multiple members normally reside in different cellular compartments such as the cytosol, endoplasmic reticulum and mitochondria; and (iii) physiological functions of a molecular chaperone could be recruited for protein folding or repair of damaged intracellular proteins [83-85]. Several studies have reported that during myocardial ischemic tolerance up-regulation of stress protein levels correlates with increases in the enzymatic activity of catalase suggesting potential additive or synergistic interactions against oxidative stress [86-88]. *Whether or not the evolutionarily-conserved functions of stress proteins, as molecular chaperones, complement the unique functions of antioxidants, and, together, lower oxidative stress/damage is a testable hypothesis under active investigation, as shown in Figure 10.*

Figure 10



Heat Shock Protein synthesis is impaired during aging

Recently, we have undertaken studies to determine if the heat shock family of stress proteins plays a role in the aging process. Heat shock proteins have been unambiguously demonstrated to minimize the deleterious effects of various -endogenously or exogenously-induced stress, including oxidative stress. A decrease in the expression of heat shock protein genes and the DNA-binding activity of the regulatory heat shock transcription factor are reported to occur during the aging process in rodents [89]. Whether the increased amounts of molecular oxidative damage, observed during the aging process, are causally associated with a decreased expression of heat shock transcription factor and by implication the stress-inducible heat shock proteins, is presently unknown.

Overexpression protects against oxidative stress/damage during myocardial ischemia.

There is a substantial amount of literature concerning the induction of hsp70 by ischemia [90-92], and the potential role of Hsp70 in ischemic preconditioning [86, 93]. *A common finding of these studies is that overexpression of the major 70 kD heat shock protein, Hsp70, improves myocardial function [94-96], preserves metabolic functional recovery [96], and reduces infarct size [97] after ischemia/reperfusion in transgenic mice.*

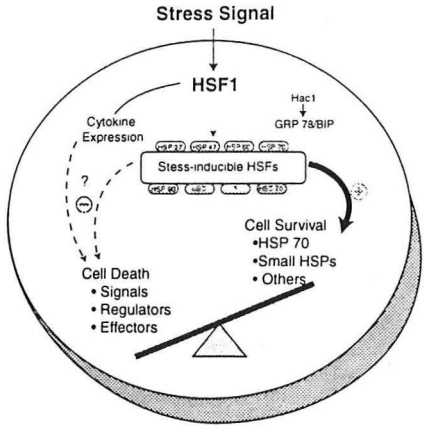


Figure 11 illustrates schematically the key features of the stress-inducible synthesis of the heat shock family of stress proteins that effects physiological recovery after pathophysiological stress. Various cardiac diseases are heavily tilted towards cell death either by apoptotic or necrotic mechanisms. Chaperones can reverse these deleterious effects through specific molecular interactions with the regulators and effectors that result, ultimately, in cellular and organ survival.

Cell Death in the Cardiovascular Aging

Absence of timely restoration of myocardial blood flow produces cellular necrosis and subsequent reparative myocardial fibrosis [98-100]. Unlike necrosis after ischemic injury, programmed cell death or apoptosis has been recognized as an important mechanism for maintaining stable cell numbers in normal tissues with varying proliferative capacities [101, 102]. Apoptosis is triggered in response to diverse physiological stimuli including oxidative injury [103, 104]. In humans, results of several studies have demonstrated the *in situ* markers (bcl-2, fas ligand, bax) of apoptosis in patients with heart failure, restenotic lesions [102], and following acute myocardial infarction, which occurs primarily at the ischemic border zone [105](see review [106].

Although a distinguishing feature of apoptosis appears to be absence of an inflammatory response, myocardial fibrosis is an ubiquitous feature of the aged heart even in the absence of overt disease [17, 107, 108]. Age-related changes from pressure overload, transient ischemia, oxidative injury, and drugs can trigger damage of myocardial cells to which the physiological response depends on their intrinsic proliferative and structure-function relationships. Cardiomyocyte hypertrophy is thought to be triggered in the remaining myocyte pool to compensate for myocyte loss from apoptosis or cell injury [107]. An alternative hypothesis is that the predominant proliferative pool of proliferative myocardial cells may do loose their replication capacities and senesce [101].

