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SKELETAL MUSCLE AND AGING

Ana Navarro [1], José M ª López-Cepero [2] and María Jesús Sánchez del Pino [3]

[1] Department of Biochemistry and Molecular Biology, [2] Department of Cell Biology and [3] Department of Biochemistry and Molecular Biology, Faculty of Medicine. University of Cádiz. Plaza Fragela nº 9. 11003-Cádiz. Spain

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1. ABSTRACT

Age-related changes in muscle mass (sarcopenia) and functional properties are the result of a very complex hierarchical system of basic cell aging processes and cell adaptive responses. A basic aging mechanism pertains to mitochondrial production of free radicals and their associated secondary effects. From this basic aging mechanism many other cellular changes can be explained as direct effects or compensatory changes. Altered skeletal muscle cell biochemical and functional properties during aging resulting from intrinsic mechanisms and from changes in hormonal or local signals that influence phenotype maintenance, are reviewed. The effect of skeletal muscle cell senescence on the cellular response to exercise, and the effect of dietary restriction on muscle cell senescence can also be explored from this perspective.

2. INTRODUCTION

Skeletal muscle tissue is developmentally organized as a set of anatomical units, specifically innervated by selected groups of neurons in spinal cord motor columns. Motor units are established through axonal competition for myotubes. Each muscle cell phenotype is highly dependent on the firing pattern of the axon innervating a single muscular end plate in most muscles (1). Fibre types arising from different neural influences can be classified according to their myosin heavy chain expression detected by immunohistochemistry or myofibrillar ATPase (mATPase) activity. Seven fibre types have been described in human muscle on the basis of the pH dependent stability/lability of mATPase activity that can be correlated with the proportion of myosin heavy chain isoforms detected by immunohistochemistry (2).

Muscular differences in fibre architecture, fibre type distribution and motor unit size, reflect enormous variability between anatomically defined skeletal muscles. Each muscle is postnatally remodelled through secondary hypertrophy by loading and daily working schedules. Disuse atrophy, due to reduced movement, immobilization, or unloading during space flight, is rapidly established (within a week) reflecting the strong dependence on a certain level of permanent loading or daily total work for structural maintenance. Muscular senescence is the functional progressive loss of individual muscular groups and is variable between anatomical entities. Many factors account for the progressive loss of muscular mass, strength, endurance and velocity of contraction. The neural factors that contribute to muscle senescence result in a progressive denervation, partially compensated by collateral reinnervation, and hence a reduced motor unit number and increased motor unit size (3,4). Motor axon loss exceeds the fibre number reduction. An inevitable consequence of central nervous tissue neural plasticity is that movement patterns lead to a remodelling of motor control neural circuits resulting in a desynchronisation of recruitment which contributes to neural fatigability or peak strength. Muscular tissue remodelling also includes vascular and connective tissue changes of lesser importance compared to other muscular senescence processes (5).

The gradual and sustained loss of total muscular mass after the peak development in the years that follow adolescence is referred to as sarcopenia. This condition accounts for the reduced strength and endurance of muscular performance (6-8). Interindividual variability, in total muscle mass related to exercise levels, and non-repaired cumulative effects of minimal lesions resulting from exhaustive exercise or intrinsic aging mechanisms, contribute to wide differences between different muscles in aged individuals.

In neuromuscular senescence, a general trend reflects a set of underlying mechanisms at very different organisational levels. The general mechanisms of aging for long lived cells contribute at several levels, including the mechanotransduction response for structural maintenance; altered response to regulation from systemic factors associated with global effects of aging; and local muscular tissue mechanisms for structural maintenance and cellular biochemical changes within muscle cells. So many factors are involved at so many organization levels, that no simple model can integrate data ranging from biochemical stoichometric changes or dynamic metabolic data at the cellular level, to clinical evidence of reduced or impaired function (neuromuscular senescence). A useful model must relate to the cumulative effects of a particular muscle with cellular impairment or reduced mechanisms of tissue maintenance. A more limited approach is to centre the analysis at the cell level, and to elucidate the changes in cellular mechanisms of skeletal cell phenotype maintenance and their age associated evolution i.e.: the balance between disuse atrophy and adaptive responses to mechanical stress of activity and their historic drift for a selected muscle. A key practical question is: to what extent are the structural and biochemical properties of a muscle sample useful predictors of functional performance levels or clinical disease threshold? Only longitudinal studies, a well controlled muscle history and how it affects skeletal muscle tissue adaptability *versus.* demands over time, can provide a clinically oriented interpretative framework.

