Chapter 7

CLINICAL AND BIOCHEMICAL EVALUATION CHANGES OVER AGING

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Aging is associated with susceptibility and reduced ability to respond to internal and external stressors. A reduction of functional reserve occurs in many physiological systems and determines increased vulnerability to diseases and high risk of functional dependence. While these modifications can be observed in most persons, particularly in the context of longitudinal studies, they are characterized by extreme variability across individuals and only modest synchronism with chronological aging. As a result, the degree of susceptibility to stressors and exhaustion of functional reserve is dispersed over a wide spectrum in persons of the same age, and the amount of dispersion becomes even greater when we consider older age groups.

By convention, geriatricians define “frail” as those individuals that are at the extreme edge of the severity spectrum in this process. Because of this conventional attitude, frailty is often used as an exchangeable term for disability, comorbidity and poor health status, and it is also considered as an

irreversible condition leading to adverse health outcomes. On the contrary, it is important to conceptualize "frailty" as a continuous, other than a discrete process, where several stages or degrees of severity can be defined as they become useful in research and clinical practice, and that below a certain degree of severity it can be reversed with appropriate interventions.

The aging process is probably associated with the development of some unavoidable degree of "frailty" which in the literature is often referred to as "normal aging". Even the healthiest octogenarian is more sensitive to the effects of stressors than the healthiest teenager. Diseases and behavioral risk factors contribute to frailty in ways cannot be completely explained by traditional biomedical expectations. The effect of environment on frailty is complex. An environment that is too challenging causes a rapid exhaustion of functional reserves and leads to an overt instability of the biological homeostasis. On the other hand, an environment that is not at all challenging, leads to a progressive "atrophy" of the homeostatic mechanisms and makes the individual more susceptible to future stressors.

Clinicians who care for older patients face several times daily the complex implications and questions posed by the age-associated frailty. For example, it is clearly difficult to prescribe a pharmacological treatment, make decisions about rehabilitation, give advice to patients about the risk/benefits of a specific surgical procedure or establish the prognosis of diseases, without having information regarding the degree of functional reserve and ability to respond to stress. On the other hand, there is still much disagreement and discussion of the criteria that should be used for the operational definition of frailty, and most have no idea as to how to grade its severity.

There is some consensus that the basic clinical features of the frailty syndrome should include the following domains: a) mobility, such as lower extremity performance and gait abnormalities; b) muscle weakness; c) poor exercise tolerance; d) unstable balance; e) factors related to body composition, such as malnutrition, and sarcopenia (loss of lean body mass), and weight loss. Validity of these factors as critical elements of the frailty syndrome is provided by studies showing that in older, non-disabled persons, individual components are associated with the classical geriatric syndromes (e.g. falls, symptomatic depression, urinary incontinence and functional impairment) and are strong and independent risk factors of disability and death.

In 1999, Walston and Fried [1] developed an interpretive framework that combines the elements of the "body composition" and "mobility" domains of the frailty syndrome into a pathophysiologic pathway where sarcopenia and poor muscle strength, by limiting mobility and physical activity, reduce total energy expenditure and nutritional intake, which, in turn, lead to weight loss and further aggravate sarcopenia. Using data from

the Cardiovascular Health Study the elements of the pathway were as follows: 1) unexplained weight loss; 2) poor grip strength; 3) self-reported exhaustion; 4) slow walking speed; and 5) low physical activity. After adjusting for significant confounders, participants with 3 or more of these characteristics were at significantly increased risk of disability, hospitalization and death. The work of Walston and Fried [1] demonstrates that aggregating measures in the domains of physical function and body composition are an effective initial basis for developing screening criteria for an intrinsic vulnerability that have predictive validity. However, without understanding the pathophysiologic pathway that leads to frailty as a syndrome that justifies the aggregation of the domains proposed by Waltson and Fried [1], we lack the critical information to envision any serious attempts to apply the concept of frailty into clinical practice. In this chapter we explore some of the biological mechanisms that tend to become dysregulated with aging and may contribute to the pathophysiology of the frailty syndrome. Some of this information concerns biological markers of frailty that can be already measured. So the possible use of these measures in current clinical practice are pointed out in the various sections of this chapter. As our understanding of the pathophysiology, clinical presentation, and consequences of the frailty syndrome improves, many additional uses of the measures will emerge, and will help identify new potential targets for intervention.

1. HUMAN AGING

Gradual physiological changes that often parallel the aging process contribute to the conventional view of “normal” aging. Normal aging implies a progressive decline of the physiological reserve and the ability to compensate, but it is compatible with autonomy over the entire life span. In frail, older persons the decline in functional reserve is accelerated and compensatory mechanisms start failing with consequent negative health outcomes as the functional reserves are depleted.

A better understanding of physiologic changes that proceed and accompany frailty and, over time, lead to disability is needed if we want to capture this pathological process in an early stage, and develop targeted interventions that will delay or postpone the onset of disability. Unfortunately, we have very little information on this topic and, worse yet, what is known is sparse and difficult to reconnect to an overall paradigm. This chapter attempts to address this problem. We will focus on body composition changes, chronic inflammation, oxidative stress and hormonal changes that often occur in older persons and are accelerated over the aging process. Additionally, in the final part of our discussion, we provide our

view on how this information can be used in clinical practice to provide better care to frail older persons.

2. OVERVIEW OF BIOCHEMICAL MARKERS AND AGING (OR FRAILTY)

Many efforts have been made to identify biochemical markers of aging in both normal and frail older individuals [2,3]. Traditionally, clinical chemistry results obtained from laboratory testing are compared with the corresponding reference values in order to determine whether such values fall within the central 95% area under the Gaussian symmetric bell-shaped curve, or the "normal range". Reference values calculated using this method are reported, for example, in the recommendations of the Expert Panel on Theory of Reference Values of the International Federation of Clinical Chemistry and the published guidelines of the National Committee for Clinical Laboratory Standards [4]. In spite of this generalized trend, several lines of research indicate that a purely statistical approach to the identification of "normal" values can be misleading, and methods based on predictive validity in relation to health outcomes should be explored. This is particularly evident for reference values in geriatric patients. Tietz et al. [5] obtained data from 236 individuals, ages 60 to 90 years, 22 individuals, ages 90 to 99 years, and 69, 100 years of age or older. As shown in Table 1 (Tietz et al), plasma levels of dehydroepiandrosterone (DHEA) and DHEA sulphate (DHEAS) were lower in individuals over the age of 90 compared to those of young adults. Other sex hormones, estradiol, estrone and testosterone were found to be much lower in persons over the age of 90 than those of young adults. Interestingly, insulin levels tended to increase in adults aging from 60 to 90 years of age, while a decline in insulin levels was observed in persons over the age of 90.* (Table 1)

The need for biological markers of pathology in the evaluation of older persons is justified by the peculiar relationship that exists between diseases and health status in old age. Because aging is associated with an increment in the global susceptibility to diseases, multiple morbidities are very common. Analogously, diseases that are not clinically overt are often associated with pathological processes that already affect the health status but have not reached the severity threshold that makes them identifiable as "diseases". Thus, the global burden of comorbidity can be captured only indirectly, using functional or biological markers.

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* Note that age-adjusted "normal" values for most of these hormones are lacking and the values prepared in the literature are highly variable from author to author.

Table 1. Laboratory ranges (95th percentile) and mean values of some hormones in young adults, 60- to 90-year olds, and > 90 years of age

		Young adults		60-90		>90	
Analyte	Sex	Range	Mean	Range	Mean	Range	Mean
DHEA, mg/L	M + F	1.60-8.00				0.17-1.69	0.79
DHEAS, mg/L	M	1800-4500				40-750	287
	F	1200-3150				20-600	231
Estradiol, ng/L	F	Follicular:30-100 Luteal:70-300				<5-20	6
Estrone, ng/L	F	Follicular: 30-100 Luteal: 60-160				<5-58	29
Insulin (mU/L)	M+F	6-23	11.8	6.6-36.7	16.4	2.4-19.0	7.20
Testosterone total (mg/L)	M	3.50-10.30				2.15-6.71	3.40

As reported by Tietz et al. (5)

Impaired glucose, protein and lipid metabolism in older individuals is common. Aging is associated with impaired glucose handling, mainly due to a decline in insulin activity [6-8]. There is strong evidence that increased resistance to insulin activity is one of the main components of diminished homeostatic glucose regulation in older persons. Insulin-mediated glucose uptake, measured using a glucose clamp technique combined with H^3-glucose infusion, was shown to progressively decline over aging [9]. The response to insulin resistance is increased insulin production. When the ability to compensate for insulin resistance by increasing insulin production is exhausted, the glucose homeostasis becomes dysregulated, and type 2 Diabetes Mellitus occurs. Therefore, insulin resistance along with the reduced ability to secrete insulin, as seen during glucose tolerance test administration in elderly subjects, also contributes to impaired glucose homeostasis [10,11]. However, the dysregulation in the glucose metabolism due to peripheral insulin resistance is important long before diabetes can be

diagnosed and cannot be overlooked in the clinical evaluation of older persons.

