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REVIEW ARTICLE

SARCOPENIA: AGE-RELATED SKELETAL MUSCLE CHANGES FROM DETERMINANTS TO PHYSICAL DISABILITY

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Human aging is characterized by skeletal muscle wasting, a debilitating condition which sets the susceptibility for diseases that directly affect the quality of life and often limit life span. Sarcopenia, i.e. the reduction of muscle mass and/or function, is the consequence of a reduction of protein synthesis and an increase in muscle protein degradation. In addition, the capacity for muscle regeneration is severely impaired in aging and this can lead to disability, particularly in patients with other concomitant diseases or organ impairment. Immobility and lack of exercise, increased levels of proinflammatory cytokines, increased production of oxygen free radicals or impaired detoxification, low anabolic hormone output, malnutrition and reduced neurological drive have been advocated as being responsible for sarcopenia. It is intriguing to notice that multiple pathways converge on skeletal muscle dysfunction, but the factors involved sometimes diverge to different pathways, thus intersecting at critical points. It is reasonable to argue that the activity of these nodes results from the net balance of regulating mechanisms, as in the case of the GH/IGF-1 axis, the testosterone and cortisol functions, the pro- and anti-inflammatory cytokines and receptors. Both genetic and epigenetic mechanisms operate in regulating the final phenotype, the extent of muscle atrophy and reduction in strength and force generation. It is widely accepted that intervention on lifestyle habits represents an affordable and practical way to modify on a large scale some detrimental outcomes of aging, and particularly sarcopenia. The identification of the molecular chain able to reverse sarcopenia is a major goal of studies on human aging.

Sarcopenia (i.e. loss of muscle mass and strength) is a highly prevalent condition in older people.

Several cross-sectional and longitudinal studies have shown a relationship between sarcopenia and physical disability which, in turn, carries on the risk of more severe health outcomes, such as hospitalisation, nursing home admission and mortality. The definition of sarcopenia as a pathological entity is still a matter of debate, and its pathogenesis is also far from being completely elucidated. In recent years, several contributions have been produced on sarcopenia, and therefore, in this paper, we attempt to critically review the more recent observations in this important field of clinical research.

Key words: sarcopenia, elderly, inflammation, signaling pathways

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Characteristics and definitions of sarcopenia

At a morphological level, sarcopenia is characterized by a reduction in number and size of skeletal muscle type IIa (fast-twitch) fibers, while the size of type I (slow-twitch) fibers is much less affected (1). The loss of muscular tissue is usually associated with an increase of adipose tissue outside and inside the muscular bellies (Fig. 1). Obese subjects may be sarcopenic, and their muscles may be heavily substituted by adipose tissue. Computed tomography has been used to evaluate the percentage of muscle and the infiltration of fat outside the muscle fibers (1); the attenuation of the signal has been considered a measure of fat inside the fibers (2).

Whole body muscular mass has been evaluated *in vivo* using different methods. Dual Energy X-ray Absorptometry (DEXA) provides precise measures of lean body mass, total fat mass and total body mineral content. Appendicular lean mass (aLM) is calculated as the sum of lean mass in arms and legs, assuming that all non-fat and non-bone tissue is skeletal muscle. Alternatively, Bioelectrical Impedance Analysis (BIA), although less accurate, provides simple and inexpensive estimates of whole body muscle mass (2).

On the basis of these measures, indexes of sarcopenia have been obtained, which, unfortunately, differ widely among Authors. Some have defined sarcopenia as a pathological entity, when a LM (muscle mass Kg / height m^2) is more than 2 standard deviations below the sex-specific young-normal mean of a reference population. Others argue that this method is misleading because it does not take into account the fat mass (3). Usually, absolute lean mass in an obese individual is higher than in a lean person, because both fat and lean mass increase with weight gain. Obese individuals, who have both higher lean and fat mass, may not appear to be sarcopenic, when absolute muscle mass is calculated, even though their muscle mass may be inadequate for their size and their physical functioning. Therefore, sarcopenia could better be evaluated, adjusting lean mass for fat mass in addition to height, so obtaining the Skeletal Muscle Index (SMI). The residuals of linear regression are used to identify the relative muscle mass and the 20^{th} percentile of distribution of residuals used as cut-off point for sarcopenia.

Another issue of debate is the definition of "sarcopenic obesity" (i.e. the pathological reduction of muscle mass in obese subjects). It may be diagnosed according to SMI, when obese subjects (BMI >30) have a relative reduction of muscle mass (below the 20^{th} percentile). Others, however, use different criteria. For example, Baumgartner et al. (4) define separately sarcopenia and obesity. Subjects are classified as sarcopenic if their relative muscle mass is <2 SD below the mean of a sample of healthy young adults and are classified as obese if their percentage body fat is above 60% percentile of the study sample. Based on the combination of sarcopenia and obesity cut-off points, the subjects are classified as sarcopenic obese, sarcopenic non-obese, non-sarcopenic obese and non-sarcopenic/non-obese. Zoico et al. (5) use a similar method, they divide the subjects on the basis of quintile scores and define "sarcopenic obesity" as high body fat (IV-V quintile of fat) with low muscle mass (I-II quintile of muscle).

The different methods of evaluation and the characteristics of the populations studied, account for the discrepancy among the prevalence values of sarcopenia, which range from 6% to 12%, in persons older than 60 years, to 30- 50% and more in the very aged (6).

A large sample of elderly subjects (2278 women and 2224 men >60 years old) in good health were evaluated in the Third National Health and Nutrition Examination Survey (7). Using whole body BIA measures, they were classified as "moderate sarcopenic" (class I) when muscle mass was between 1 and 2 SD below the reference values obtained in young people, and as "severe" (class II) when the muscle mass was below 2 SD of the reference values. The prevalence of moderate sarcopenia was 59% in women and 45% in men, whereas the prevalence of severe sarcopenia was respectively 10% and 7%.

Newman et al. (7) studied sarcopenia in 3075 well-functioning men and women, aged 70 to 79, enrolled in the Health ABC Study. Appendicular muscle mass was evaluated by means of DEXA and sarcopenia was defined according to two methods, the first adjusting only for height (aLM/ht^2), and the second for height and fat. The prevalence values were 8.9% in overweight men and 7.1% in overweight women, but 0% in obese subjects of both sexes; however, when the values of muscle mass were adjusted for height and fat mass, the prevalence

rose to 15.4% and 11.5% for the overweight and to 21.7% and 14.4% for the obese (males and females, respectively).