One intriguing hypothesis is based on recent studies that senescent fibroblasts upregulate the anti-apoptotic protein bcl-2, which normally prevents cell death [109]. Thus, the failure to eliminate damaged, but aged, fibroblasts is a potential mechanism that has been proposed to contribute to the age-related organ dysfunction [101, 110]. Efforts to identify the causal factors and mechanisms responsible for cell death in post-mitotic, terminally differentiated cardiomyocytes and proliferative population of myocardial cells is important future avenue for research that could lead to cell-specific strategies to combat age-related mortality in humans.

In addition to alterations in phenotype, senescent human fibroblasts exhibit both repression and up-regulation of certain genes as well as post-translational modification of their gene products. Distinct changes of cellular proteins in aging fibroblasts include: transcription factors (*c-fos*, Id, and pollα); cytokines (Il-6); regulatory proteins (cdk2, cdc2, and PCNA) as well as molecular chaperones (hsp70 and hsp90) .

Replicative senescence and oxidative damage hypotheses: A reconciliation?

Growth arrest and altered cellular function are common features of both replicative senescence and terminal differentiation. Mechanistically, the replicative senescence theory has potential appeal to the biology of cardiovascular aging. If senescence is a tumor suppressive mechanism, then senescent cells (fibroblasts, interstitial cells) that accumulate may contribute to cellular and organ (systolic and diastolic) dysfunction. Therefore, terminally-differentiated cardiac myocytes, which do not proliferative, respond to growth signals (e.g. hypertension, oxidative stress) with increased cell size or hypertrophy. Myocardial hypertrophy, in turn, leads to ventricular dysfunction and congestive heat failure, which disproportionately affects the elderly. Reduced replicative capacities of senescent fibroblasts and interstitial cells in aging heart could be causative factors in increased collagen deposition and interstitial fibrosis, well-established pathologies associated with myocardial dysfunction. These are testable hypothesis for future research. Thus, it is plausible that measurement of events of the predominant fraction of cardiomyocytes, which comprise the minor fraction of myocardial cells, has overshadowed the potential mechanisms of cellular senescence in the majority of myocardial cells.

Klotho mutant mice mimic aging in humans

A stunning discovery of an aging model of mutant mice for an unknown gene, called klotho, was reported in a recent issue of *Nature* [111]. A research team in Japan, led by investigator Makato Kuro-o, serendipitously found an insertional mutation of a transgene in which homozygous mice stopped growing after 3 to 4 weeks, and died after 8 to 9 weeks instead of the usual 2 to 3 years. Positional cloning indicated the transgene had disrupted an unidentified gene, called klotho-named for a Greek goddess who spins the thread of life-which has sequence homologies with members of the β-glucosidases, an enzyme that is expressed in bacteria and mammals and functions to digest fat-soluble glycolipids. Klotho mutant mice resemble aging in humans including a short life span, atherosclerosis, infertility, skin atrophy, osteoporosis, ectopic calcification, and emphysema [111]. The phenotype exhibited 100% penetrance, no perinatal mortality and was unaffected by gene background. Thus, genetically identical mice except for the homozygous mutation differed significantly in their rate of aging that mimicked several age-related diseases in humans. One major concern is whether klotho mutant mouse model represents an uncharacterized metabolic syndrome, which promotes premature death rather than accelerated aging.

Nonetheless the team is actively pursuing an intriguing hypothesis, based on gain of function studies that rescue the phenotype. The enzyme's anti-aging properties may be linked to an active secreted form of the enzyme, which breaks down glycolipids to release ceramide, a compound implicated in pathways for cell survival. This novel finding is hitherto unsuspected and is unrelated to other forms of premature aging in humans, produced by mutant helicase, such as Werner's Syndrome and progeria. The undeniable excitement, however, is that a laboratory model can now be used to test whether the second criterion of generality can be satisfied through a universal mechanism in other species including age-related human diseases. In addition, avenues also exist in such animal models to examine the behavioral, physiological, pathologic, and genetic influences in the absence or presence of age-related diseases.