An ideal model must consider: a) the basic aging mechanisms for long-lived cells; b) mechanisms underlying muscular cell structural maintenance and c) the interplay between the above and their responses (hypertrophy or disuse atrophy) in aged individuals. A gradual change in the interaction between these factors is presumed. The age-associated systemic changes in hormonal or general metabolic parameters which can regulate muscular cell phenotype maintenance, functional properties or adaptational responses must also be considered. The effects of excessive strength demands (relative to usual level), their repair mechanisms and their cumulative effects over time are difficult to evaluate by their dependence on muscular previous history and because it is difficult to modelize how the stimulus is distributed to the cell population of a muscle. Excessive endurance demands (e.g. exhaustive exercise) also induce local tissue lesions followed by inflammatory and reparative responses, which are influenced by the exercise itself, and by the age-associated changes in immune system (9-11). Their cumulative effects also affect muscular tissue structure with advancing age. At the cellular level, the subcellular mechanisms of excitation-contraction coupling, contraction and relaxation mechanisms, and their associated biochemical changes in muscular aged cells are related to energetic systems, free radical production and other basic cellular changes associated with aging.

3. SARCOPENIA OF AGING

The age-related decrease in total skeletal muscle mass has been referred to as sarcopenia. This reduction in muscle mass is a direct cause of the age-related decrease in muscle strength and power, i.e.: muscular senescence (8). Other factors in addition to mass reduction contribute to the age-related motor disability, and affect specific groups of muscles, motor units and muscle cell types differently (4,12). The global reduction in muscle mass occurs relative to body mass, and encompasses other changes in body composition, i.e. an increase in fat tissue and reduced fat-free mass, and hence a lower basal metabolic rate in the elderly. The energy expenditure declines by about 15% between the third and eighth decades of life. Such changes are correlated with reduced bone density, insulin sensitivity, and aerobic capacity, but the causal links remain to be elucidated (6). Progressive resistance exercise can produce substantial increases in strength and muscle size, even in the oldest patients (13). In aged patients, exercise protocols are a way to lose fat-mass, improve glucose tolerance, and maintain muscular mass (7,14). The systemic metabolic, physiological and functional secondary effects of sarcopenia and muscular senescence show a great interindividual variability, even between sexes (15), and are not discussed in this review. Statistical variation between human groups is presumed to be dependent on social and nutritional habits, as well as on underlying genetic

differences (16). More epidemiological data, and a complete assessment of the pathophysiologic consequences of sarcopenia, would be necessary to evaluate the environmental factors involved and their public health impact (17). Wasting secondary to an imbalance between reduced caloric intake, appetite disregulation, and energy loss, in spite of reduced physical exercise in the elderly, could also contribute to sarcopenia (18). Weight losses in obese patients have effects on fibre type composition, fibre size, capillarity and succinate dehydrogenase activity, so dietary restriction alone may alter the structural properties of muscular tissue (19).

Sarcopenia of aging is the final result of many changes including: a) progressive denervation with perhaps a reduced ability for collateral reinnervation (1,4,20); b) reduced motor unit number and increased motor unit size (3,4); c) decreased protein synthesis of myofibrillar proteins (21); d) disuse atrophy mechanisms (22); e) altered equilibrium of locally produced growth factors and/or systemic trophic factors for muscular cell phenotype maintenance (23).