At an individual level, the extent of sarcopenia is determined by two factors: the initial amount of muscle mass and the rate at which it declines with age. The muscle mass is determined by genetic and environmental factors; the decline is caused by several pathogenetic components, which are discussed in detail hereafter. The age-associated decline in muscle mass begins in the fifth decade, but, it may be also appreciated from 30-40 years of age. The rate of muscle loss, as expected, has large individual variability, but it appears fairly consistent, approximately 1%-2% per year past the age of 50 (8). The loss of mass is associated to a parallel decrease in muscle strength, although some authors argue that there may be a discrepancy between the two. In fact, experimental studies show that the force produced by single muscle cells is greater in young men versus old men, with a decline in force production with age at a cellular level (8).

Pathogenesis of sarcopenia

The pathogenesis of sarcopenia is complex and, in general terms, can be explained by an imbalance between the rates of muscle protein synthesis and breakdown, in which the net muscle protein balance is negative. In fact, the *in vivo* fractional rate of muscle protein synthesis, measured by determining the incorporation of intravenously administered 13C-leucine into skeletal muscle, has been found to be reduced in elderly sarcopenic subjects (9).

Several factors, which influence the muscle protein metabolism, are involved in the pathogenesis of sarcopenia (Fig. 2). This knowledge is very important on a clinical level for tailored preventive and therapeutical strategies. It is very difficult to establish the hierarchical order of all the factors which have been studied so far. In our opinion, it is likely that some of them are common to all subjects during the aging process, whereas others may have relevance in individual subjects.

Hypertrophy, atrophy of the muscle and senile sarcopenia

Two different growth factors are expressed by muscle, derived from the insulin-like growth factor 1 (IGF-1) gene by alternative splicing: a systemic IGF-1 of liver type and a variant called "Mechano Growth Factor (MGF)", which is expressed in response to physical activity (10). These forms of IGF-1 have different roles, but both are important regulators of muscle growth: the systemic IGF-1 is important as the provider of mature IGF-1 and upregulates protein synthesis; the MGF activates muscle stem cells and cause them to proliferate. MGF production in response to exercise declines with advanced age, paralleling the decline in circulation GH levels (11). More recently the IGF-1/PI3K/Akt muscle pathway has been better characterized (Fig. 3) (12). Physiologically the release of Insulin-like growth factor 1 activates the phosphatidylinositol 3 kinase (PI3K), that leads to activation of the serine/ threonine kinase Akt, and of the mammalian target of rapamycin (mTOR) kinase, via phosphorylation. The activation of mTOR can induce the proteic synthesis through the p70S6 kinase and through the eukaryotic translation initiation factor 4E binding protein 1 (4E-BP-1) and lastly, by the activation of the protein initiation factor eukaryotic translation initiation factor 4E (eIF-4E).

Tor is a Ser/Thr kinase which acts as a regulator of cellular response to nutrient conditions and integrates different signals, including growth factors and hormones. Activation (phosphorylation) of Tor promotes growth, and when it is inhibited by Rapamycin, atrophy ensues. Growth factor receptor signaling via the Akt pathway as well as other inputs (e.g. energy status, hypoxia, DNA damage via p53) act on a GTPase which is a molecular switch for Tor (13). In spite of this evidence, skeletal muscle atrophy is not merely the converse of skeletal muscle hypertrophy and/or synonymous with Sarcopenia in the elderly (14). At least four different systems are involved in protein degradation during muscle atrophy [the lysosomal, calpain, caspase and ubiquitin proteasome systems] (15).

The relative contribution of each system to the specific process in which muscle atrophy develops, i.e. renal disease, cancer, sepsis, aging, microgravity, AIDS, etc. is unclear

In many disease states or in nutrient deprivation, muscle mass is markedly reduced, with serious health implications, since muscles are utilized for energy production at times of food deprivation. The

discovery that atrophy-related genes are induced and controlled by a signalling network regulated by Akt, revealed an unsuspected double-edged role for a key molecule which is involved in cellular growth, but when activated, at the same time negatively affects the catabolic process (15). The stimulation of muscle with anabolic agents (e.g. IGF-1) indirectly activates Akt1 and the protein synthetic machinery, while inhibiting protein synthesis repressors (4E-BP-1). Catabolic agents vice versa inactivate Akt leading to FOXO dephosphorylation and nuclear transcription of atrogenes.

In clinical conditions such as uremia, cancer cachexia or diabetes, a common set of genes (termed atrogins) is induced or suppressed. Among the induced genes many are involved in protein degradation, including ubiquitin (Ub), Ub fusion proteins, the Ub ligases Atrogin-1/MAFbx (muscle atrophy F-box; also known as *Atrogin-1)* and MuRF-1 (muscle ring finger 1). The messenger RNA (mRNA) levels, of Atrogin-1/MAFbx and MuRF-1 are reported unchanged (human), slightly increased or decreased (rat) in aged muscle (16).

The RING-finger proteins (MuRF1, MuRF2, MuRF3) belong to the RING-B-box-coiled-coil subclass, characterized by an NH_2-terminal RING-finger followed by a zinc-finger domain (B-box) and a leucine-rich coiled-coil domain (17).

The physiological role of this group of proteins was associated with skeletal myoblast differentiation, myotube fusion, microtubule stabilization and homo-oligomerization, in other words, the MuRF-protein shared the role of myogenic regulator of the microtubule network (17).

In particular, MuRF1 has been demonstrated to have ubiquitin-ligase activity, and MuRF2 could bind and potentially regulate the turnover of a structural myofibrillar protein (titin) (18). Finally, MURF-3 is developmentally upregulated, it associates with microtubules, the sarcomeric M-line and Z-line, and is required for microtubule stability and myogenesis (19).

Atrogin-1/MAFbx is very strongly induced in many catabolic states and contains a functional F-box domain that binds to Skp1 and thereby to Roc1 and Cul1, the other components of SCF-type Ub-protein ligases (E3s), as well as a nuclear localization sequence and PDZ-binding domain (20).

Both these genes, MuRF1 and MAFbx, encode E3 ubiquitin ligases and are regulated by a family of transcription factors termed Fork-head box O (FOXO).

The FOXO sub-family of transcription factors is composed, in mammalians, by at least three different isoforms which have many biological functions, such as cellular metabolism, differentiation and apoptosis. During skeletal muscle atrophy, an increase in FOXO1 mRNA, associated to several other atrophy related genes, has been observed. In two models of *in vitro* atrophy, in which differentiated myotubes were either deprived of nutrients or treated with dexamethasone, a reduction in myotube diameter occurred, together an increase in atrogin-1/MAFbx mRNA (21).