Model Systems for Human Aging Research

With minor exceptions, genetically engineered animal models are rapidly becoming the established standard to test important hypotheses about the regulation and functions of mammalian genes in human health and disease. In other words, the model exhibits several features that are closely related to abnormalities observed in the cross-section of human conditions (e.g. atherosclerosis, cancer and Alzheimer's type neuropathologies). For example, Bev Paigen and

coworkers, at the Jackson Laboratories, have pioneered the use of recombinant inbred strains of mice to elucidate the genetic factors responsible for the development of atherosclerosis. Contrary to popular opinion, inbred strains of mice, fed an atherogenic diet of 15% fat and 1% cholesterol, do develop atheromatous plaques in the aorta and coronary arteries and cholesterol gallstones [112, 113]. Although laboratory rodents and non-human primates have been the mainstay of aging research, the mouse model is rapidly gaining notoriety as the model of choice [114]. Murine models of genetically inbred mice circumvent the prohibitive costs of larger non-human primates, which are long-lived, inaccessible to genetic manipulation, slow to sexual maturity and produce litter of small size. A stable mouse colony allows the following advantages: (1) studies can be conducted during the life-span (2-3 years) of the laboratory mouse; (2) an established colony can be maintained in which environmental, nutritional, exercise and other factors are held constant; and (3) the development of cardiovascular diseases and age-related illnesses can be assessed.

How to Prevent & Treat the Aging Blues (Grumpiness)

As the fastest growing segment of the population, elderly individuals with chronic diseases are often cited as major contributors to the soaring costs of medical services. In a recent review of hospital discharges (n=678, 954) in Massachusetts (nonfederal), hospitalization costs were highest for the 70- to 79-year old group and declined thereafter among age groups between 60 to 100 years [115]. Moreover, patients 80 years and older tended to be hospitalized in non-teaching hospitals contributing to the overall cost per admission than in younger elderly individuals. Because the relationship between health-care, morbidity and age is not straightforward, the cost of dying and not old age has been cited for high costs rising health-care costs, which consume ~ 40 percent in the last period of life,[116]. Besides issues of health-care costs, there is increasing evidence that health prevention services and cost-effective therapies are under-utilized in the elderly individuals.

MANAGEMENT OF OLDER CARDIAC PATIENTS

Anger, hostility and coronary artery disease

A growing number of epidemiological studies have found a positive correlation exists between anger and hostility and the incidence of coronary artery disease [117-119]. Gene-environment interactions, which are presently poorly defined, play important roles in physiological adaptation including cardiovascular aging. Recommendations for reducing cardiovascular risk such as diet, psychological profile, and particularly exercise seem prudent even the oldest old [120, 121].

Exercise training retards physiological aging in older individuals.

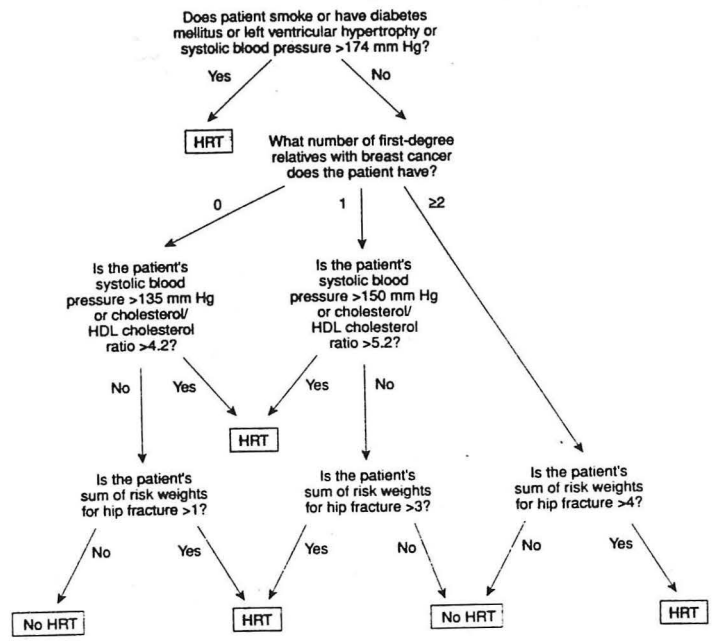
Results of recent studies indicate that older individuals should be encouraged to participate in aerobic exercise programs. A well-established benefit of exercise programs is the retardation of physiological aging in individuals with advancing chronological age. Endurance training, as measured by Vo_{2max} is affected by both intensity and the duration of exercise. Prior levels of cardiovascular fitness does influence the observed benefits of lifestyle intervention. Shulman and colleagues at the Gerontology Research Center in Baltimore enrolled ten sedentary men, age 60, without overt cardiac disease, for exercise training for 24 to 32 weeks and eight age-matched endurance-trained men who agreed to stop their exercise program for twelve weeks (deconditioning). This intervention abolished all the initial differences in cardiovascular performance such that sedentary individuals achieved indices of endurance trained men including increase Vo_{2max} , which correlated linearly with increase in cardiac index, stroke volume, end diastolic index, end systolic index [122]. Detraining of the endurance-trained older men completely reversed the training effects in twelve weeks . By design, these investigators could measure the effects of aerobic training from opposite ends of the fitness spectrum: sedentary individuals

