

Wednesday Morning (8am-Noon)
Aging and Osteoporosis
Continental Room of the Sun Valley Inn

(1) The (Brief) Epidemiology and Biology of Aging
Steven R. Cummings, MD

The incidence of overall mortality and several degenerative diseases, including hip fracture, stroke, heart failure and Alzheimer's disease increase exponentially with aging. Mortality begins to rise exponentially at about age 28 reaching a maximum human lifespan of about 120 years for women and 115 for men.

The mass of several systems, including muscle, bone, brain and kidney decreases with age, but the amount of fat in organs, such as muscle and bone marrow increases with aging. Many physiological functions decline steadily with age, including total energy expenditure, amount of spontaneous movement, gait speed, muscle strength and endurance, maximum heart rate, cognitive function, glomerular filtration rate, decline steadily with age. Decreased amount and speed of movement, decrease in muscle mass, accumulation of damaged proteins, cellular and nuclear disorganization, and wrinkling are features of aging in many species, from worms to mice to humans.

Caloric restriction, rate of living, free radicals, and entropy

In model organisms, caloric restriction consistently prolongs lifespan and slows many physiological changes with age. (In poikilotherms, similar changes are produced by decreasing temperature.) This observation spawned and supports the 'rate of living' theory of aging: production of energy damages proteins, DNA and other essential molecules and this damage accumulates to cause dysfunction of cells and organs.

The most popular explanation for aging is we 'rust.' The *free radical* or *oxidative damage* theory proposes that cells progressively degenerate because aerobic metabolism, that is essential to life, necessarily generates oxygen radicals that damage other macromolecules. Indeed, oxidative phosphorylation generates one oxygen radical (such as O₂⁻) for every 50 to 1,000 molecules of ATP produced from ADP. Theoretically, these oxygen radicals would preferentially damage mitochondrial (where the >90% of these reactions take place), but may also other parts of cells. Most, but not all studies in all tissues, find that mitochondrial DNA damage increases and mitochondrial energy production decreases with aging. The theory would predict that bolstering anti-oxidant defenses, such as increasing levels of superoxide dismutase (SOD) and treating with antioxidant vitamins, would slow aging, decrease the incidence of age-related diseases and prolong lifespan. Increasing SOD expression has prolonged lifespan of some model systems, but human trials of antioxidants have consistently produced no or even harmful effects on risks of diseases.

The 2nd law of thermodynamics (essentially, entropy increases with time) says that all macromolecular systems become less organized with time. Faster rates of metabolism and generation of heat will inexorably decrease the order and organization of molecular structures. This process –entropy – will limit or decrease the efficiency of cellular functions and cause errors of DNA and protein transcription and other processes without invoking molecular damage from oxygen radicals. Systems require considerable energy to maintain order, mainly be replacing disordered parts. Stem cells play this role in organisms, but they cannot continuously renew and replace all organs.

Insulin signaling

Caloric restriction also decreases insulin production as well as IgF1 production and experiments in worms, flies and mice have shown that decreasing insulin signaling and production of growth

hormone and IgF1 (and their homologues) consistently prolongs lifespan and slows many features of aging. This might work by reducing the 'rate of living' or generation of oxidative damage, but other experiments show that decreased insulin signaling has other effects that may mediate its benefits, including increasing heat shock proteins that clear damaged macromolecules.

Neuroendocrine control of aging

Besides the effects of growth hormone, some have postulated that aging may result, at least in part, by decreasing hypothalamic-directed production of other hormones, such as sex hormones, DHEA or prolactin. There has been meager to no support for this hypothesis in humans, but recent experiments in *c. elegans* showed that ablation of neurosensory cells prolonged lifespan by decreasing production of a yet-to-be-identified hormone.

Cellular 'clock' and cell senescence

Discovery of the 'Hayflick' phenomenon – that fibroblasts double about 30 times then become senescent – gave birth to the theory that some built in system limited the lifespan of cells, and animals. Telomeres (nucleotide 'caps' at the end of chromosomes) shorten with each cell division reaching a critical length that induces cell senescence. A finding that short telomeres predicted higher mortality popularized this theory but it has not been replicated and people with mutations causing extremely short telomeres (DKC) have only a few features of aging (including osteoporosis).