Thus muscle mass atrophy, independently from the etiological promoter, shares a common transcriptional pathway activated in many systemic diseases: the Ub–proteasome pathway. Ubiquitin is a short peptide that can be conjugated to specific protein substrates. A chain reaction might build a poly-ubiquitin complex onto the substrate; this ubiquitin chain targets the substrate proteolyzed into small peptides by the proteasome (22). The addition of ubiquitin to a protein substrate is a modulating process which requires three distinct enzymatic components that confer substrate specificity: an E1 ubiquitin-activating enzyme, an E2 ubiquitin-conjugating enzyme, and an E3 ubiquitin-ligating enzyme. Thus, the regulation of ubiquitin activation-polymerization appears to be a coordinate signalling pathway, similar to a phosphorilation one.

Altogether the data reported suggest that an increased rate of substrate ubiquitation and increased proteasome activity occurs during muscle wasting; information about the activity of this pathway during human aging is scanty, or even controversial, in experimental animals. Since degradation of damaged or misfolded proteins is essential for cellular homeostasis and renewal, the process of ubiquitation, which marks aged or denatured proteins for proteasome digestion, needs to be successfully maintained throughout life and requires energy expenditure (23). The selectivity of ubiquitin conjugation resides in the Ub ligases (E3) which are specific for different proteins.

Atrogin-1/MAFbx belongs to a class of E3s

whose target in muscle wasting is at present unknown, and another E3, MuRF1, is induced in atrophying muscle. Multiple ubiquitin conjugation pathways seem to be therefore induced in muscles undergoing atrophic changes, leading to targeting distinct protein components for removal. This might explain why knocking out a single gene does not affect the capacity to undergo atrophy, since only when protein degradation of critical factors exceeds protein synthesis, skeletal muscle wasting may occur.

Synthetically, the activity of the two pathways in aged skeletal muscle seems to be unaltered or decreased while there is some evidence that the efficiency of myoprotein synthesis decreases (14).

In conclusion, the loss of muscle mass during aging seems to be substantially different from the muscle atrophy of adults occurring in a variety of clinical situations.

Chronic low level inflammation and pro-inflammatory cytokines

Proinflammatory cytokines, TNF-α in particular, have been implicated in cachexia and other catabolic conditions, although the effect seemed to be mediated by growth factor inhibition, mainly IGF-1. Only recently, selective targeting of skeletal muscle myosin and its degradation through the Ub-proteasome pathway has been directly related to TNF action (24).

Induction of Ub ligase E3α mediates, with other atrogene products (atrogin-1 and MuRF-1), the muscle atrophy seen in cachectic conditions. However, this pathway may differ from that linking chronic TNF-α stimulation with sarcopenia; the possible involvement of NFκB in these effects has surfaced, and transcriptional activation of atrogenes was found to be a consequence of inhibitor degradation allowing nuclear translocation of NFκB. Local induction of TNF-α, both in the myofibers and in the surrounding tissues (adipocytes for instance) has been demonstrated in many chronic inflammatory diseases, including sepsis, cancer, COPD and CHF. Tissue expression of IL-1β and TNF-α is triggered by a receptor-mediated activation of NFκB, and this self-perpetuating mechanism may underlie both progressive atrophic changes of chronic disease states and sarcopenia in ageing subjects. However only circumstantial evidence and data on circulating cytokine levels support this interpretation, and no detailed mechanistic studies have been conducted in different experimental models.

A condition of chronic low level inflammation, due to an increased production of proinflammatory cytokines and other mediators, may cause reduced muscle protein synthesis. The serum levels of several cytokines increase with age. Among postulated causes may be the presence of chronic ailments that are highly prevalent among older people, such as angina pectoris, myocardial infarction, congestive heart failure, diabetes mellitus and cancer (25), but a certain lifestyle predisposing to these conditions, including sedentary habits, or a primary dysregulation of the cytokine response threshold, have also been suggested (26). Raised levels of C reactive protein (CRP) have been assumed to reflect an inflammatory status underlying most age-associated conditions. The absolute dependency of CRP production on proinflammatory cytokines including IL-1, IL-6 and TNF-α has opened the still unresolved question of which may constitute the most robust indicator of low grade inflammation.

There is abundant evidence implying these cytokines in the pathogenesis of sarcopenia. In experimental animals, Ciliary Neurotrophic Factor (CNTF), a member of the gp130 family of cytokines which includes IL-6, causes loss of muscle mass (27). Both preclinical and clinical studies indicate that IL-1, IL-6 and TNF-α released by macrophages and endothelial cells, promote muscle wasting in cancer and chronic infection by activating the ubiquitin-proteasome pathway (25).

Higher amounts of inflammation markers are detected in association with low levels of physical activity, and they are significantly reduced by exercise training of different duration and intensity. In this context it is crucial to bear in mind that IL-6, but not TNF-α, shows a rapid early peak during physical exercise. The muscle itself is the source of this increased circulating IL-6, whose gene is actively transcribed during contractile activity. Recent evidence indicates that IL-6 mediates several beneficial effects of physical activity also through TNF-α suppression. Therefore, IL-6 should be viewed as a counter-inflammatory cytokine, and raised levels of both IL-6 and CRP merely as

Table I. *Musculo-tendinous and neural adaptations to resistance training.*

Muscular Adaptations
– Muscular hypertrophy – Potentially increased force or contractile velocity of individual fibers – Changes in the expression of slow and fast twitch myosin heavy chains – Increased tendon stiffness which increases power through enhanced transmission of muscle force
Neural Adaptations
– Increased neural drive to the primary agonist resulting in greater motor unit recruitment and higher motor unit firing rates – Improved coordination between agonist /synergist muscles – Decreased agonist-antagonist coactivation

indicators of the attempt of the organism to dampen inflammation fuelled by IL-1 and TNF-α as well as other factors (28).

The role of cytokines is particularly intriguing in sarcopenic obesity. The fat is an active producer of cytokines, so that an increased production at local and systemic level could contribute to aggravating sarcopenia. The association could be even more complex, taking into account also the reduced physical activity which is frequently observed in obese subjects.

Finally, it must be acknowledged that cytokines are strong predictors of incident disability, which is a surrogate index of sarcopenia, independent of other known risk factors (29).