Most of the reduction in fibre size in sarcopenia can be explained in terms of their myofibrillar content, as a result of reduced synthesis of myofibrillar proteins with aging (21), although other protein fractions may also be affected. The synthesis of both mitochondrial proteins and myosin heavy chain declines with age, whereas the sarcoplasmic protein pool is unchanged (21,24-26). A reduced synthesis rate of proteins suggests changes in sarcomere protein remodelling that may help explain the loss of skeletal muscle mass and function with aging (27). Resistive exercise produces muscle hypertrophy, and increases mixed muscle and myofibrillar protein synthesis in both young (28-30) and old individuals (28, 31-33).

In spite of reduced myofibrillar content, the hypertrophy mechanisms in aged muscle are possibly limited by the reduced pool of satellite cells, and/or reduced activation and fusion by deficits of local growth paracrine factors (34,35). The regenerative potential of skeletal muscle, and overall muscle mass, declines with age. This regenerative potential may be influenced by autocrine growth factors intrinsic to the muscle itself (36). Extrinsic host factors that may influence muscle regeneration include hormones, and growth factors secreted in a paracrine manner by macrophages (37). Eccentric strength exercise, which involves strong mechanical overload of myofibres, provides a convenient method for studying muscle regeneration in both humans and animal models. An inflammatory response ensues in which distinctive populations of macrophages infiltrate the affected tissue: some of these macrophages are involved in phagocytosis of damaged fibres; other macrophages arriving at later times may deliver growth factors or cytokines that promote regeneration (37). These include fibroblast growth factor, insulin-like growth factor, interleukin-1 and tumour necrosis factor, which are important regulators of muscle precursor cell growth and differentiation (38), as well as nerve growth factor, which is essential for maintenance or re-establishment of neuromuscular synaptic contact. Several cytokines (23), including interleukin-1 and tumour necrosis factor (from mononuclear cells), interleukin-15 (from skeletal muscle itself), and ciliary neurotrophic factor (from Schwann cells), as well as growth hormone and insulin-like growth factor-I (31), have a strong influence on the balance between muscle protein synthesis and breakdown. The functional activity of invading macrophages can be influenced by age, by factors from myofibres (glutamine) (23), influences from extracellular matrix (39), and by the antioxidant status of the host (40).

4. ENERGY INTAKE PATTERN IN AGING

Both a progressive decline in energy intake and a reduction in daily energy needs are associated with aging (41). The decline in energy intake may be less than the age-associated decline in energy expenditure, resulting in a positive energy balance and gains in fat mass in humans (42), but the inverse situation is also common. Three distinct components constitute the daily energy requirements of muscle of the older individual: the resting energy expenditure, thermogenesis from shivering muscle (43), and exercise (44-47). The resting energy expenditure comprises about 65-70% of the total daily energy expenditure (44-46). Long-term caloric restriction in monkeys causes a long-term reduction in energy expenditure, although motor activity level is maintained (48). Inadequate energy intake can be an important contributor to sarcopenia in older individuals, because negative energy balance induces negative nitrogen balance, independent of nitrogen intake (49).

Intake control is altered and appetite is reduced in older human subjects (50). Changes in hypothalamic neuropeptides associated with appetite regulation (51,52), serotonin metabolites in cerebrospinal fluid (53), and peripheral monocyte cytokine production (IL-6, TNF-[alpha]) (54) have been described. Caloric restriction is the only intervention that appears to slow the intrinsic rate of aging (55) and can markedly prolong the life span (56). The life-extending benefits of caloric restriction depend on the prevention of malnutrition and a reduction in overall caloric intake, rather than any particular nutrient (55,57,58). Some responses to caloric restriction are quite rapid, for example in rats, blood glucose and circulating insulin concentrations are decreased after only five days and three weeks respectively, (59), but the long lasting effects are presumed to be mediated trough respiratory basal changes and will be further discussed.