Total body protein, lean body mass and the rates of protein synthesis decline with increasing age. More importantly, such changes are components of an impaired homeostatic phenomenon, which is not always balanced with adequate dietary protein intake. Furthermore, an altered state of hepatic protein synthesis with reduced fibrinogen and other protein carriers, such as thyroxine-binding protein and iron-binding protein may result in an altered coagulatory state, reduced thyroxine plasma concentrations and an anemic state. In addition, reduced plasma concentrations of albumin have been correlated with a higher degree of oxidative stress.

Lipid and lipoprotein concentrations vary over an individual's lifespan [12]. In particular, total cholesterol and triglyceride levels tend to increase up until 50 years of age and then a gradual decline starts to occur. Interestingly, a positive correlation exists between total cholesterol and/or triglyceride levels with the incidence of cardiovascular disease up to the age of 50 years. However, the ability of total cholesterol to predict coronary heart disease in very old individuals remains controversial. Raiha et al [13] reported that an elevated level of total cholesterol was not a cardiovascular risk in older persons, but predicted survival for non-cardiovascular disease mortality, while Manolio et al [14] did not find any correlation between total cholesterol and all-cause mortality in older subjects [13]. Interestingly, studies have reported that persistently low cholesterol levels increased the risk of mortality in males aged 71 to 93 [15]. Low total cholesterol levels have also been associated with all-cause mortality in elderly Italian men and women, thus underscoring the potential importance of low levels of cholesterol as a warning sign of rapidly declining health [16].

Such discrepancies can be explained by one of the major differences between middle-aged and older populations, which is the presence of an increased prevalence of poorer health of older individuals. In fact, older frail persons with low total cholesterol levels are more likely to have a decreased survival rate than older persons with little or no disease in the presence of chronically low cholesterol values [17]. Interestingly, after adjusting for frailty markers in a large sample of older persons, elevated total cholesterol levels predicted an increased risk for death from CHD, and the risk of death from CHD decreased as cholesterol levels declined [18]. These authors also emphasized the finding that frailty markers were consistently associated with low cholesterol levels, thus confirming similar previous reports. Only further investigations aimed at evaluating controlled clinical trials with lipid-lowering therapy in non-frail older persons can shed light on the risks or the benefits of such treatment.

Regarding lipoproteins, high-density lipoprotein cholesterol (HDL-C) is a considered a protective factor for CHD [19]. In particular, HDL-C levels

have been associated with good health status, while reduced HDL-C values are recognized as risk factors for CHD in both middle-aged and older persons. Furthermore, it has been shown that reduced HDL-C also predicts non-CHD/stroke mortality in older persons [20]. Thus, low HDL-C may also be considered a valid biomarker for chronic disease and poor health status in old age.

Ueno et al[3] have recently described biomarkers of aging in women. In particular, these authors suggest that five variables should be considered specific biomarkers for aging in women: forced expiratory volume in 1.0 s (FEV_1), systolic blood pressure (SBP), glucose (GLU, mg/dl), ratio of albumin to globulin (A/G) and mean corpuscular hemoglobin (MCH, pg). Such multiple physiological variables reflect the function of diverse vital functions, in particular, pulmonary function, blood pressure, glucose handling, protein metabolism and hematological functioning. Biological age scores (BAS) were calculated using the parameters mentioned above. Ueno et al [3] concluded that the rate at which women age is relatively slow up until 65 years of age. Then after 65 years of age their rates of aging rapidly increase. Therefore, the biological processes aimed at maintaining a stable homeostasis correctly function up to age 65; after 65, false signaling of such a complex system occurs causing it to lose its effectiveness. This observation is of extreme importance as altered biomarkers are highly correlated with mortality [21] and the frailty syndrome is commonly observed in persons over the age of 65.

The aggregation of variables in global indices based on their predictive role for specific outcomes is very appealing for clinical use, but adds very little to our understanding of the global burden of disease in old age. Recently, authors have also suggested that the involvement in multiple physiological systems that is characteristic of older patients with comorbidity should be interpreted in the context of the "frailty syndrome". According to current views of frailty, homeostasis is disrupted when the ability of individuals to respond to internal and external changes declines below the threshold of effective compensation. When this occurs, abnormal concentrations of specific biomarkers of frailty become detectable in the biological fluids, and structural changes take place in cells and tissues. Unfortunately, serum biomarkers are not currently used to identify frailty, which still remains a clinical diagnosis based on medical history, symptoms, and signs. Clinically, the frailty syndrome is characterized by an excessive reduction in lean body mass, in walking performance and in endurance, associated with a perception of exhaustion and fatigue [22]. Several lines of evidence, however, show that this syndrome is often paralleled by important changes in physiological systems accompanied by changes in serum levels of biomarkers.

3. FRAILTY AND THE NEUROMUSCULAR SYSTEM

There is growing evidence that the core target of the frailty syndrome is motor organization, specifically the muscular and nervous systems. Disease, disuse and aging trigger a mechanism that impoverishes the redundancy of muscular and nervous backup systems, leading to a measurable decline of motor performance. Once the process is activated, its consequences follow a common pathway leading to a more generalized loss of motor functioning. There is good evidence that measures that are related to mobility and motor performance are interpretable as proxy markers of frailty. However, the "diagnosis" of frailty, as a syndrome, hides an array of different pathologic processes that may involve the integrity and functionality of selected physiological subsystems implicated in motor performance [2]. Some of these subsystems include: bone, joints, muscles, peripheral nerves, metabolic efficiency, aerobic capacity and energy production. Clinically, the best criteria for screening of frailty are tests of mobility, gait, balance, manual dexterity, activities of daily living (ADLs) [23], instrumental activities of daily living (IADL) (24) and the Barthel Index [25]. However, it is conceivable that specific biomarkers could be measured in order to identify the involvement of each one of these physiological systems in the early stages of the disablement process.

Lower extremity performance in non-disabled persons is an excellent predictor of poor quality of life, deterioration of health status, incident disability, health care utilization, nursing home admission and death. Thus, physical performance measures have been considered "vital signs" of functional decline in older persons [26]. In particular, gait speed and the short performance battery, developed in the context of the EPESE study, have been identified as quantitative estimates of future risk for functional decline and hospitalization [26] Observational studies provide good evidence that performance-based measures of mobility are valid proxy measures of frailty and global susceptibility to adverse health outcomes.

In older persons, poor muscle strength and poor physical performance often coexist. Midlife handgrip muscle strength has been recognized as an important factor that predicts old age functional ability [27]. Observational studies have consistently shown that chronic conditions such as coronary heart disease, diabetes and pulmonary obstructive disease are associated with lower muscle strength. These findings suggest that a core mechanism exists that is responsible for changes in body composition and disease susceptibility in old age and ultimately to the age-associated changes in functional capacity. Possible links between diseases in old age and "frailty" are: nutritional depletion, inflammation, reduced physical activity or inactivity. These mechanisms are, in turn, risk factors for mortality. Thus,

in persons afflicted with chronic illness, reduced muscle strength could be considered an important marker of disease severity. Indeed, handgrip muscle strength has also been associated with overall mortality, independently of poor nutritional status, inflammation and physical inactivity [28]. These findings suggest that muscle strength has a direct effect on mortality or increases the risk of mortality through a mechanism that is still unclear.

4. BODY COMPOSITION CHANGES

The two main components of body composition are fat mass and lean (fat-free) mass. Fat-free mass consists of body cell mass, extracellular fluid and the extracellular solids such as collagen and bone mineral [29]. The body cell mass may be further subdivided into the fat-free portion of cells within muscle, viscera and the immune system. The body cell mass is functionally the most important compartment in determining energy production and expenditure, protein needs, and metabolic response to stress (acute phase response).