Oxidative stress

The oxidative stress hypothesis of sarcopenia, which affirms that the continuous exposure to oxidative injury contributes to muscle atrophy and lowered functional capacity, is gaining growing acceptance. In this context, mitochondria play a crucial role, because they are not only the main generators of the primary reactive oxygen species and the most immediate targets of the oxidative damage, but also because mitochondria regulate stress response and apoptosis (30).

Moreover, the oxidized proteins may not be as efficiently removed by the proteolytic system (ubiquitination and lysosomal degradation), resulting in the accumulation of lipofuscin and cross-linked damaged proteins. An age-related accumulation of non-functioning proteins that are not efficiently removed could increase the amount of non contractile material in the muscle, which might explain why muscle strength declines to a greater degree than total muscle mass in sarcopenia (31).

Neurogenic factors

Also neurogenic factors could be considered as universally involved in the pathogenesis of sarcopenia. In fact, there is nearly a 70% reduction of the number of motor neurons in spinal cord by the age of 90 in previously healthy individuals, compared with younger subjects (32). Because neurons have a relevant trophic role, this big change could well be the most important single factor driving the loss of muscle. However, the loss of motor neurons may occur as the result, rather than the cause, of muscle loss, because of retrograde atrophy of these neurons.

Although the role of neurogenic factors in the pathogenesis of sarcopenia is uncertain, no doubt exists about the relevance they have in explaining the decreased strength and power in older adults. The reduced function is mainly due to changes in the pattern of neural activation of muscles, such as the decreased neural drive to agonist muscles, the potentially disturbed coordination between agonist/synergist muscles and the increased agonist-antagonist coactivation.

Moreover, the degeneration in the cerebellum and

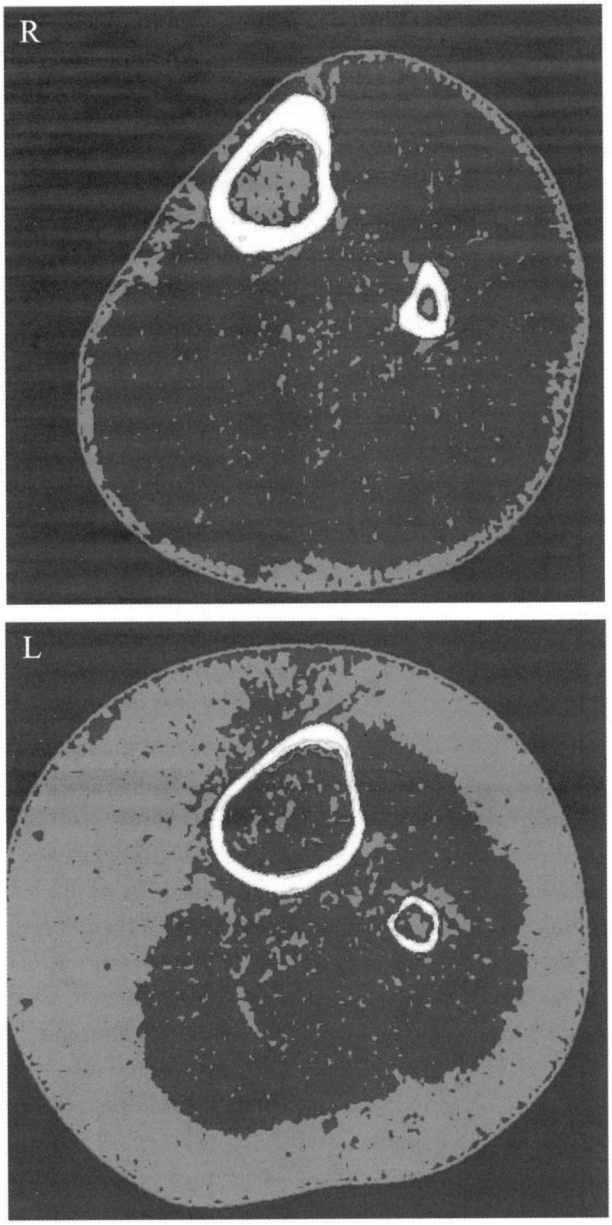

Fig. 1. *Peripheral quantitative computerized tomography: calf muscle area (more intense grey level) of a young (R) and an elderly (L) person. The less intense grey level represents the perimuscular and intramuscular fat tissue. (Images taken from the InCHIANTI Study Database).*

other supraspinal centres may forbid the formation and generation of the descending command to muscles; a decrease in the number of corticospinal fibers synapsing onto spinal motoneurons may limit the effectiveness of transmission of the descending motor command; the reduced afferent input to the spinal motoneuron pools from peripheral sensory receptors can decrease motoneuron excitability; the axonal degeneration, demonstrated by the inverse relationship between calf muscle density and compound muscle action potential (CMAP), may increase the fat infiltration of muscles (33).

The loss of muscle force in old age due to altered Ca^{++} regulation is a complex process occurring in parallel with atrophy (34); the reduced response to action potential-triggered muscle contraction may also be a result of structural damage to the sarcoplasmic reticulum and low expression of membrane fusion proteins. Although denervation induces muscle atrophy (without cytokine mediation), the relations between these two events in the pathogenesis of sarcopenia of the elderly are still far from being dissected.

Satellite cells and muscle regeneration

The adult skeletal muscle is able to undergo extensive regeneration in a matter of days after acute damage, through differentiation and cell fusion of specific precursor cells contained within mononuclear "satellite" cells positioned between the plasma membrane and the basal lamina of mature muscle fibers (35).

These cells may be considered as "dormant myoblasts", capable of extensive proliferation and differentiation, in response to exercise and injury. The stem cell nature of satellite cells comes from different data. Firstly, their capacity of self-renewal has been shown, because the regenerative capacity remains unchanged across multiple cycles of injury and repair; secondly, in *ex vivo* explant cultures, they give rise to intermediate progenitor cells expressing the myogenic transcription factor Pax-3; finally, satellite cells transplanted, even in small numbers, into the radiation-ablated muscle of immuno-compromised, dystrophic hosts, give rise to large amounts of new myofibers, which, in turn, persist in the skeletal muscle for several weeks and can be reactivated and expanded in response to additional muscle injury.