5. HORMONAL FACTORS THAT INFLUENCE SKELETAL MUSCLE

5.1. Growth hormone and insulin-like growth factor I

Growth hormone (GH) is secreted episodically by the anterior pituitary somatotrophs in response to hypothalamic signals (mainly growth hormone releasing hormone and somatostatin), and is modulated by several signals from the periphery, including insulin-like growth factor I (IGF-I), thyroid hormones, glucocorticoids, and gonadal steroids (60). A sexually dimorphic pattern of GH secretion in aged humans has been suggested, and could be

partly responsible for sexually dimorphic muscle mass. In rodents, there is a significantly higher mean 24 hour serum GH concentration in females, compared with males (61). The number and amplitude of growth hormone secretory bursts declines with aging (62). Growth hormone stimulates the production and secretion of IGF-I (somatomedin C) by several tissues ("somatomedin hypothesis") (63). Circulating IGF-I levels also decline with ageing, as a consequence of reduced GH secretion. IGF-I concentration in the seventh decade of life is approximately half of that in the third decade (64). The functional consequences for muscle of the age-related decline in growth hormone secretion and IGF-I levels remain unclear. In a recent review the therapeutic role of recombinant human GH was analysed (65) and beneficial anabolic effects of IGF-I in elderly humans have been suggested (66). In addition, GH retards muscle damage due to immobilization in older rats (67). Preliminary results of GH treatment in the normal elderly suggest beneficial effects on body composition, but with a high incidence of side effects. Long term administration of GH on muscle mass, myofibrillar protein synthesis (31), or strength associated with resistance training in older individuals (68,69), does not seems to be beneficial. IGF-I administration increased whole body protein synthesis and breakdown by the same amount (66). Other reports with GHRH treatment detected a net increase in muscle mass and bioenergetics that reduced anaerobic metabolism during exercise in elderly individuals (70). hrGH therapy in conjunction with resistance exercise does not increase muscle mass or strength in aged patients (71). Questions of cost, benefit, dosage, safety and tolerance need to be critically addressed before GH can be considered for sarcopenia treatment in the aging (72). It is possible that the effect of GH and IGF-I on aged skeletal muscle depends on other hormones that decline with aging.

5.2. Glucocorticoids and muscle protein synthesis

The glucocorticoids may play an important role in muscle atrophy in old age. Glucocorticoids have catabolic effects, reducing protein synthesis and stimulating protein degradation in skeletal muscle (73,74). Recently, the cellular mechanisms and proteolytic pathways involved in the catabolic effects of glucocorticoids have been revised (75). The effects of glucocorticoid treatment on muscle protein balance were compared in adult and old rats. The results showed that muscle wasting occurred more rapidly in old rats, and the recovery of muscle mass was impaired (76). Protein turnover in muscle cells is differentially affected by glucocorticoid treatment, with adult rats showing an increased protein breakdown and muscle atrophy following dexamethasone treatment. Ubiquitination and proteasomal activity were increased and cathepsin B expression upregulated. Aged rats showed a reduced protein synthesis response. Biochemical events downstream from the glucocorticoid receptor regulated promoters differs in adult and aged muscle cells, and mRNA expression and/or translational changes lead to different effects on protein turnover (75,77). Glutamine synthetase (also regulated by glucocorticoids receptors) is expressed equally in adult and old rats (78). A decrease in nitrogen balance is induced more slowly in old rats, but this effect is more pronounced and long lasting. Glutamine homeostasis is impaired in aged rats (79). Muscle cell types have specific gene transcription responses to dexamethasone treatment; white fast-twitch muscle of aged rats is resistant to inhibition of protein synthesis by glucocorticoids (80). Integrated responses of several growth factor signals are also changed by corticoids in aged muscle cells. Increased protein synthesis induced by insulin and IGF-I is depressed by dexamethasone to a greater degree in aged rats. Glucocorticoid effects on protein turnover (by regulation of synthesis or breakdown) are more pronounced in old muscle cells, by neutralizing anabolic signals, or by a more profound and long lasting effect on the ubiquitin proteasome pathway of myofibrillar protein degradation.