There are substantial changes in body composition that accompany the aging process [30]. In particular, the fat mass increases and accumulates preferentially in the abdominal area, while a parallel decline in muscle mass and bone density occurs. Interestingly, the changes in body composition that begin to manifest during adulthood may be partially explained by an imbalance of energy intake and expenditure. In older adults, however, these changes are extremely accelerated compared to younger cohorts, and cannot be explained simply as an imbalance between energy intake and expenditure.

In most older persons, fat mass constitutes a greater percentage of total weight than individuals at younger ages. A population-based study in which anthropometric parameters were measured over the entire life span (age range: 20-103 yrs.) demonstrated that the accumulation of abdominal fat with age occurs primarily during middle age [31] but is different for men and women. In particular, the greatest change of waist circumference seems to occur in men between 20 and 55 years of age, while in women, the waist circumference tends to increase progressively across the entire life span.

Many studies have shown that increased visceral fat is a risk factor for age-related diseases such as hypertension, type 2 diabetes, cardiovascular disease and some types of cancer [20,32]. Adipose tissue has also been correlated with oxidative stress, reduced glucose uptake, and reduced insulin clearance. Understanding how changes in body composition and, in particular, fat distribution, affect the risk for many disease states and mortality is one of the most important research questions that should be addressed in future studies.

The simplest clinical indicator of visceral fat is the waist circumference. A number of studies have shown that waist circumference is an independent risk factor for cardiovascular disease in adults, including those 65 years and older. On the contrary, the relationship between body mass index (BMI), cardiovascular disease and all-cause mortality is controversial. The highest mortality rates have been found in older persons with very low BMI, while in middle-aged persons, BMI was positively associated with mortality [33]. These data suggest that the relationship between body composition and health-related outcomes in older persons cannot be evaluated simply in conventional terms of body fat, but rather fat distribution and type of fat accumulated, both providing essential information for assessing such risk.

The notion that aging is associated with gradual reduction of lean body mass is also generic. In fact, selected tissues seem to be more affected by aging than others. In particular, the decline in non-fat mass is largely attributed to sarcopenia. Sarcopenia has been increasingly used to describe the age-related decline in both muscle mass and muscle strength. However, despite the term "sarcopenia", the precise criteria that define such a state have still not been agreed upon.

Changes in body composition that parallel the aging process are strongly associated with a decline in physical function and mortality risk. The underlying mechanisms responsible for the excess age-associated decline of muscle mass and function compared to other sections of lean body mass are still unknown. Several hypotheses have been proposed, which include: i) intrinsic biochemical and physical changes leading to muscle atrophy [34]; ii) reduced neuronal stimulation due to reduction in the number of α-motorneurons or their activity [35]; iii) oxidative damage of mitochondrial DNA with accumulations of mutations that reduce the efficiency of the metabolic pathways aimed at energy production [36,37]; iv) influence of external factors such as malnutrition, sedentary life-style and disease typically observed in older persons (38); v) loss of endogenous hormone production [39,40]; and vi) dysregulation of catabolic cytokines [41,42]. Over the aging process, both changes in the contractile efficiency of muscle fibers and changes in tissue quality, such as an increase in connective tissue and pericellular fat infiltration, may also contribute to altered muscle function.

There are many methods that simultaneously measure body fat and fat-free components of body composition. Some important measures are as follows: 1) skin-fold measurements are obtained using hand-held calipers, which exert a standardized pressure at various body locations. The sum of these measurements is used to derive body fat percentage. The caliper method is based on the idea that the thickness of subcutaneous fat reflects a constant proportion of the total body fat and that the sites selected for measurements represent the average thickness of the subcutaneous fat [43,44].

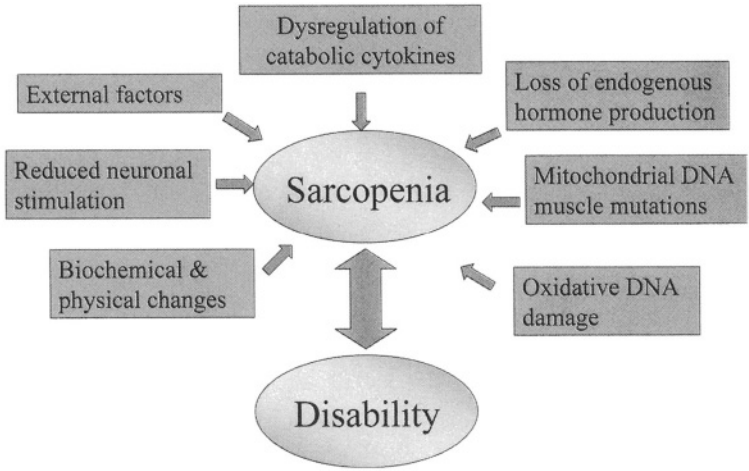
Also, the mean arm circumference with triceps skin-fold thickness is used to calculate muscle area and thus, derive fat-free mass; 2) hydrodensitometry or underwater weighing is another instrumental method that measures whole body density by determining body volume. This technique is based on the two-compartment model (fat and fat-free mass). The densities of bone and muscle are higher than water. Body fat percentage is then calculated from body density using standard equations (sirk or brozek); 3) bioelectrical impedance analysis (BIA) is measured utilizing a safe electrical signal that passes through the body. Impedance is greatest in fat tissue (10-20% water), while fat-free mass (70-75% water) allows the signal to pass much more easily. The measurement obtained is entered into a formula along with height, weight, and gender to determine lean and fat mass. However, in order to obtain correct evaluations from the BIA it is necessary that the body is within normal hydration ranges.

Newer more sophisticated methods for the assessment of body composition include the Dual Energy X-ray Absorptiometer (DEXA), computed tomography (CT), magnetic resonance imaging (MRI) and air displacement plethysmography . DEXA is a relatively new method that uses three compartments (total body mineral, fat-free mass, and fat mass). DEXA consists of a dual energy beam (two low dose x-ray sources) that scans bone and soft tissue simultaneously. DEXA is currently considered the "gold standard" measure because of the high degree of precision in a single measurement and the ability to provide the exact location of fat tissue distribution. CT scanning produces cross-sectional scans of the body. As the beam rotates data is collected, stored and applied to algorithms to build images that describe body composition. MRI utilizes a magnetic field that "excites" water and fat molecules, producing a measurable signal, which is then measured and analyzed. Whole body air displacement plethysmography (trade name BOD POD) is a new technique that is similar to the underwater method, but uses air displacement instead of water. It is based on Boyle's law, which states that volume and pressure are inversely related. All the methods described above are summarized in Table 2 (shown on page 154), with information on their reliability, advantages and disadvantages.

It is interesting to speculate on the consequences of sarcopenia that are not directly related to poor muscle strength. A number of physiological functions that take place within muscle tissues have a critical effect on human metabolism: muscles are a reservoir of body proteins and energy that can be utilized in periods of extreme stress or malnutrition; amino-acids can be mobilized during acute infections and are used as building blocks for antibodies; hormones are produced and catabolized in muscle tissue. Thus, age-related muscle mass reduction may explain the lower metabolic adaptation and immunological response to disease. Indeed, poor muscle strength is a strong predictor of mortality, independent of any other known

risk factors for poor muscle strength. The rate of decline varies among individuals and is influenced by factors that modulate the balance between catabolic and anabolic processes. There are several possible mechanisms that may be involved in the genesis of sarcopenia (Fig. 1). The most important of these mechanisms are prolonged pro-inflammatory state, change in hormone secretion signaling activity, and unopposed oxidative stress. However, recent data suggest that these three pathophysiological mechanisms are highly interconnected and should be interpreted as components of a unique process leading to frailty and disability in old age [45].

Figure 1. Possible factors involved in the genesis of sarcopenia



5. INFLAMMATION

Increased circulating and tissue levels of inflammatory markers have been observed in older persons, especially those who are frail and/or affected by comorbidity. Normally, cytokines or other biomarkers of inflammation initiate and regulate the acute phase inflammatory response during an infection, a trauma or any other type of stress. However, studies have suggested that a primary dysregulation of the mechanisms that initiate, modulate and shut off an inflammatory response often occurs with aging [46,47]. Such a dysregulation is mainly testimonied by high plasma levels of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), Interleukin-6 (IL-6) (48-50), (Interleukin-1) IL-1 and acute phase proteins in older persons [46]. In extreme situations, such as diseases that cause prolonged hypercatabolic states, severe muscle "wasting" may develop over a short period. However, a certain degree of muscle "decline" has been attributed to the reduced capacity of skeletal muscles to synthesize new proteins in the

aging process [29]. An imbalance between muscle protein synthesis and degradation occurs, ultimately leading to reduced muscle mass, protein content and strength. Such imbalance has been linked to pro-inflammatory cytokines capable of inducing proteolysis or inhibiting protein synthesis. TNF-α induces muscle proteolysis and plays a significant role in muscle wasting (cachexia). TNF-α and IL-6 can also inhibit protein synthesis, either directly or by interfering with IGF-1 signaling[51].