Cytokines, growth factors and several important signaling pathways regulate muscle remodeling and regeneration. The myogenic potential is increased by some inflammatory cell products, including TGF-beta, TNF-alpha and IL-6. Recent studies, however, indicate the alternatively spliced form of IGF-1 which is muscle-specific, MGF, as the main activator of division for muscle satellite cells. An age-related loss of expression of MGF in response

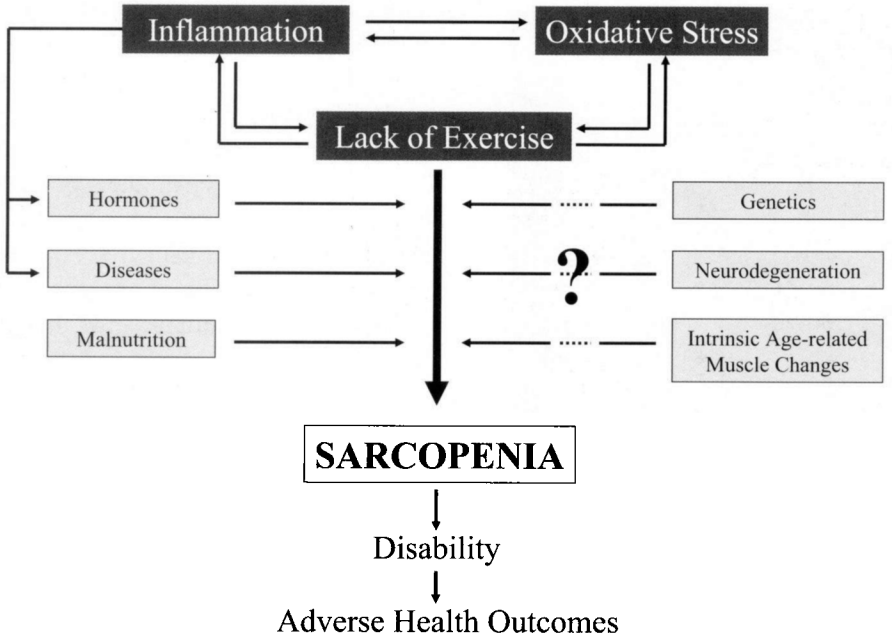

Fig. 2. *Pathogenesis of sarcopenia, from inflammation and oxidative stress to disability and adverse health outcomes.*

to mechanical overload has been reported, and the MGF pulse following exercise was blunted in elderly subjects compared to young ones (11). GH treatment, however, upregulates IGF-1 expression and in conjunction with exercise pushes towards more splice variant MGF being produced (36).

Mechanical stimulation upregulating MGF in conditions of adaptation is lost in certain diseases, such as muscular dystrophies (id.). Negative regulatory factors also play a role, and a tissue specific factor, myostatin/GDF8, was discovered (37). It belongs to the TGF-beta family and is secreted as a propeptide. The main activity of the mature peptide seems to maintain satellite cells in a quiescent state (id.) and to antagonize MyoD (a myogenic regulatory factor) expression and activity. Myostatin antagonists, including the propeptide itself and follistatin, have been advocated for promoting muscle hypertrophy and opposing progressive sarcopenia.

The proliferation of satellite cells in response to muscle injury is positively regulated by the Notch pathway: the Notch receptor is expressed in quiescent satellite cells, and Notch signal transduction responsible for satellite cell activation is initiated by upregulation of its ligand, Delta, on the fibers adjacent to the damaged muscle and on the satellite cells themselves (38).

With aging, the regenerative potential of skeletal muscle rapidly declines. However, it is unknown whether it depends on intrinsic molecular changes in the stem cells or in alterations of the aging environment, or both.

It is worthy of note that Notch continues to be expressed in aged satellite cells, while the induction of Delta after injury falls with age. This results in a defective regeneration of old muscle tissue.

On the other hand, some experiments suggest that also the cell environment may be important.

When small fragments of muscle from young or old rodents are transplanted into either young or old muscle beds, the efficiency of muscle regeneration is clearly determined by the age of the host environment, rather than by the age of the muscle donor. Therefore systemic factors may influence muscle repair, and these factors change with age (39).

Sexual hormones

Both androgen and oestrogen levels decrease with age, and their decline is related to decreased muscle mass. However, real deficiency states are found only in a small fraction of aged subjects.