Cellular defenses against damage

Damaged proteins can interfere with normal cell function. Heat shock proteins (Hsps) increase in response to stress and serve as chaperones for shuttling damaged proteins to degradation. Werner's syndrome of acceleration of some aspects of aging is due to a mutation of the WRN gene involved in DNA repair, but there is little other evidence that normal aging is due to decreased DNA repair.

Inflammation and aging

Circulating levels of many pro-inflammatory cytokines, such as IL-6, increase with aging. They cause insulin resistance and have been associated with an increased risk of mortality, especially from cardiovascular disease, and have been associated with poor neuromuscular function and frailty. These cytokines are produced from inflammatory responses to infections, foreign antigens, and damaged native proteins. They are also produced by visceral fat and by senescent cells. It has been proposed that this is a particularly important 'cause of aging' in humans because we live long lives exposed to many infections and environmental insults.

Miscellaneous 'causes' of aging The availability of energy in cells controls gene expression through Sirtuins, enzymes whose functions can be replaced or augmented by resveratrol in red wine. DNA becomes increasingly methylated with aging which can suppress transcription of numerous genes, but its relationship to features of aging and mortality are not known. Progeria is very early onset of many features of aging due to mutation of the LMNA gene that causes abnormal protein scaffold around the inner edge of the nucleus and build-up of LMNA protein within the nucleus.

Slowing aging

A small (CALERIE) trial in humans showed that 25% reduction in caloric intake decreased metabolic rates, improved insulin sensitivity, increased mitochondrial biogenesis and decreased markers of oxidative damage of DNA. Our research group hopes to show that treatment with resveratrol will slow aging without having to cut calories.

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(2) Aging and Fragility of Bone

Charles H. Turner
Indiana University

Bone aging is a collection of largely irreversible changes to its micro-architecture and functional capacity. These include non-enzymatic cross-linking of bone collagen, which makes it more brittle, and the disintegration of the trabecular network. Biomechanical analyses have demonstrated that not all trabecular bone loss is equivalent. Rapid bone loss associated with high bone turnover can cause trabecular perforation and removal, whereas slower bone loss may result in thinning of trabeculae. The former is far more detrimental to bone strength. Bone loss that results in trabecular perforation reduces bone strength by 2- to 5-fold more than loss caused by trabecular thinning. Interesting, normal adaptive and reparative processes may contribute to trabecular perforation. Typically perforations occur when osteoclasts target the narrowest region of a trabecula. This region is also under the most stress and may accumulate microdamage more rapidly, which in turn attracts osteoclasts.

With aging, the outside dimensions of bones increase due to periosteal apposition. This is accompanied with loss of bone on the inner surfaces. Bone loss is not uniform throughout the skeleton but may be much worse in specific regions. This regional bone loss appears to contribute to femoral neck fragility. The superior region of the neck loses bone at a much higher rate than the inferior region, probably because of the stress distribution imposed on bone tissue due to daily activities. Unfortunately, loss of bone in the superior region makes the hip much more susceptible to failure during a fall.

Bone marrow stem cells lose their ability to differentiate into bone cells with age. Similarly stem cells within the periosteum lose the ability to differentiate into chondrocytes, which may explain why fracture healing becomes less efficient with age. The ability of mechanical loads to generate new bone formation declines with age and this might be due to a loss in the functional capacity of the osteocyte network. It is well known that the number of osteocytes within bone tissue decreases with age, but we do not yet know the functional significance of this decline.

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(3) Aging and sarcopenia

D. D. Thompson

Pfizer Global Research and Development, New London, CT

Sarcopenia is the loss of skeletal muscle mass resulting in a reduction of physical strength and ability to perform activities of daily living. Loss of muscle and strength with aging results in frailty leading to an elevated risk of suffering a fall, difficulty recovering from illness, prolongation of hospitalizations, and long-term disability requiring assistance in daily living. Further, the reduction of muscle mass and physical strength leads to diminished quality of life, loss of independence, and mortality (1). This loss of independence represents a high economic healthcare burden and area of high medical need. Sarcopenia also results when rapid muscle loss and reduced physical strength occurs due to disease-induced cachexia, immobilization, or drug-induced sarcopenia. The Institute of Medicine, a division of the National Academy of Sciences declared "frailty associated with old age" a priority area for national healthcare and an area that required increased research to deal with this healthcare issue (2).