5.3. Testosterone and muscular mass and strength

Serum testosterone concentrations decline in older men. The age-dependent decline in androgens may contribute to concomitant changes in body composition i.e. a lower percentage of muscle mass and an increase and redistribution of body fat (81-83). Many studies have focused on the use of testosterone to reduce age-associated sarcopenia in men (84). When testosterone was administered to older men for 12 months, it was found to significantly increase handgrip strength (85). In the lower extremitis (quadriceps and hamstring), testosterone administration in older men increased muscle strength after only 1 month of treatment (86). In addition, the supraphysiological replacement of testosterone in young eugonadal men has also been shown to increase muscle mass and improve strength (87). On the other hand in young patients, testosterone administration preserves protein balance, but not muscle strength, during 28 days of bed rest (88). Testosterone has been used in postmenopausal women to improve muscle strength (89). There is no evidence that the increase in muscle strength resulting from testosterone administration could be mediated by the conversion of testosterone to estradiol (90,91). Testosterone increases muscle protein synthesis, intramuscular mRNA concentrations of IGF-I, and decreases the concentration of the inhibitory IGF binding protein 4. This indicates a stimulation of the intramuscular IGF-I system during testosterone administration (86,92).

With regard to body composition, no significant changes were found in weight or fat mass after 3 (93) or 12 (85) months supplementation treatment with parenteral testosterone. However, an increase in weight has been found, caused by an increase in fat free mass (94). Some age-related differences in body composition due to androgen decline, such as nutritional status and lipoproteins, are not influenced by androgen supplementation in older men (95).

5.4. Thyroid Hormone and phenotypic muscle effects

Thyroid hormone (T3) acts on nuclear receptor proteins which, in skeletal muscle cells, regulate the gene expression of myofibrillar protein isoforms (96). Other nuclear receptors for oestrogen and androgen can also interact with thyroid hormone receptors on the same promoter of (alpha) MyHC isoform (97). This isoform is expressed during fast to slow MyHC transitions in experimental models (98). During aging, the circulating T3

levels are decreased (99), and the age associated fibre type shift from fast to slow is attributable, at least partly, to this change (100). The ratio of myosin light chain isoforms MLC1Sa/MLC1Sb is increased during ageing and, after thyroid hormone treatment, has some effect on contraction velocity of type 1 (slow) fibres, although the effect of aging and thyroid hormone treatment on the myosin light chains ratio is supposed to be mediated through a different pathway (101).

5.5. Insulin and muscle glucose uptake

The dependence of skeletal muscle on insulin for glucose uptake is well established. A well-known observation of aging is the development of impaired glucose tolerance, which begins in the third or fourth decade of life and progresses thereafter (102). However, elderly individuals typically have normal or slightly elevated serum insulin levels (103). In isolated perfused pancreas or isolated islets of Langerhans from rats, age-related changes in insulin secretion in response to glucose have not been observed (104,105). However, isolated perfused pancreas from aged rats show a reduced insulin release in response to low, but not high, concentrations of glucose (106). In isolated perfused pancreas from aged rats, somatostatin induced a greater inhibition of glucose-stimulated insulin release than seen in young animals (107). An age-dependent decrease in islet insulin, but not glucagon or somatostatin mRNA levels has been reported (108). The senescent pancreas has an impaired insulin response to glucose (109), but other factors contribute more to the impaired glucose tolerance observed in the elderly (110). One of these factors is that insulin-mediated skeletal muscle cell glucose uptake declines with advancing age (111,112). IGF-I enhances glucose uptake in rodent skeletal muscle by stimulation of its own receptor (113,114) and this effect is also reduced in adult rats (115). In humans, decreased plasma levels of IGF-I precedes insulin resistance (116), although the skeletal tissue contribution is not established. Triglyceride accumulation in skeletal muscle cells, and membrane phospholipid composition, has been suggested to increase insulin resistance (117,118). Also, insulin induces NO-dependent vasodilatation in the vascular bed of skeletal muscle, which is reduced in aging, and has been proposed to contribute to insulin resistance (119,120). Insulin stimulation induces the translocation from their intracellular compartment to the cell surface of Glu-T4 (adipose/muscle type glucose transporter) (121). Chronic exercise increased both IGF-I production and IGF-I receptor, as well as Glu-T4 content, even in old animals (122) and man (123,124). It has been reported that Glu-T4 concentration does not increase in old Fisher-344 rats (125). Differences in exercise protocols are relevant in explaining these discrepant results. Glu-T4 protein content is decreased in aged rats, attributable to a diminished translational efficiency, since Glu-T4 mRNA level is maintained (126). Insulin resistance of muscle cells is attributed to impaired translocation of intracellular Glu-T4 to the sarcolemma (127). A general deficit of translocation of proteins to surface membranes in aged animals has not been reported.