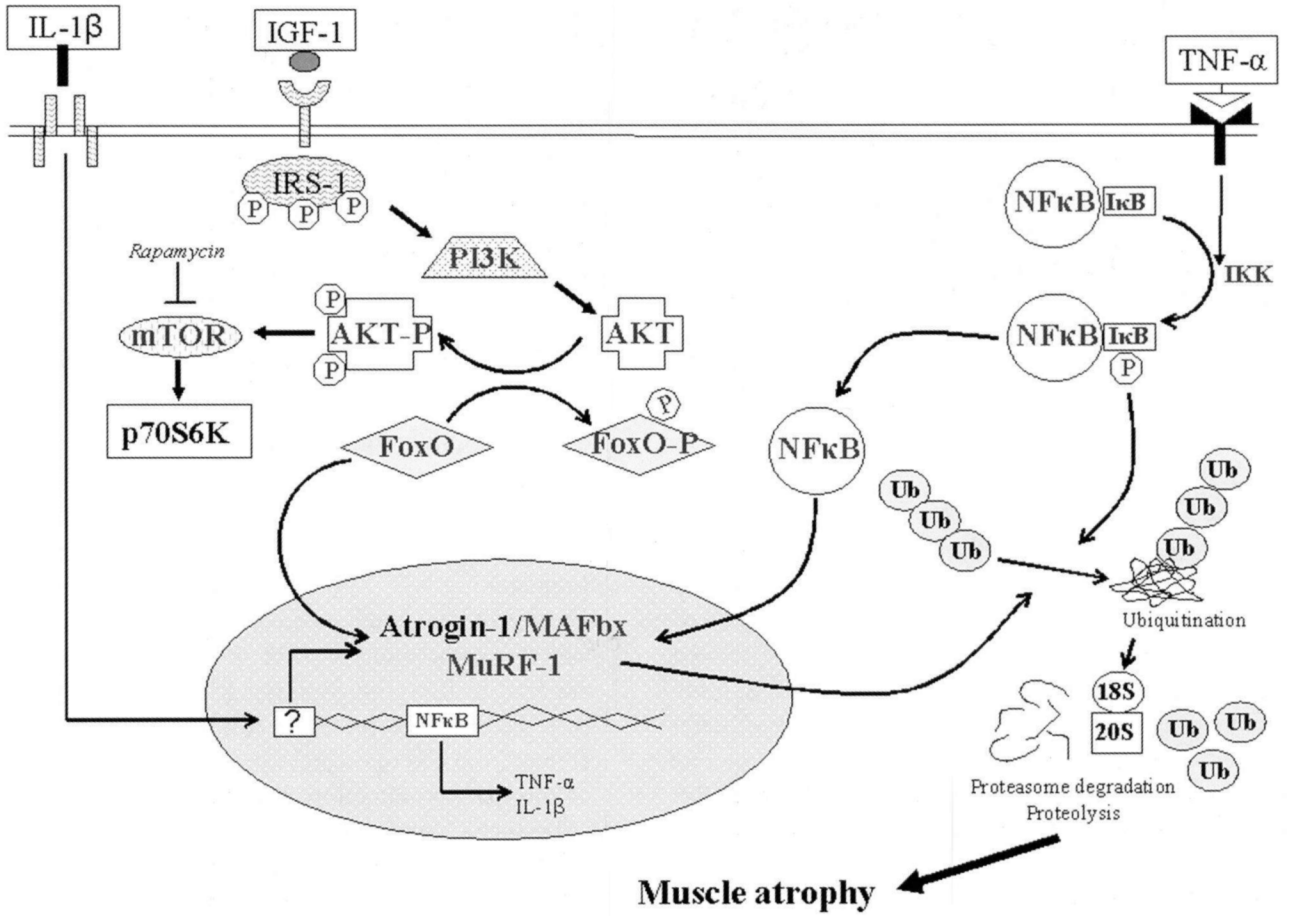

Fig. 3. *Signaling pathways relevant to atrophy-hypertrophy. The recruitment of the insulin-receptor substrate (IRS-1) leads to the activation of the phosphatidylinositol 3-kinase (PI3K)–Akt pathway. The Akt controls directly the transcriptional activation of mTOR and FoxO. The TNF-α, on the other hand, trigger IKK [IκB (inhibitory κB) kinase complex] which activates the IκB/NF- κB signaling pathway. All those events lead to an increased transcription of Atrogin-1 and MURF-1 which leads to increased ubiquitination and proteolytic muscle protein breakdown.*

Cross-sectional and longitudinal studies (40) have convincingly demonstrated that testosterone levels decrease with normal aging, even when taking into account the confounding influences of sampling time, chronic illnesses and multiple medications. The average decline of serum testosterone is about 35%; however, because Steroid Hormone Binding Globulin increases, there is an even greater decline in free or bioavailable testosterone. Estimates of the crude prevalence of "true" androgen deficit (defined using both signs/symptoms plus total and calculated free testosterone) range between 6.0 and 12.3% in elderly subjects and the crude incidence rate of androgen deficiency is 12.3 per 1000 person-years, the rate increasing significantly with age (41). The net effects of oestrogen on muscle mass and muscle strength has been poorly investigated. Oestrogen receptors have been found in muscle cells and therefore estrogens are also likely involved in the metabolic control of this tissue (42).

Adrenal steroids

Alterations in adrenal steroid levels have been proposed to contribute to muscle wasting in several chronic diseases, including CHF and chronic pulmonary disease. Reduced levels of dehydroepiandrosterone and its sulfate (DHEAS), the main adrenal androgens produced in humans, and a high cortisol/DHEAS ratio are thought to induce an imbalance of protein synthesis relative to degradation, favouring a catabolic pattern (43). The ratio between cortisol and DHEAS is commonly assumed as

being an index of adrenal steroid metabolism, and it increases with age. In addition to displaying opposing effects on muscle protein synthesis, these steroids exert opposed actions on the immune system, partly due to cytokine regulation (44).

Hormonal dysfunction, namely a decrease of anabolic hormones, may synergize with the catabolic effects of proinflammatory cytokines, and conversely high levels may protect from these effects. In this respect, it is more important to investigate the balance between all contributing factors than to study each one in an isolated fashion.

GH/IGF-1 axis

The age-related changes of the GH/IGF-1 axis activity are mainly dependent on age-related variations in the hypothalamic control of somatotrophin function. The term "somatopause" indicates the potential link between age-related decline in GH and IGF-1 levels and changes in body composition, structural functions and metabolism which characterize aging (42). Also, in some sarcopenic individuals a reduction in GH and IGF-1 secretion has been demonstrated.

Leptin

Leptin, a protein of 146 aminoacids, stimulates food intake and regulates the sense of satiety, thus contributing to the maintenance of body weight and energetic balance. This hormone works through the hypothalamic-pituitary axis and reduces the secretion of GH. Leptin is produced mainly by fat, and to a lesser extent by other tissues, such as muscle, stomach and brain. The production of leptin is modulated by cytokines, steroids and catecholamines. The hypercatabolic state of congestive heart failure is associated to high levels of leptin and of its soluble receptor, as well to high levels of cytokines, and particularly of TNFα.

Disuse, malnutrition, cognitive dysfunction

The wasting of muscle mass which occurs with immobility or disuse is a widely recognized phenomenon. Muscle atrophy is associated with a negative protein balance and a loss of muscle fiber nuclei, most likely through apoptosis (32).

In some elderly subjects sarcopenia is mainly dependent on inadequate overall dietary intake and suboptimal variety of foods eaten. For muscle to maintain its mass, the rate of protein synthesis must be in balance with the rates of degradation to aminoacids, in combination with dietary absorption, maintaining the difference in amino acid utilization. For sarcopenia to occur, only small imbalances between synthesis and degradation over many years are necessary to eventually result in a significant loss of muscle mass. In young adults, muscle mass accounts for approximately 30% of the whole body protein turnover, whereas in elderly persons muscle mass only represents approximately 20% or less of the whole body protein turnover. With advancing age, illness, trauma, or inadequate dietary intake of aminoacids can all decrease the rates of protein synthesis and ultimately exacerbate the onset of sarcopenia.

Experimental studies have shown that feeding healthy elderly women with 0.4 g/kg of protein, compared to 0.8 g/kg (optimal RDA recommended dose), for 9 weeks, produces a marked reduction in cell mass, muscle mass and nitrogen balance (9) .

Cognitive dysfunction, for example, may lead to sarcopenia, either directly or indirectly, due to anorexia, decreased food intake and lack of physical activity. A decreased blood flow to muscles, due to atherosclerosis of the lower limb arteries or to reduced cardiac output for cardiac heart failure, can aggravate sarcopenia (8).

Genetic factors

Notwithstanding the large body of research performed on humans, the pathogenesis of sarcopenia still remains incompletely understood. Recent experimental data coming from studies in the non parasitic worm Caenorhabditis Elegans shed additional doubts about the relevance of some biological factors (45). The aging worms develop sarcopenia starting in the post-reproductive period, but sarcopenia does not develop in a stereotyped fashion. It develops at different ages within a cohort of genetically identical worms, but this heterogeneity is observed also for life span. It is supposed that the causes of sarcopenia may lie within the aging muscle itself. In fact, the hormonal milieu of the worm is substantially different from that present in mammals, being devoid of sex hormones and some cytokines such as IL-6. Moreover, in contrast to

mammals, low levels of IGF-1 have been associated with an increase in longevity and in prevention of sarcopenia. Interestingly, the worm's nervous system remains largely unaffected by the aging process.