Elevated plasma levels of each of the pro-inflammatory cytokines mentioned above have also been observed in many age-related diseases, such as anemia, osteoporosis, sarcopenia, atherosclerosis, cancer, type 2 DM, impaired cognitive functioning, and Alzheimer's disease. This further supports the theory that a core mechanism contributes to overall age-associated changes in functional capacity.

6. HORMONES

Anabolic agents shift the anabolic/catabolic balance of protein metabolism toward the synthesis of new proteins, which is needed to replace the proteins that are continuously catabolized, therefore maintaining muscle integrity and volume. Hypertrophy requires the proliferation of muscle nuclei (hyperplasia) in order to maintain the nuclear/cytoplasmic ratio (52). Hormonal factors shown to be related to muscle hypertrophy are: Insulin-like Growth Factor-1 (IGF-1), Growth Hormone (GH), testosterone and dehydroepiandrosterone. High IGF-1 concentrations are associated with characteristics that are opposite to those typical of aging, including decreased body fat content, increased muscle mass and improved metabolic homeostasis of glucose and lipids. At the muscular level, IGF-1 stimulates protein synthesis and satellite cell differentiation, thus, playing a crucial role in the maintenance of muscle mass and function. Many studies have provided insight into the signaling pathways by which IGF-1 affects muscle anatomy and function [53-55]. Circulating IGF-1 concentrations decrease with advancing age. The age-associated decline in IGF-1 plasma concentrations is influenced by reduced GH levels, and also by nutritional status, insulin and inflammatory cytokines. Specifically, the biologic activity of IGF-1 on muscle strength can be inhibited by IL-6 [55], suggesting that the detrimental effect of inflammation on muscle functioning may be mediated by IGF-1. Furthermore, studies provide evidence that the higher concentrations of pro-inflammatory cytokines found in older persons directly interferes with the IGF-1 gene protein expression and receptor sensibility in muscles [55,56]. High IL-6 and low IGF-1 plasma concentrations are considered risk factors for poor muscle strength, poor lower extremity performance and disability.

The aging process is associated with the loss of many anabolic signals to muscle function. Recent studies have shown that age is not only accompanied by a decline in anabolic activity, but an increase in catabolic signals as well. In fact, impairment of the anabolic IGF-1 signaling pathway may have several negative effects:

1) Reduced physical activity that is often observed in advanced age causes decreased stretch-activation stimulation of different muscle isoforms of IGF-1;
2) An age-related decline of GH influences IGF-1 muscle response;
3) The progressive loss of appetite with reduced food intake can result in malnutrition and eventual "wasting";
4) Loss of motoneurons that are essential for skeletal muscle functioning leads to atrophy and increased proteolysis.

There is evidence that the age-associated decline in GH levels in combination with lower IGF-1 levels also contributes to the development of sarcopenia [57,58]. The reduced pituitary secretion of GH is probably due to age-related changes in the GH-releasing hormone (GHRH). Unfortunately, treatment with GH has demonstrated many adverse effects, such as peripheral edema, arthralgias, glucose intolerance and type 2 diabetes [40]. Investigations have demonstrated that therapy with GHRH (somatorelin) in older persons is capable of restoring the age-related decline of the GH response [59]. More studies attempting to verify whether such pharmacological approaches can restore muscle functioning as well as the metabolic homeostasis in elderly persons while minimizing side effects are underway.

Testosterone affects muscle mass and muscle strength both directly and indirectly. It has been reported that testosterone increases protein synthesis and intramuscular mRNA concentrations of IGF-1 and decreases inhibitory IGF binding protein 4 concentrations [60]. Due to evidence that testosterone levels decline with advancing age, a negative impact on muscle function is not surprising. Older men with low circulating levels of testosterone tend to have lower muscle strength than men of the same age with normal testosterone, and studies utilizing supplemental therapy with testosterone have shown an increase in muscle mass and strength in elderly males. Testosterone has also been linked to body composition changes such as an increase in muscle mass and a decrease in fat mass [61]. The widespread use of testosterone replacement remains controversial due to safety concerns and inconsistent reports regarding clinically important outcome measures.

The production and the circulating levels of adrenal sex hormone precursors, dehydroepiandrosterone (DHEA) and DHEA sulphate (DHEAS), decline significantly with aging [62]. DHEAS serum levels have been correlated with parameters of body composition. Some clinical trials have shown that supplementation with DHEA resulted in increased muscle strength and decreased body fat[63]. However, more recently these findings

were not confirmed in a large randomised controlled trial performed in men 60 to 80 years old. The mechanism by which DHEA acts on muscle function is probably related to the peripheral conversion to testosterone and dihydrotestosterone, but a direct effect of DHEAS cannot be excluded since specific receptors have been identified in muscle tissue.

Estrogen levels also decline with aging. Although estrogen has a direct anabolic effect on muscle cells in vitro, several authors believe that the effect of estrogen on muscle is mediated by their conversion to testosterone [64]. Interestingly, both estrogen and testosterone are capable of inhibiting IL-6 production, suggesting that an age-related decline of such hormones would play a pivotal role in catabolic signaling on muscle tissue. However, the available information regarding the effects of supplemental therapy of estrogen on muscle function is limited and the results are inconclusive. While some studies have concluded that estrogen therapy in postmenopausal women does not significantly affect muscle mass or strength [65,66], others suggest that estrogen therapy has a positive effect on body composition. For example, Sorensen et al [67] demonstrated that estrogen replacement therapy was significantly associated with an increase in lean body mass and also a decrease in total body fat.

As previously mentioned, advancing age is associated with impaired glucose handling mainly due to a reduction of insulin peripheral activity. Since insulin plays a pivotal role for muscle contraction by increasing glucose uptake and promoting intracellular glucose metabolism, it is plausible that age-related insulin resistance (IR) may be an important cause of poor muscle strength in old age. Furthermore, a reduction of insulin peripheral activity may reduce the muscle tissue anabolic rate leading to a relative catabolic state and in turn, facilitating sarcopenia. The contraction of Type I fibers is especially dependent on glucose entry and metabolism compared to contraction of Type IIa (fast twitch, oxidative, glycolytic) or IIb (fast twitch, glycolytic) fibers [68]. Type I fibers are more responsive to insulin, and are more representative of the muscle in older persons [69].

Over the aging process, changes in both the contractile efficiency of muscle fibers and changes in tissue quality, such as an increase in connective tissue and pericellular fat infiltration, may contribute to altered muscle function [70]. Moreover, insulin resistance (IR) could be further worsened by the occurrence of pericellular fat accumulation both directly and through the increased production of pro-inflammatory cytokines, such as IL-6 and TNF-α. Furthermore, a recent study demonstrated that a decline in aged skeletal muscle force might also be due to a reduction of L-type calcium channels, resulting in excitation-contraction uncoupling and less Ca^{2+} release by the sarcoplasmic reticulum (SR) [71]. Insulin has a stimulatory effect on intracellular calcium uptake [71]; thus, an age-related state of IR may negatively affect muscle contraction via this mechanism. It is well known

that IGF-1 actively stimulates insulin receptors. Since IGF-1 levels decline throughout aging, the decline in muscle strength that is associated with aging may be mediated by decreasing plasma IGF-1 levels that contribute to IR. Studies will be needed in order to verify if the impact of IR on specific muscle tissue and functioning in aged individuals exists.

Certain changes typically occur in muscles of older adults. The quantity of muscle declines, although this varies between individuals, but the composition of the muscle changes with aging as well. Increased infiltration of fat deposited in skeletal muscle tissue may affect muscular function. Much of the existing data on the association between intramyocellular lipid (IML) content has been obtained directly from muscle tissue biopsies. However, the use of muscle attenuation through computed tomography (CT) scanning, as a measure of IML, has been validated [72]. In 45 men and women, the muscle fiber lipid content determined histologically with oil red staining was correlated with muscle attenuation. Thus, the use of CT-derived muscle attenuation should be considered a non-invasive method of measuring IML. In fact, Visser et al [73] demonstrated that increased skeletal muscle fat infiltration measured by CT scanning was associated with poorer lower extremity performance independently of total body fat and muscle area in older men and women.