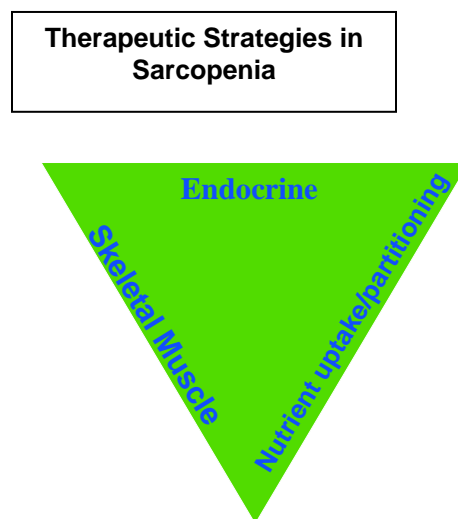
Today, there are ~34M persons aged 65 and over – almost 13 percent of all Americans – and this number will grow to ~70M by 2030, representing 20% of the population (3). Worldwide, the individuals who will become the frail elderly will more than double from ~321M in 1990 to ~799M in 2025. As a consequence of the expansion of this population segment along with increased longevity, the number of the elderly who will become sarcopenic and frail and require long-term institutionalization will consume an ever-expanding share of healthcare funds. In the US, 1.5M persons aged 65+ years are institutionalized each year, and 33% of these individuals are put into long-term healthcare facilities solely due to their physical frailty and their inability to maintain

perquisite activities of daily living, with most frail elderly facing >2 years of self-care disability at the end of life.

No effective and safe therapy is now available to prevent or restore muscle loss in these conditions. Currently, the standard of care for sarcopenia is either nutritional supplements and appetite enhancers or exercise to maintain or improve muscle strength. In spite of these treatment options, many elderly still lose muscle strength and function and are subsequently at risk for the deleterious outcomes of frailty. Anabolic steroids are also occasionally prescribed but are limited due to a poor efficacy and safety profile.

Emerging therapeutic strategies to prevent and treat sarcopenia can be divided into three categories: 1) nutrient uptake/partitioning, 2) skeletal muscle, and 3) endocrine (Figure 1)

Figure 1



Improved nutrient uptake is first line therapy in the treatment of sarcopenia, but has very limited efficacy. Significant breakthroughs are occurring in focusing on skeletal muscle targets that will treat sarcopenia, for example, myostatin (4). Also, the loss of neuromuscular junction integrity in aging may be a major contributor to sarcopenia and extends the focus beyond the muscle to also include innervation (5). Devising new therapies to maintain neuromuscular innervation with aging may provide substantial clinical benefit. Finally, endocrine strategies for the treatment of sarcopenia have been delineated. Two notable endocrine approaches include selective androgen receptor modulators (SARMs) and growth hormone secretagogues.

Growth hormone secretion and pulsatility decline with age, most notably after the age of 50 years of age. Growth hormone replacement to subjects with growth hormone deficiency has been shown to improve muscle mass. However, it is less clear if growth hormone replacement is efficacious in elderly sarcopenic subjects. With the discovery of orally active compounds that selectively stimulate growth hormone secretion, it is feasible in elderly sarcopenic subjects to test the hypothesis that restoring growth hormone secretion and pulsatility to young adult levels may increase muscle mass and physical performance.

CP-424,391 is an orally active growth hormone secretagogue or ghrelin receptor agonist (6). In preclinical models, CP-424,391 stimulates growth hormone secretion in pituitary cells and increases growth hormone secretion in rats and dogs. CP-424,391 was evaluated in single and multiple dose clinical trials and demonstrated increased growth hormone secretion leading to increased IGF-1 levels. Further, CP-424,391 was evaluated in longer term trials in elderly frail subjects where body composition and physical performance were evaluated.

In conclusion, therapeutic strategies for the treatment and prevention of sarcopenia are under investigation. Future studies will ascertain the ability of these strategies to alter the course of sarcopenia to determine if improvement in body composition and physical performance will have a beneficial outcome in the growing elderly population.

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(4) Vascular calcification.

Y. Tintut, S. Morony, M. Huang, J. Lu, Z. Zhang, W. Tseng, and L. L. Demer.
Departments of Medicine, Physiology, and Biomedical Engineering, UCLA, Los Angeles, CA, USA.