6. EXERCISE, STRUCTURAL MAINTENANCE OF SKELETAL MUSCLE, AND AGING

Mammalian skeletal muscle is well suited for intermittent bouts of activity and develops and maintains its structure by mechanotransduction pathways that influence the skeletal muscle cell phenotype modulation and the hypertrophy level. Disuse atrophy is one of the most important and rapidly establishing contributors to sarcopenia of aging (7,17). Progressive resistance exercises and strength training can produce substantial increases in strength and muscle size, even in the oldest cases. For many older patients, exercise represents the safest, least expensive means to lose body fat, decrease blood pressure, improve glucose tolerance, and maintain long-term independence (14,128). Types of strength exercise (eccentric or concentric) daily schedule and duration differ widely in published studies and are not reviewed here. The general consensus is that whilst clinical effects are beneficial, cellular mechanisms of muscle structural and functional restoration have not been completely analysed (129). Individual variability in total muscle mass and body composition, history of their muscle-skeletal system, energetic balance effects of the type of exercise and intrinsic ability of muscle to hypertrophy have not been analysed as separate factors. The chronic cumulative and secondary effects of exercise on muscle senescence are presumed to derive from incomplete reparation after very intense bouts of exercise, or an age-related decline in reparative muscle mechanisms.

7. SKELETAL MUSCLE CELL CHANGES ASSOCIATED WITH AGING

7.1. Postsynaptic changes, sarcolemma channel alterations and depolarisation properties

The end plate postsynaptic action potential corresponds to the opening of membrane voltage-dependent sodium channels (130-132). These channels belong to a large multigene family of closely related isoforms (133). The molecular structure and function, and the role that abnormal sodium channels play in human disease, are well known (134,135). The expression of the adult phenotype isoforms requires the interaction of muscle fibres with active motor neurons (136-138). Modification of sodium channel gating can modify sarcolemma excitability and, as a consequence, can alter contractile properties of skeletal muscle (139). Extrajunctional sarcolemma of aged-rat fibres presents a higher sodium current density than that of young-rat fibres, which results from the presence of a higher number of available channels per membrane area (140). Aged-rat fibres can be discriminated on the basis of channel conductance. Some present a conductance of 18 pS as in young-adult rats and others exhibit a conductance of 9 pS, while ensemble average currents activate and inactivate more slowly. Biophysical properties, such as open probability, mean open time, steady-state inactivation and use-dependent inhibition are very similar in both fibres and the differences are not due to dennervation (139). Chronic treatment of aged rats with either GH or hexarelin restored current kinetics, but not channel conductance and density. These results confirm the specific age-related changes in

sodium channel behaviour, and show that treatment with either GH or hexarelin has partial restorative effects (141).