towards endurance levels whereas endurance trained individuals were allowed to be decondition, albeit in substantially shorter period (a potential criticism of the study).

Hormonal therapy in postmenopausal women.

Hormonal replacement therapy is an important intervention, which reduces the relative risk of developing cardiovascular disease in postmenopausal women [123], although this approach is not shared by all [124]. Presently, over 30 million postmenopausal women have an *additional* average life expectancy of 28 years [124, 125]. Epidemiologic studies have found the protective benefit against coronary heart disease in premenopausal women is abolished in the postmenopausal years [126-128]. Potential protective mechanisms of estrogen attributable to its antioxidative and vaso-protective properties including the lowering of blood lipids and lipoproteins and direct effects on the vessel wall [129], which now appears to be unrelated to nitric oxide [130]. Estrogen and progestins therapy, which retards the risk of endometrial cancer from unopposed estrogen action [131], restore this cardioprotective effect in postmenopausal women at no added risk of stroke [132]. Older women with major risk factors for coronary artery disease should be considered for hormonal therapy, which reduces cardiovascular morbidity and mortality (in addition to hip fractures and osteoporosis). Figure 12 shows a potential scheme for patients whose life expectancy is extended by at least 6 months by hormonal replacement therapy [133].

Figure 12



Thrombolytic therapy in older men and women.

Thrombolytic therapy effectively restores blood flow to the myocardial wall rendered ischemic by occlusion of a coronary vessel, and improves the mortality of patients after acute myocardial infarction. Multiple factors, however, exclude the majority of patients with an acute myocardial infarction from receiving thrombolytic agents with estimates between 21-23% in the

United States [134, 135] to 49% in the UK for various reasons, were under-represented in earlier trials.

ACE in the older cardiac patient: Angiotensin converting enzyme inhibitors are of proven benefit for reducing morbidity and mortality in patients with congestive heart failure and after myocardial infarction [136, 137]. Older cardiac patients seem to miss out on therapeutic advances, which substantially affects morbidity and mortality. For example, among eligible Medicare recipients, without a contraindication for ACE inhibitor therapy from postMI and abnormal LVEF, almost half (45 percent) did not receive the medication at discharge [138].

ACE vs Angiotensin II receptor blockade inhibitors (ELITE Trial). The Evaluation of Losartan in the Elderly Study [139], ELITE, randomly enrolled 352 patients, mean age 73.5 (33 percent women), with systolic LV dysfunction and congestive heart failure to Losartan (50 mg /day) or captopril (up to 50 mg TID) for 48 weeks follow-up [140]. An attractive feature of this study was that in elderly individuals found significantly less side effects from losartan. Therefore, fewer patients discontinued therapy, and there was a significant decrease in all-cause mortality in patients on losartan compared to patients on captopril treatment. Further studies using larger number of patients are needed to determine whether losartan provides a clear-cut survival benefit over captopril, which is associated with development of number of side effects (altered taste, cough, rashes, and angioedema) limiting its proven use.

Digoxin therapy for congestive heart failure in old people

In a geriatrics practice (mean age 81 yr.) at a teaching hospital, the prevalence of digoxin use was 19 percent with rate control of atrial fibrillation the leading indication [141]. Despite its widespread use for treatment of heart failure, the effects of digoxin therapy on morbidity and mortality remained controversial until recently. Baseline characteristics of patients in the Digitalis Investigation Group (DIG) study assigned to digoxin (3397 patients) or placebo (3403 patients) included normal sinus rhythm (100 percent), the mean age 63 yr. (26 percent > 70 yr.), 22 percent female sex, ~ 15 percent nonwhite race, the majority NYHA class II (54 percent), previous MI (65 percent), primary etiology of CHF (70 percent IHD), ejection fraction (28 percent), and daily average digoxin dose, 0.25 mg (70 percent). Overall mortality was unaffected on digoxin therapy (~35 percent death in 37 months follow-up); although digoxin treatment reduced the risk for hospitalization for heart failure ($p<0.001$) [142].