Globally, all these considerations and the presence of a high number of mitochondria suggest the prediction that muscle mass in C. Elegans may be more susceptible to oxidative damage and mitochondrial dysfunction.

Genetic studies have been the object of intense interest for the identification of molecular pathways involved both in longevity and aging, and in aging-related phenotypes of specific organ systems, such as skeletal muscle. Single-gene loci which directly affect longevity seem to differ from those regulating muscle growth and degeneration (46). Transgenic mice with muscle-restricted expression of IGF-1 seem to escape age-related loss of muscle mass and function (47). However, the overexpression of, or a mutant, transcription factor, FOXO, downstream of IGF-1 signalling, blocks the inhibition of muscle protein degradation (48). Since diverse isoforms of IGF-1 are described, it will be important to explore their selectivity of action on muscle cells. A central role of myostatin, a muscle-restricted member of the TGFβ super family, as a negative regulator of muscle mass (49), has prompted studies on its interaction with the IGF-1 pathway and changes during aging.

In this context, it is interesting to note that caloric restriction, the only known environmental manipulation able to extend the life span in a broad spectrum of life forms, is likely mediated by a gene, *SIR2*, whose mammalian ortholog encodes a protein deacetylase, Sirt1, regulating glucose metabolism, stress resistance and apoptosis, and acting through inhibition of the insulin-IGF1 pathway (50).

Despite these impressive discoveries, genetic differences affecting the process of muscle atrophy in senescent human beings are still under scrutiny, and no firm conclusion has been established so far.

The conclusion may be that the mechanisms of sarcopenia vary across species, and in the same species can differ from one subject to another.

Outcomes of sarcopenia

As an obvious consequence of the age-associated loss of muscle mass and strength, gait may be severely impaired. Another significant problem is the increased tendency to fall, because of inadequate muscular response to loss of balance; however other problems complicate this event, since bone maintenance against continuous resorption needs mechanical strain afforded by physical activity, and osteoporosis may set in. Moreover, heat generation provided by muscles is impaired, as is the metabolic homeostatic and tissue repair response to trauma, so that a frail elderly subject may not survive the consequences of a fall due to lack of muscle tissue. Several clinical studies confirm that sarcopenia is a strong risk factor for disability.

In a cross-sectional analysis performed by means of BIA method, Janssen et al.(2) found that the likelihood of functional impairment and disability was approximately two times greater in older men and three times greater in older women with class II (severe) sarcopenia than in older men and women with a normal Skeletal Muscle Index, respectively. The association remained significant also after adjustment for several confounders.

The same Author, in the Longitudinal Cardiovascular Health Study, which enrolled 5036 men and women aged 65 and older, found that at baseline the likelihood of disability was 79% greater in those with severe sarcopenia, but was not significantly greater in those with moderate sarcopenia than in those with normal muscle mass. During the 8-year follow-up, the risk of developing disability was 27% greater in those with severe sarcopenia, but was not statistically greater in those with moderate sarcopenia than in those with normal muscle mass. In conclusion, in this study severe sarcopenia was a modest independent risk factor for the development of physical disability. The effect of sarcopenia on disability was considerably smaller in the longitudinal analysis than in the cross-sectional analysis. Zoico et al. (5) found that subjects with low muscle mass only and those with high body fat and low muscle mass (sarcopenic obese) showed a lower isometric leg strength and a significantly higher prevalence of functional limitation in ADL and IADL compared to women with normal fat and muscle mass. In the study of Baumgartner et al. (51) sarcopenic obese subjects were two to three times more likely to report onset of IADL disability during an 8-year follow-up period than lean sarcopenic or non-sarcopenic obese subjects and those with normal

body composition. Villareal et al. (52) observed that obese elderly subjects, although reporting absolute greater values of free-fat mass, had lower FFM in percentage of body weight, lower muscle strength and a reduced performance, evaluated with subjective and objective measures. The Health Aging and Body Composition Study, including 3075 well-functioning subjects of both sexes aged 70-79 years, found that, after a mean period of 2.5 years, muscle cross-sectional area, muscle tissue attenuation (a measure of a fat infiltration), measured by TC at mid-thigh, and knee extensor strength were associated with an increased risk of mobility loss. Therefore, all these studies show that it is possible to identify a subset of older sarcopenic subjects, who are in a highly transitional stage and at high risk of developing difficulty in mobility tasks in the near future. These subjects in such rapid transition may represent an optimal target group for intervention to prevent disability.

Other studies, using surrogate measures which are strictly dependent from sarcopenia, indirectly support its role as risk factor for disability (8).

The phenotype of frail elder adults, described by Fried et al. (53), is characterized by the presence of a critical mass of three or more "core frail elements" among the following: weight loss > 10 lbs in the past year, weak grip strength (lowest quintile), exhaustion (by self-report), slow gait speed (lowest quintile) and low physical activity (lowest quintile). All these are surrogate parameters of sarcopenia. In the Cardiovascular Health Study, frailty, defined according to Fried's criteria, was predictive of a 3-year incidence or progression of disability in both mobility and ADLs, independent of comorbidity, health habits and psychosocial characteristics. Compared with fit older subjects, those who were moderately or severely frail had a relative risk for institutionalization of 8.6 (95% confidence intervals 4.9-15.2) and for death of 7.3 (95% CI 4.7-11.4). Frailty was also associated with poor self-ratings of health, more comorbidity and more social isolation. These risks persisted after adjustment for age and sex. In the Women's Health and Aging Study II, 436 community-dwelling women,70-80 years of age at baseline, not cognitively impaired and reporting difficulty in no areas of physical function, were recruited (54). In these subjects, the self report physical disability was assessed by means of the National Health Interview Survey; the objective measures of performance were the maximal isometric strength of the knee extensor muscles, the Functional Reach Test, the time taken to walk 4 m and the time to climb up and down a flight of 14 stairs at usual pace. After an 18 months follow-up, almost half of these high-functioning women became disabled in mobility; both the self report and performance measures were, independently and jointly, predictive of incident mobility disability; the slowest walking speed and stair climbing speed resulted as being the more sensitive predictors (2).