7. OXIDATIVE STRESS

The accumulation of lipofuscina [74] and increased cross-linking of collagen [75] were the first observations reported on the effect of the aging process at the cellular level. At that time it was unknown that these modifications are, at least in part, related to oxidative stress. More recently, researchers have focused on the progressive changes that occur in the DNA structure and the underlying causes and potential consequences of these mutations. For example, a number of studies suggest that excess and unopposed oxidative stress is the main cause of increasing mitochondrial DNA (mtDNA) mutations with aging and in several age-related diseases. Accordingly, oxidative stress characterized by an uncontrolled production of free radicals is considered a major factor in the aging process. In aerobic biological systems, free radicals are primarily derived from oxygen and are produced by splitting a covalent bond into atoms or molecules with an unpaired electron, therefore forming highly reactive oxygen species (ROS). In normal physiological conditions, the intra-mitochondrial environment is characterized by a substantial equilibrium between the production of ROS and the activity of anti-oxidant mechanisms, such as glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD). Several lines of research suggest that the endogenous production of ROS increases with age and, in

parallel, the activity (but not the tissue concentration) of anti-oxidants declines, therefore increasing the risk of damage due to oxidative stress, especially at the level of the mtDNA. In addition to its effect on mtDNA, oxidative stress also adversely impacts other vulnerable targets, including lipid and protein components of membranes. Free radicals can cause lipid oxidation with a consequent reduction in transmembrane transportation. Age-related overproduction of ROS may also lead to the activation of apoptosis. Therefore, the accumulation of oxidatively damaged mtDNA, together with enhanced apoptosis act synergistically to cause the general decline of biochemical and physiological function of tissues over the aging process. The underlying mechanisms by which these events accompany the aging process remain to be identified and merit further investigation.

Studies suggest that the degree of unopposed oxidative stress is predictive of mortality. In particular, the production of free radicals in the heart, kidney and liver is inversely proportional to the maximum lifespan [76] and rate of mitochondrial oxygen radical generation is negatively associated with animal longevity. In animal models, caloric restriction, which decreases the rate of aging, also decreases mitochondrial oxygen radical production and oxidative damage to mtDNA.

The mitochondrial DNA/oxidative stress hypothesis can explain certain age-related disease states such as Parkinson's disease, Alzheimer's disease and skeletal muscle myopathies. Recently, epidemiological studies have suggested that dietary anti-oxidants may have a significant impact on age-related disease states [77,78]. This remains unproven in clinical trials. The clinical implications of oxidative stress are complex, and intervention studies are needed to further clarify the role of dietary and supplemental antioxidants in the prevention of age-associated frailty.

8. SUCCESSFUL AGING

The possibility of reaching the extreme end of the human lifespan results from the continuous adaptation of the body to respond to negative insults over the aging process. Healthy centenarians are a very selective group of persons representing one of the best living models of "successful aging". Many studies have focused on centenarians' anthropometric, endocrine and metabolic characteristics in order to formulate a clearer clinical picture of successful aging. They report that the average fat free mass (FFM) of healthy centenarians is similar to that of other aged subjects but lower than middle-aged adult subjects [79]. However, most healthy centenarians do not undergo the usual anthropometric derangement found in elderly persons. For example, the waist/hip ratio has been found to be lower in healthy centenarians than in other aged individuals. Regarding endocrine

factors, total plasma IGF-1 concentrations were similar in both healthy centenarians and aged subjects, but the molar ratio IGF-1/IGF binding protein-3, an expression of free plasma IGF-1 concentration, was observed to be significantly elevated in healthy centenarians compared to elderly subjects [80]. This ratio is negatively correlated with body mass index, body fat content, plasma triglycerides, and FFA and LDL concentrations[80].

While serum markers may be useful for helping identify successful aging, caution should be used since the interpretation may be different in younger adults than in older persons. For example, in older persons, the ability of total cholesterol to predict age-related diseases such as coronary heart disease (CHD) has been challenged. In middle-aged adults, total cholesterol levels have been shown to have a direct association with CHD and mortality, but such a relationship in individuals over the age of 65 remains controversial. In older persons, a J or U-shaped association has been reported, suggesting that extremely high or low concentrations have an increased risk of death [81,82]; total cholesterol levels have also been shown to have a positive association, an inverse association, and no association with mortality in older persons.

Up to now, most studies have considered the association of total cholesterol on CHD in subjects under the age of 85 years. Interestingly, a recent study reporting data on fractionated lipoprotein levels among persons over the age of 85 years, concluded that low HDL cholesterol, but not high LDL cholesterol, is a risk factor for mortality from CHD and stroke in persons over the age of 85 [83]. Lipoprotein (a) [Lp(a)], a genetically controlled cholesterol-rich lipoprotein, has been hypothesized as an independent risk factor for premature CHD, stroke, and peripheral artery disease in elderly persons [84,85]. This observation may be due to the presence of Lp(a) in atherosclerotic plaques and its ability to stimulate smooth muscle proliferation[86].

The physiological and pathological roles of Lp(a) probably change with aging. Support for this comes from a study by Baggio et al [87], which reported no significant differences in Lp(a) serum concentrations among healthy centenarians, persons <65 and >65 years of age, even though Lp(a) has been proposed as an independent risk factor for cardiovascular disease. Centenarians with high Lp (a) levels had significantly higher IL-6 levels, thus characterizing the paradox of successful aging. Such data questions the idea that Lp(a) is under strict genetic control and suggests that environmental factors may play a significant role in older adults, including subclinical inflammatory states. Thus, a continuous remodeling of lipid metabolism may occur with aging and may be critical for successful aging. The deleterious reshaping of serum lipids and lipoproteins in young, adult and elderly individuals are considered risk factors for age-related diseases, while their biological significance in healthy centenarians remains unknown. Thus, only

future investigations highlighting age-related changes in lipid physiology of healthy centenarians on mortality rates will resolve such discrepancies.

Healthy centenarians have a lower degree of oxidative stress. In fact, it has been shown that healthy centenarians have greater plasma antioxidant defenses than aged individuals. According to the remodeling theory on aging, the body continuously and correctly adapts to deleterious changes over time. As previously mentioned, an age-related up-regulation of the inflammatory response takes place over the aging process. In both sick and healthy elderly individuals, peripheral blood markers of inflammation (albumin, cholesterol, IL-6 and CRP) have been associated with increased risk for mortality. Interestingly, the age-related increase of serum IL-6 levels has been seen in both elderly and centenarian individuals [49,87]. IL-6 dysregulation has been suggested to play a role in the pathogenesis of a variety of age-related diseases, such as diabetes and atherosclerosis [88]. Indeed, healthy centenarians have elevated pro-inflammatory cytokine concentrations, but do not have the same high incidence of most age-related disease states in other elderly persons. Thus, in healthy centenarians such abnormal cytokine levels may reflect a state of subclinical inflammation. The reason why healthy centenarians adapt correctly to such insults remains unknown.

Whether healthy centenarians have some protective genetic factors that can protect against deleterious changes or facilitate the remodeling process remain unknown. Future investigations will be needed in order to provide the necessary answers.

Tables 3 and 4 summarize some of the clinical and biochemical evaluations described in the text above, and that can be used to assess the degree of “successfulness: of the aging process. Note that these are only examples. An exhaustive list would be very large, and out of the scope of this chapter.

Table 2.

Table 2. Diverse Techniques for Body Composition Evaluations

Method	Reliability	Advantages	Disadvantages
Hydrodensitometry	++	Traditional reference method for body composition research	High cost; long test duration (15-60'); difficult for persons who dislike, can't be submersed in water, or have difficulty expelling air from their lungs; reading errors if air remains in lungs; reading may vary due to body hydration.
Skin-fold Measurements	++	Low cost; test duration: 10-20'	Precision depends on the skill of the technician; accuracy depends on sites measured; difficulty in grasping skin-fold of obese; multiple readings are required for accuracy.
BIA	++++	Test duration: 10'; Moderate cost; low to moderate technician skill.	Electrolyte gel can be uncomfortable; accuracy depends on minimal variability caused by body hydration level; measures derived by the equation used.
DEXA	+++++	Test duration: 10-20';subjects only have to lie still; measures fat distribution throughout the body in a single scan; no need to account for air mass in the lungs.	Very high cost.
MRI	++++	Very useful for high quality images for body fat distribution.	Very high cost; requires a highly skilled technician.
CT	++++	Very useful for ratio intra-abdominal fat to extra-abdominal fat.	Very high cost; exposure to radiation
BOD POD	++++	Brief test duration: 20 sec;	Very high cost; limited in availability; requires further testing in order to verify test accuracy in measuring body composition.