Previous studies have indicated withdrawal of digoxin in patient with congestive heart failure worsens functional capacity, ejection fraction and exercise capacity [143-145]. Thus, digoxin is clearly an effective inotropic agent in older patients, with atrial fibrillation and/or congestive heart failure. Careful attention in older individuals is needed to circumvent toxicity, given co-morbid factors such as renal dysfunction and reduced body mass index [146].

Cardiovascular Surgery in older men and women

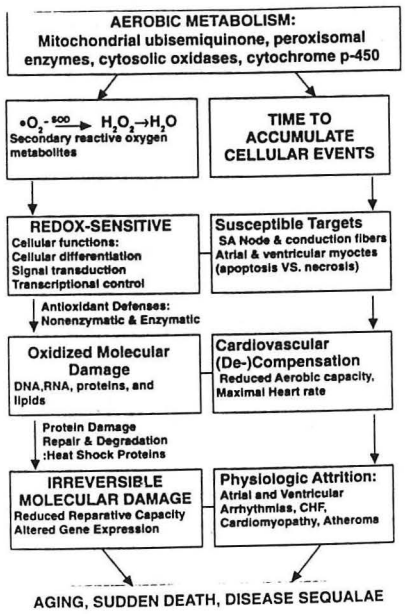
Surgical morality increases with advancing age of both men and women, moderately severe congestive heart failure (Canadian Class III-IV and NYHA IV, loss of sinus rhythm (atrial fibrillation), and the need for urgent and emergency operation. In a small series from the Brigham and Women's Hospital of 222 patients, 75 years and older, the operative mortality was 3.6 percent for elective, ~14.9 percent for urgent and 35 percent in emergencies for coronary artery bypass grafting [147]. Although 83 patients (37 percent) had postoperative complications, the actuarial probability of survival was 75 percent at 48 months [147], which was similar to others [148].

An operation for aortic stenosis is the most common reason for surgical correction of valvular heart disease in older men and women. Bioprosthetic valves, which avoid anticoagulation unless indicated otherwise, are often implanted [149]. Even with correction of occlusive coronary

artery disease, the surgical mortality is 12.4 percent [149] Placement of prosthesis in older patients, mostly women, with a small aortic root without annulus enlargement, can be circumvented with the 19-mm St. Jude Medical heart valve prosthesis [150]. The absence of thromboembolic (~96 percent) and anticoagulation-related hemorrhage (~ 91.8 percent) were extremely favorable over 16 yr. follow-up. Additional benefits include increased functional capacity, periods free from angina, and reduced hospitalization.

CONCLUSION

The medical profession can no longer view older men and women as a homogeneous group nor as individuals worthy of our sympathy but not our fullest attention given their diverse lifestyles as caregivers, parents, grandparents, scholars and 'carriers of the flame'. The booming business of aging research will likely provide insights into the fundamental mechanisms of the aging process, which, in turn, will guide the development of novel therapeutic strategies that retard or ameliorate several age-related diseases. In the interim, the increasing risk of cardiovascular disease in the aging population places a substantial burden on the entire medical community to intervene swiftly with the most cost-effective treatment schemes currently available. Sudden death at 85-year on the golf course or tennis course may be viewed by some as a swift and happy ending after a wonderful, productive life. Not so for the majority! The absolute number of older individuals with chronic illnesses will increase with disproportionate numbers coming from increased survival and aggressive management of acute myocardial infarction, heart failure, and lethal arrhythmias. Cardiovascular medicine has brought the most significant advances in the past decades and is poised to play an even greater role in the future. Missed opportunities to effectively treat older patients can impart great costs to the patient, to the family, and, ultimately, to society. It is time to dispel the myth that "the medical profession is to blame for the burgeoning number of aged people", and to celebrate the richness of our national resources during this remarkable period in the history of humankind.



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