In conclusion, sarcopenia and the reduced performance of the muscular system seem to be predictors of disability and adverse health outcomes; therefore, prevention and therapy of these conditions are mandatory to delay frailty, increase healthy life span and reduce the costs of hospital and nursing home care.

Prevention and therapy

Many observational studies show that physical activity from middle age onwards results in a slower progression of muscle mass reduction and related functional limitations and has a strong role in the prevention of disability. In the 3-year Amsterdam Longitudinal Aging Study, Visser et al. (55) studied the association of physical activity and sports participation with mobility performance in 2109 men and women, aged 55 to 85. A regular active lifestyle was associated with the smallest decline in mobility and a beneficial effect was observed for sports and non-sports activities, independent of demographic variables, depression, cognitive status and chronic diseases. Recently, Landi et al. (56) have shown that the type and level of physical activity performed in every day tasks can be sufficient to achieve significant positive effects, also in the very old.

Resistance exercise is a far more powerful stimulus of muscle hypertrophy than is endurance exercise. In a cross-sectional study of elderly men with different training backgrounds, Klitgaard et al. (57) found that elderly weightlifters maintained youthful muscle mass and strength while swimmers did not. More directly, the efficacy of exercise in preventing or reversing sarcopenia has been demonstrated by intervention trials. In healthy

older adults, high intensity Progressive Resistance exercise Training (PRT) induces significant increases in fat-free mass, muscle fiber area and muscle cross-sectional area. Moreover, these studies have not shown that PRT can decrease total fat mass and visceral fat.

Limited information is available for physically frail elderly populations, who are at the greatest risk of sarcopenia and reduced mobility. Some evidence suggests that the muscle hypertrophic response to PRT may be impaired in very old individuals (58). In a study enrolling 91 community-dwelling frail >78 year old subjects of both sexes, a 3-months supervised PRT induced improvement in maximal voluntary thigh muscle strength and whole body free-fat mass, compared to control subjects who underwent home-based exercise (59). However, the supervised exercise program was not sufficient to reduce the whole-body or intra-abdominal fat area in this population. In other studies the effects of PRT have been evaluated, taking into account functional parameters, which are directly related to sarcopenia. The meta-analysis of 41 randomized controlled trials with PRT in older adults shows that moderate to large increases in mass and strength have usually been obtained. Compared with younger subjects, resistance training in elderly people produces increases in muscle mass that are smaller in absolute terms, but similar in relative terms (60). Acute resistance exercise increases myosin heavy chain actin synthesis rates to similar absolute levels in both 78-84 and 23-32 year-old men and women, suggesting that the process is not influenced by advancing age (61).

Low-moderate intensity resistance training programs have been advocated for frail elders as a way to increase the practical dissemination and acceptability of this modality of training. Randomized trials indicate that even the frailest nursing home patients can benefit from resistance exercise, with almost two-fold increases in lean body mass and resulting improvements in strength, exercise tolerance and walking speed. These positive results come, however, not only from the increase in muscle mass, but also from muscolo-tendinous and neural adaptations, such as the improved coordination between agonist/synergist muscles, the decreased agonist-antagonist coactivation and the greater motor unit recruitment (62) (Table I).

Biohumoral changes following exercise mostly depend on the type, duration and intensity of exercise and the baseline conditions which are also influenced by adaptation to training. Changes in plasma volume and haemo-concentration are also observed, but the extent of measured levels of mediators and cell number grossly exceeds that of the haematocrit increase (mean 9%). Leukocytosis occurs during exercise, through mobilization of cells from marginated or confined pools, their possible activation with release of mediators and finally, migration to exercising tissues during the recovery phase. However, these events, which can be seen as belonging to the adaptive response to exercise, do not necessarily involve tissue damage or inflammation. In fact, training induces long term anti-inflammatory effects, despite a burst of increase of certain inflammatory markers in the short term.

Nutritional supplementation may be useful in sarcopenic undernourished individuals. In elderly men, undergoing 12 weeks of resistance training, dietary protein-calorie supplements were followed by gains in muscle mass, but not in strength (63). However, the effect of nutritional supplement is not significant for all types of exercise, and it has been reported that oral protein supplementation caused about 25% increase in the quadriceps muscle cross-sectional area when taken immediately after the training session, whereas no increase was observed if the supplement was taken 2 hours after training.

The effects of hormone replacement therapy in elderly subjects is a matter of debate. GH, associated to resistance training, increases strength in younger subjects via muscle hypertrophy (increase of cross-sectional areas of both type I and type II muscle fibers), but is less effective in the same experimental conditions in elderly subjects.

The treatment with GH upregulates IGF-1 gene expression both in the liver and in the skeletal muscle; when the treatment with GH was combined with resistance training, a significant increase in MGF was observed, also in the oldest old (over 85 years) subjects. GH, associated to testosterone, produces an increase in lean mass, but not in strength. Blackman et al. (64), in a randomized, double-blind, placebo-controlled study lasting 26 weeks, treated 65-88 year old men and women with GH and/or testosterone for

men and hormone replacement therapy for women. GH reduced fat mass and increased lean mass in both sexes. Again, little or no increase in strength was observed.

Taking into account these data and considering the possible adverse effects of hormone replacement therapy, on the basis of current knowledge, testosterone and GH therapy should be considered only for elderly subjects, who are truly hypogonadal and/or have a documented significant reduction of GH.

CONCLUSIONS

Human skeletal muscles are a mosaic of different types of specialized myofibers whose activity is determined by the pattern of motoneuron firing. The tonic pattern of contractile work is obtained through endurance training, the kind of physical exercise which has been seen to contrast obesity, diabetes, hypertension or heart disease. However, building up and maintaining muscle mass requires phasic contractile activity and a continuous minimal level of physical exercise.

The pathogenesis of sarcopenia is complex: lack of exercise, inflammation and oxidative stress, which are all common to aging, whereas hormonal and nutritional deficits are important only in selected cases. The role of the nervous system and of genetic components is at present unclear. The search for pathogenetic factors of sarcopenia in the clinical setting is relevant, because the vast majority of studies show that this condition may be prevented or reversed. Particularly structured exercise programs and an active lifestyle are effective in contrasting sarcopenia and the progression of frailty. The slow but inexorable loss of muscle mass, typical of advanced age, is an essential component of frailty. There is therefore clear evidence that the prevention and treatment of frailty in the elderly must be founded on a multidimensional approach and that the exercise prescription must be included within the mainstream of the medical practice, rather than an optional adjunct to standard care of the frail elderly.

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