Table 3. Summary of diverse techniques described in the text in older persons

Test	Purpose	Clinical Situation	Specific Examples
Glucose Clamp	To assess insulin action and secretion	Impaired glucose handling	Measurement of insulin-mediated glucose uptake and glucose stimulated insulin secretion (11)
Isometric Muscle strength (upper limb)	To assess handgrip muscle strength	Sarcopenia, predictor of disability and mortality	Handheld dynamometer (27)
Isometric Muscle strength (lower limb)	To assess lower extremity muscle strength	Sarcopenia	Hip Flexion, Knee Extension, Ankle dorsal flexion, Hip abduction
Balance	To assess static balance	Screening for falls	FICSIT balance score (89)
ADL	To assess self-care, mobility and incontinence	Disability screening	Katz (ADL) (23) Barthel Index (25)
IADL	To assess the ability to shop, cook, perform household activities and finances	Disability screening	Lawton (IADL) (24)
Physical performance tests	Quantitatively assess gait, balance, and risk of falls	Valid proxy for frailty and global susceptibility to adverse health outcomes	Tinetti performance oriented mobility (90) assessment Short physical performance battery (91)

Table 4. Summary of some laboratory tests utilized in older people

Test	Values	Clinical Importance
Fasting glucose (mg/dl)	≥126	Diabetes mellitus
OGTT 2 hr glucose (mg/dl)	≥200	Diabetes mellitus
Fasting glucose (mg/dl) +	<110 + <140	Normal glucose homeostasis
OGTT 2 hr glucose (mg/dl)	110-125 + <140	Impaired fasting glucose (IFG)
	<110 + 140-199	Impaired glucose tolerance (IGT)
	110-125 + 140-199	Combined IFG + IGT
Total cholesterol (mg/dl)		
	>200	Hypercholestolemia with increased cardiovascular risk
	<50	Frailty marker
HDL	≤ 35	Risk of atherosclerosis
	≥160	Risk of atherosclerosis
LDL		
Albumin (g/dl)	<3.5	Malnutrition
C-reactive protein	>0.5mg/dl	Inflammatory state
Hemoglobin	men: <12g/dl	Anemic state
	women: <13g/dl	Anemic state
Red blood cells	men: <4.3 x 10^6	Anemia, hemorrhage, hemolysis
	women: <4.0 x 106	
	men: >5.5 x 10^6/µl	Polycythemia
	women: >5.1 x 106/µl	
White blood cells	neutrophils: >7500/µl	Infection, inflammatory state, neoplasms, metabolic disease states
	eosinphils: >500/µl	allergies, neoplasms,
	monocytes: >1000/µl	infection, sarcoidosis
	lymphocytosis: >4000/µl	mieloproliferative disorders
	neutrophils:<1500/µl	neutropenia: altered production, excessiva destruction
	lymphocytes: <1000/µl	lymphopenia: altered immune response

8. CONCLUSIONS

It is widely recognized that the assessment of diseases status performed according to the traditional dichotomy "no disease vs. disease" is insufficient to understand the complexity of problems that influence health and well being in older persons. This concept was recognized long ago by geriatricians and implemented in the paradigm of "Comprehensive Geriatric Assessment". Accordingly, many researchers and clinicians have proposed that the direct assessment of physical and cognitive function provides the essential information that is needed to design effective interventions in frail older persons. However, this approach has never been completely translated

into clinical practice and many geriatricians claim that the administration of any available medical treatment is still conditioned to a previous diagnosis of specific diseases and hypotheses about specific pathophysiological pathways. Furthermore, significant changes in health status may occur and be amenable to effective treatment long before any clear effect on physical and cognitive function is detected.

We propose that the concept of frailty – a condition that involves impairment in multiple physiological systems and is characterized by exhaustion of functional reserve, massive use of compensatory strategies and high risk of homeostatic breakdown – can be used by clinicians to gain a better understanding of the global burden of disease and reduced physical function in older persons and their interaction with the "pure" effect of aging. Unfortunately, there is still no agreement on the criteria that should be used in order to identify frail older persons. However, there is general consensus that comorbidity, disease susceptibility and risk of developing multiple health outcomes are commonly associated with the detection of abnormal circulating levels of several biomarkers and changes in body composition. Thus, composite measures of mobility, body composition, strength, circulating hormones and biomarkers of inflammation may help clinicians understand the severity of health status deterioration in their patients over and beyond the information provided by the simple diagnosis of diseases. Aggregate measures of these outcomes should be developed in future studies and are likely to replace the current criteria for the definition of frailty, both in research projects and in clinical practice.

REFERENCES

1) Walston J, Fried LP: Frailty and the older man. Med Clin North Am, 1999 Sep;83(5): 1173-94
2) Ferrucci L, Cavazzini C, Corsi A, Bartali B, Russo CR, Lauretani F, Ferrucci L. Cavazzini C, Corsi AM, Bartali B, Russo CR, Lauretani F, Bandinelli S, Bandinelli S, Guralnik JM: Biomarkers of frailty in older persons. J Endocrinol Invest, 2002;25;10-5
3) Ueno L, Yamashita Y, Moritani T, Nakamura E: Biomarkers of aging in women and the rate of longitudinal changes. J Physiol Anthropol, 2003; 22: 37-46
4) NCCLS: How to define, determine, and utilize reference intervals in the clinical laboratory; proposed guideline. NCCLS document C28P (ISBN 1-56238-143-1), NCCLS, 940 West Valley Road, Suite 1400, Wayne, Penn 19087, USA, 1992
5) Tietz NW, Shuey DF, Wekstein DR: Laboratory values in fit aging individuals--sexagenarians through centenarians. Clin Chem, 1992 Jun;38(6):1167-85
6) Fink RI, Kolterman OG, Griffin J, Olegsky JM: Mechanism of insulin resistance in ageing. J Clin Invest, 1983; 71: 1523-35
7) Rowe JW, Minaker KL, Pallotta JA, Flier JS: Characterization of the insulin resistance in aging. J Clin Invest, 1983; 71: 1581-7
8) Gumbiner B, Thorburn AW, Ditzler TM, Bulacan F, Henry RR: Role of impaired intracellular glucose metabolism in the insulin resistance of aging. Metabolism, 1992;41:1115-21