In humans and experimental models, vascular calcification causes hypertension, left ventricular hypertrophy, and congestive heart failure. The distribution of vascular calcification maps closely with atherosclerosis. Surprisingly, vascular calcification also correlates age-independently with osteoporosis. We previously identified and isolated, from the artery wall, multilineage cells that produce a mineralized matrix in a regulated process nearly identical to osteoblastic differentiation and mineralization, governed by most of the same transcription factors and signaling pathways. These cells, which are derived from single cells by dilutional cloning, have a lineage potential similar to that of mesenchymal stem cells, and, thus, have potential use in tissue bioengineering. But cells alone are not sufficient to produce tissue; architectural organization is essential to function. Thus, we were intrigued to observe that 5-10 days after uniform monolayer plating, these cells spontaneously organize into localized aggregates in macroscopically-visible patterns, such as evenly-spaced spots or stripes. We hypothesized that the patterns were arising from the uniform monolayer through a general phenomenon known as reaction-diffusion.

To test this hypothesis, we developed a mathematical model in which parameter values were determined experimentally. Results provided evidence that the pattern formation resulted from a reaction-diffusion process driven by interaction between two morphogens, bone morphogenetic protein-2 (BMP-2) and its inhibitor, matrix GLA protein (MGP). The model prospectively and accurately predicted experimental outcomes of treatment with exogenous MGP as well as of treatment with warfarin, which is known to inhibit MGP function. The former converted the pattern from stripes to spots, and the latter from widely-spaced stripes to a dense labyrinthine pattern (stripe-doubling).

To assess whether these cells retained multilineage capacity *in vivo*, which would be important for therapeutic use, we performed preliminary studies in which diffusion chambers containing the cells were implanted subcutaneously in hyperlipidemic (*Idlr*^{-/-}) and wild-type mice (n=4 of each). After 56 days, the cellular products were explanted from the chambers and examined histochemically and immunohistochemically. Although the cells implanted in wild-type mice remained quiescent, cells implanted in hyperlipidemic mice produced substantial matrix and aggregated into nodules with essentially all features of atherosclerotic plaque, including excess matrix, ectopic mineral, fibrous cap, foam cells, cholesterol crystals, and necrotic core. Immunohistologically, we found evidence for collagens type I and II, collagen I. These findings suggest that artery-derived, mesenchymal stem cells and reaction-diffusion principles may contribute significantly to the development and distribution of vascular calcification and that these cells may be useful in bioengineering because of their unique ability to self-organize into architectural patterns in a manner open to control by specific biochemical treatments.

(5) Aging and Falls: Causes and Prevention

S.R. Lord,

Prince of Wales Medical research Institute, Randwick, Sydney, Australia

Studies on age-related changes in neurophysiological and sensory systems date back to the early 19th century. In general, the many studies undertaken since then have found that for each system studied, age-related functional declines commence in the third decade, and accelerate in older age. However, the extent of such changes is markedly affected by the varying test procedures and definitions of the normal aged individual used in each study. If the selection criteria include only older subjects who are not disadvantaged by poor health or inactivity, are at ease in a laboratory setting and have a high level of motivation for the task, only small age-related increases are observed. Small age-differences are also observed if the tasks required are relatively simple. If the selection criteria are relaxed, or if the tasks are more difficult, greater changes in performance with age have been observed.

This paper reviews the published studies on age-related changes in vision, vestibular function, peripheral sensation, strength, reaction time and balance. It also presents findings of work we have undertaken on the inter-relationships among these physiological systems, their contributions to stability and gait, and the role that impairments in these systems play in predisposing older people to fall. A range of screening and assessment tools will be described including the Physiological Profile Assessment (PPA). The PPA makes use of normative data derived from large population studies for assessing vision, peripheral sensation, lower limb strength, reaction time and balance. This information is then used to derive a falls risk index score and a physiological profile for identifying specific physiological deficits that require targeted interventions for reducing fall risk. Examples of how the falls risk index score and components have been used in falls prevention randomised control trials and health care settings will be described.

Our findings indicate that it is feasible to obtain quantitative measurements of important neurophysiological and sensory systems, and to use these assessments to identify older people at risk of poor outcomes such as falls and institutional placement, and to evaluate the effectiveness of intervention programs aimed at maximising physical functioning and independence in old age.