9) Ferrannini E, Vichi S, Beck-Nielsen H, Laakso M, Paolisso G, Smith U: European Group for the study of Insulin Resistance (EGIR). Insulin action and age. Diabetes, 1996; 45:947-53
10) Kosaka K, Kuzuya T, Hagura R, Yoshinaga H: Insulin response to oral glucose load is consistently decreased in established non-insulin-dependent diabetes mellitus: the usefulness of decreased early insulin response as a predictor of non-insulin-dependent diabetes mellitus. Diabet Med, 1996:S109-19
11) Defronzo 79: Glucose intolerance and aging: evidence for tissue insensitivity to insulin. Diabetes, 1979 Dec;28(12): 1095-101
12) Lipid Research Clinics Program Epidemiology Committee: Plasma lipid distributions in selected North American populations: the Lipid Research Program Prevalence Study. Circulation 1979;60: 427-439
13) Raiha I, Marniemi J, Puukka P, Toikka T, Ehnholm C, Sourander L: Effect of serum lipids, lipoproteins, and apolipoproteins on vascular and nonvascular mortality in the elderly. Arterioscler Thromb Vase Biol, 1997 Jul;17(7): 1224-32
14) Manolio TA, Ettinger WH, Tracy RP, Kuller LH, Borhani NO, Lynch JC et al: Epidemiology of low cholesterol levels in older adults. The Cardiovascular Health Study, Circulation 1993; 87: 728-37
15) Schatz IJ, Masaki K, Yano K Chen R, Rodriguez BL, Curb JD: Cholesterol and all-cause mortality in elderly people from the Honolulu Heart Program: a cohort study, Lancet. 2001 Aug 4;358(9279):351-5
16) Brescianinni S, Maggi S, Farchi G, Mariotti S, Di Carlo A, Baldereschi M, Inzitari S: Low total cholesterol and increased risk of dying: Are low levels clinical warning signs in the elderly? Results from the Italian Longitudinal Study on Aging. JAGS, 2003; 51: 991-6
17) Pekkanen J, Nissinen A, Vartiainen E, Salonen JT, Punsar S, Karvonen MJ: Changes in serum cholesterol level and mortality: a 30 year follow up. Am J Epidemiol, 1994; 139: 155-65
18) Corti MC, Guralnik J, Salive M, Harris T, Ferrucci L, Glynn R, Havlik R: Clarifying the direct relation between total cholesterol levels and death from coronary heart disease in older persons. Annuals of Internal Medicine, 1997; 126: 753-760
19) Schaefer EJ, Moussa PB, Wilson PW, McGee D, Dallal G, Castelli WP: Plasma lipoproteins in healthy octogenarians: lack of reduced high density lipoprotein cholesterol levels: results from the Framingham Heart Study. Metabolism, 1989 Apr;38(4):293-6
20) Volpato S, Leveille S, Corti MC, Harris T, Guralnik J: The value of serum albumin and high-density lipoprotein cholesterol in defining mortality risk in older persons with low serum cholesterol. JAGS, 2001; 49: 1142-1147
21) Masoro EJ: Physiological system markers of aging. Exp Gerontol, 1988;23(4-5):391-7
22) Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, Seeman T, Tracy R, Kop WJ, Burke G, McBurnie MA: Cardiovascular Health Study Collaborative Research Group. Frailty in older adults: evidence for a phenotype. J Gerontol A Biol Sci Med Sci, 2001;56(3):M146-56
23) Katz S, Akpom CA: 12 Index of ADL. Med Care May, 14 (5 suppl):116-8; 1976
24) Lawton MP, Brody EM: Assessment of older people:self-maintaining and instrumental activities of daily living. Gerontologist, 1969; 9: 179-186
25) Wade DT, Colin C: The Barthel ADL Index – a standard measure of physical disability. Int Disabil Studies, 1988; 10: 64-67
26) Studenski S, Perera S, Wallace D, Chandler JM, Duncan PW, Rooney E, Fox M, Guralnik JM: Physical performance measures in the clinical setting. J Am Geriatr Soc, 2003;51(3):314-22

27) Rantanen T, Guralnik JM, Foley D, Masaki K, Leveille S, Curb JD, White L: Midlife hand grip strength as a predictor of old age disability. JAMA, 1999; 10;281(6):558-60
28) Rantanen T, Volpato S, Ferrucci L, Heikkinen E, Fried LP, Guralnik JM: Handgrip strength and cause-specific and total mortality in older disabled women: exploring the mechanism. J Am Geriatr Soc, 2003 May;51(5):636-41
29) Roubenoff R: The Pathophysiology of Wasting in the Elderly. J Nutr, 1999;129:256S-259S
30) Williamson DF: Descriptive epidemiology of body weight and weight changes in 17,000 adults. Int J. Obes, 1988; 12:391-401
31) Bartali B, Benvenuti E, Corsi AM, Bandinelli S, Russo CR, Di Iorio A, Lauretani F, Ferrucci L: Changes in anthropometric measures in men and women across the life-span: findings from the InCHIANTI study. Soz Praventivmed, 2002; 47(5): 336-48
32) Elahi D, Dyke MM, Andres R: Aging, fat, metabolism and obesity. In Masoro EJ (ed): Handbook of Physiology. Section 11: Aging. Oxford University Press, New York, 1995:47
33) Visscher TL, Seidell JC, Molarius A, van der Kuip D, Hofman A, Witteman JC: A comparison of body mass index, waist-hip ratio and waist circumference as predictors of all-cause mortality among the elderly: the Rotterdam study. Int J Obes Relat Metab Disord, 2001;25(11): 1730-5
34) Carmeli E, Coleman R, Reznick AZ. The biochemistry of aging muscle. Exp. Gerontol, 37:477-89, 2002
35) Zeynep E, Faisal Beg M, Burke D, De Luca C: Effects of aging on motor-unit control properties. Am Physiol Soc, 2081-91,1999
36) Mecocci P, Fano G, Fulle S, MacGarey U, Shinobu L, Polidori MC, Cherubini A Vecchiet J, Senin U, Beal MF: Age-dependent increases in oxidative damage to DNA, lipids and proteins in human skeletal muscle. Free Radic Biol Med, 26:303-8, 1999
37) McKenzie D, Bua E, McKiernan S, Cao Z, Aiken JM, Wanagat J: Mitochondrial DNA deletion mutations: a causal role in sarcopenia. Eur J Biochem, 269:2010-5, 2002
38) Baumgartner R, Koehler K, Romero L, Garry P: Serum albumin is associated with skeletal muscle in elderly men and women. Am J Clin Nutr, 64:552-8,1996.Frisancho AR. Triceps skinfold and upper arm muscle norms for assessment of nutritional status. Am J Clin Nutr. 1974; 27:1052-8.
39) Kamel H, Maas D, Duthie E: Role of hormones in the pathogenesis and management of sarcopenia. Drugs Aging, 2002; 19: 869-877
40) Blackman MR, Sorkin JD, Munzer T, Bellantoni MF, Busby-Whitehead J, Stevens TE, Jayme J, O'Connor KG, Christmas C, Tobin JD, Stewart KJ, Cottrell E, St Clair C, Pabst KM, Harman S: Growth hormone and sex steroid administration in healthy aged women and men: a randomized controlled trial. JAMA, 2002 Nov 13;288(18):2282-92
41) Iovino M, Monteleone P, Steardo L: Repetitive growth hormone-releasing hormone administration restores the attenuated growth hormone (GH) response to GH-releasing hormone testing in normal aging. J Clin Endocrinol Metab, 1989;69(4):910-3
42) Roubenoff R: Inflammatory and hormonal mediators of cachexia. J Nutr, 1997;127:1014S-1016S
43) Roubenoff R: Catabolism of aging: is it an inflammatory process? Curr Opin Clin Nutr Metab Care, 2003;6(3):295-9
44) Frisancho AR: Triceps skinfold and upper arm muscle norms for assessment of nutritional status. Am J Clin Nutr 1974; 27:1052-8

45) Ferrucci L, Guralnik J: Inflammation, hormones, and body composition at a crossroad. Am J Med, 2003 Oct 15;115(6):501-2
46) Heymfield SB, McManus C, Smith et al: Anthropometric measurement of muscle mass: revised for equations for calculating bone free arm muscle area. Am J Clin Nutr, 1982; 36: 680-690
47) Brunnsgard H, Pederson M, Pederson BK: Aging and proinflammatory cytokines. Curr Opin Hematol 8: 131-136, 2001
48) Franceschi C, Bonafè M, Valensin S et al: Inflamm-aging: An evoluzinary perspective on immunosenescence. Ann NY Acad Sci, 908: 244-254, 2000
49) Ershler WB: Interleukin-6: a cytokine for gerontologists. J Am Geriat Soc, 41:176-181, 1993
50) Fagiolo U, Cossarizza A, Scala E, et al: Increased cytokine production in mononuclear cells of healthy elderly people. Eur J Immunol, 23: 2375-2378,1993
51) Albright JW, Albright JF: Soluble receptors and other substances that regulate proinflammatory cytokines in young and aging humans. J Geronto A Biol Sci Med Sci, 55: 20-25, 2000
52) Frost RA, Lang CH: Regulation of insulin-like growth factor-1 in skeletal muscle and muscle cells. Minevra Endocrinol, 2003;28(1):53-73
53) Barton-Davis ER, Shoturma DI, Sweeney HL: Contribution of satellite cells to IGF-I induced hypertrophy of skeletal muscle. Acta Physiol Scand, 1999;167(4):301-5
54) Cappola AR, Bandeen-Roche K, Wand GS, Volpato S, Fried LP: Association of IGF-I levels with muscle strength and mobility in older women. J Clin Endocrinol Metab, 2001;86(9):4139-46
55) Cappola A, Qian-Li X, Ferrucci L, Guralnik JM, Volpato S, Fried LP: Insulin-like Growth Factor I and Interleukin-6 Contribute Synergistically to Disability and Mortality in Older Women. JCEM, 88: 2019-25, 2003
56) Barbieri M, Ferrucci L, Ragno E, Corsi A, Bandinelli S, Bonafe M, Olivieri F, Giovagnetti S, Franceschi C, Guralnik JM, Paolisso G: Chronic inflammation and the effect of IGF-I on muscle strength and power in older persons. Am J Physiol Endocrinol Metab, 2003;284(3):E481-7
57) Ferrucci L, Penninx BW, Volpato S, Harris TB, Bandeen-Roche K, Balfour J, Leveille SG, Fried LP, Md JM: Change in muscle strength explains accelerated decline of physical function in older women with high interleukin-6 serum levels. J Am Geriatr Soc, 2002;50(12): 1947-54
58) Rudman D: Growth hormone, body composition and aging. J Am Geriatr Soc, 1985; 33: 800-1
59) Kelijam M: Age-related alterations of growth hormone/insulin-like-growth-factor J axis. J Am Geriatr Soc, 1991; 39: 295-307
60) Baumgartner RN, Waters DL, Gallagher D, Morley JE, Garry PJ: Predictors of skeletal muscle mass in elderly men and women. Mech Ageing Dev. 1999;107(2):123-36
61) Mooradian AD, Morley JE, Korenman SG: Biological actions of androgens. Endocr Rev, 1987; 8: 1-28
62) Baulieu EE, Thomas G, Legrain S, Lahlou N, Roger M, Debuire B, Faucounau V, Girard L, Hervy MP, Latour F, Leaud MC, Mokrane A, Pitti-Ferrandi H, Trivalle C, de Lacharriere O, Nouveau S, Rakoto-Arison B, Souberbielle JC, Raison J, Le Bouc Y, Raynaud A, Girerd X, Forette F: Dehydroepiandrosterone (DHEA), DHEA sulfate, and aging: contribution of the DHEAge Study to a sociobiomedical issue. Proc Natl Acad Sci USA, 2000;97(8):4279-84
63) Tummala S, Svec F: Correlation between the administered dose of DHEA and serum levels of DHEA and DHEA-S in human volunteers: analysis of published data. Clin Biochem, 1999;32(5):355-61

64) Grinspoon S, Corcoran C, Miller K, Biller BM, Askari H, Wang E, Hubbard J, Anderson EJ, Basgoz N, Heller HM, Klibanski A: Body composition and endocrine function in women with acquired immunodeficiency syndrome. J Clin Endocrinol Metab 1997;82: 1332-7
65) Haarbo J, Marslew U, Gotfredsen A, Christiansen C: Postmenopausal hormone replacement therapy prevents central distribution of body fat after menopause. Metabolism, 1991;40(12):1323-6
66) Gower BA, Nyman L: Associations among oral estrogen use, free testosterone concentration, and lean body mass among postmenopausal women. J Clin Endocrinol Metab, 2000;85(12):4476-80
67) Sorensen MB, Rosenfalck AM, Hojgaard L, Ottesen B: Obesity and sarcopenia after menopause are reversed by sex hormone replacement therapy. Obes Res, 2001; 9: 622-6
68) Song XM, Ryder JW, Kawano Y, Chibalin AV, Krook A, Zierath JR: Muscle fiber type specificity in insulin signal transduction. Am J Physiol, 277:R1690-6, 1999
69) Staron R, Hagerman F, Hikida R, Murrey T, Hostler D, Crilll M, Ragg K, Toma K: Fiber type composition of the vastus lateralis muscle of young men and women. Journal of histochemistry & cytochemistry, 48:623-629, 2000. Lexell J, Henriksson-Larsen K, Wilblad B, Siostrom M: Distribution of aging studied in whole muscle cross-sections. Muscle Nerve, 6:588-595,1983
70) Madsen OR, Lauridsen UB, Hartkopp A, Sorensen OH: Muscle strength and soft tissue composition as measured by dual energy x-ray absorptiometry in women aged 18-87 years. Eur J Appl Physiol Occup Physiol, 76: 239-45,1997
71) Paolisso G, Gambardella A, Balbi V, Galzeranno D, Verza M, Varricchio M, D'Onofrio F: Effects of magnesium and nifedipine infusions on insulin action. Substrate oxidation and blood pressure in aged hypertensive patients. Am J Hypertens, 920-26,1993
72) Goodpaster BH, Kelley DE, Thaete FL, He J, Ross R: Skeletal muscle attenuation determined by computed tomography is associated with skeletal muscle lipid content. Appl Physiol, 2000;89(1):104-10
73) Visser M, Deeg DJ, Lips P, Harris TB, Bouter LM: Skeletal muscle mass and muscle strength in relation to lower-extremity performance in older men and women. J Am Geriatr Soc, 2000;48(4):381-6
74) Strehler BL: Time, cells and aging. Academic Press, New York, 1981
75) Kohn RR: Aging of animals: possible mechanisms. In Kohn RR, ed, Principles of mammalian aging, Prentice-Hall, Englewood Cliffs, NJ, 1978
76) Sohal RS, Svensson I, Sohal BH, Brunk UT: Superoxide anion radical production in different animal species. Mech Ageing Dev, 1989;49(2): 129-35
77) Gilgun-Sherki Y, Melamed E, Offen D: Antioxidant treatment in Alzheimer's disease: current state. J Mol Neurosci, 2003;21(1):1-12
78) Paolini M, Sapone A, Canistro D, Chieco P, Valgimigli L: Antioxidant vitamins for prevention of cardiovascular disease. Lancet, 2003 ;362(9387):920
79) Paolisso G, Gambardella A, Balbi V, Ammendola S, D'Amore A, Varricchio M: Body composition, body fat distribution, and resting metabolic rate in healthy centenarians. Am J Clin Nutr, 1995;62(4):746-50
80) Paolisso G, Ammendola S, Del Buono A, Gambardella A, Riondino M, Tagliamonte MR, Rizzo MR, Carella C, Varricchio M: Serum levels of insulin-like growth factor-I (IGF-I) and IGF-binding protein-3 in healthy centenarians: relationship with plasma leptin and lipid concentrations, insulin action, and cognitive function. J Clin Endocrinol Metab, 1997;82(7):2204-9
81) Frank JW, Reed DM, Grove JS, Benfante R: Will lowering population levels of serum cholesterol affect total mortality? Expectations from the Honolulu Heart Program. J Clin Epidemiol, 1992;45(4):333-46

82) Stemmermann GN, Chyou PH, Kagan A, Nomura AM, Yano K: Serum cholesterol and mortality among Japanese-American men. The Honolulu (Hawaii) Heart Program. Arch Intern Med, 1991;151(5):969-72
83) Weverling-Rijnsburger AW, Jonkers IJ, van Exel E, Gussekloo J, Westendorp RG: High-density vs low-density lipoprotein cholesterol as the risk factor for coronary artery disease and stroke in old age. Arch Intern Med, 2003 14;163(13):1549-54
84) Maher V, Brown BG: Lipoprotein (a) and coronary heart disease. Curr Opin Lipidol, 1995; 6: 229-235
85) Dahlen G: Lp(a) lipoprotein in cardiovascular disease. Atherosclerosis, 1994; 108: 111-26
86) Sansoni P, Cossarizza A, Brianti V, Fagnoni F, Snelli G, Monti D, Marcato A, Passeri G, Ortolani C, Forti E, et al: Lymphocyte subsets and natural killer cell activity in healthy old people and centenarians. Blood, 1993;82(9):2767-73
87) Baggio G, Donazzan S, Monti D, Mari D, Martini S, Gabelli C, Dalla Vestra M, Previato L, Guido M, Pigozzo S, Cortella I, Crepaldi G, Franceschi C: Lipoprotein(a) and lipoprotein profile in healthy centenarians: a reappraisal of vascular risk factors. FASEB J, 1998;12(6):433-7
88) Pickup JC, Mattock MB, Chusney GD, et al: NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X. Diabetologia, 1997; 40:1286-1292
89) Judge JO, Schechtman K, Cress E: The relationship between physical performance measures and independence in instrumental activities of daily living. The FICSIT Group. Frailty and Injury: Cooperative Studies of Intervention Trials. J Am Geriatr Soc, 1996;44(11):1332-41
90) Tinetti ME: Performance oriented assessment of mobility problems in elderly patients. J Am Geriatr Soc, 1986;34: 119-126
91) Guralnik JM, Simonsick EM, Ferrucci L, Glynn RJ, Berkman LF, Blazer DG, Scherr PA, Wallace RB: A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission. J Gerontol, 1994;49(2):M85-94