In rat fast-twitch muscle fibres, the enhanced activation of protein kinase C that occurs during aging leads to reduction of macroscopic chloride conductance (142,143). Chronic treatment of aged rats with GH improves the macroscopic chloride conductance and sarcolemma excitability of skeletal muscle (144). This effect is mimicked in vitro by IGF-1, which suggests that this peptide is the mediator of GH at the muscular level in its effect on chloride channels (145). In the short term, the peptide may stimulate a serine-threonine phosphatase, that is able to counteract the enhanced age-related activation of protein kinase C, increasing the chloride conductance (145). Furthermore, IGF-1 may induce the neosynthesis of chloride channels in the long term (145**).** These results show that the skeletal muscle chloride channel is a target of impairment of the GH/IGF-I axis occurring in aged subjects (145). Taurine induces an increase of the resting membrane chloride conductance, and it has been suggested that a reduction of taurine content could play a role in the alteration of electrical and contractile properties observed during aging (146). The levels of mRNA encoding the principal skeletal muscle chloride channel (ClC-1) provide evidence that the decrease of resting membrane chloride conductance observed during aging is associated with a downregulation of ClC-1 expression in muscle (147).

The properties of ATP-sensitive potassium channels are modified by age-related redox potential change (148). The activity of calcium-activated potassium channels increases with advancing age (149). The result is an increase of the macroscopic potassium conductance (143,149).

7.2. Altered excitation-contraction coupling and calcium homeostasis

Contraction of skeletal muscle is activated by calcium released from the sarcoplasmic reticulum (SR), in response to depolarisation of the sarcolemma propagated via the transverse T tubules. The depolarisation is detected by voltage sensors (dihydropyridine receptors) in the T tubule membrane, and the signal is transmitted to sarcoplasmic reticulum calcium release channels (ryanodine receptors) at triad junctions between T tubules and sarcoplasmic reticulum release terminals. Transmission of this signal takes place by a mechanical or allosteric interaction between the dihydropyridine receptors and ryanodine receptors (150-152). It has been accepted that calcium-induced calcium release (153,154) also plays a significant role in skeletal excitation-contraction coupling (155,156). This progress in the understanding of function has taken place in parallel with the increasingly precise definition of the junction structure (157). In particular, the alternation of release channels, in apparent contact with dihydropyridine receptors and others channels devoid of this interaction, has suggested that two kinetic phases are present in the waveform of calcium release flux under voltage clamp, and that these correspond to the existence of two different control mechanisms (155,158,159). The type 3 ryanodine receptor (RyR3) is a ubiquitous calcium release channel that has recently been found in mammalian skeletal muscles. It is codistributed with the skeletal muscle isoform (RyR1) and the dihydropyridine receptor. Highly expressed during development, only some fibres of several phenotypes maintain their expression in adulthood. No age-related changes have been reported for this last receptor (160).

In aged skeletal muscle the calcium-sequestering activity of sarcoplasmic reticulum may be altered but the phosphorylation-dependent regulation and mechanisms of reduced calcium uptake remain controversial (161-163). Perhaps a modulation of RyR1 by the SR luminal protein calsequestrin, or the coupling of RyRs by FKBP-12, may be altered in ageing skeletal muscle (163,164). Impairment of excitation-contraction coupling could be due to a decrease in dihydropyridine receptors (142,163,165). Dihydropyridine-sensitive calcium currents are reduced in skeletal muscle fibres of aged humans and mice (165,166). The final effect is a significant reduction of the amount of calcium available for triggering mechanical responses in aged skeletal muscle and, the reduced calcium release is due to dihydropyridine receptor-ryanodine receptor uncoupling in fast-twitch fibres (165,167). Caloric restriction in rats can prevent the reduced expression of dihydropyridine and ryanodine receptors associated with aging (168).

The calcium ATPase of the sarcoplasmic reticulum for calcium uptake and muscle relaxation has been extensively studied in aged rats with no consistent results. It has been suggested that changes in the tertiary structures (169), more rapid inactivation of the protein *in vitro* during mild heat treatment (170), and a reduction in calcium uptake rate of SR-enriched membrane vesicles isolated from slow-twitch (but not fast-twitch) muscles, that nevertheless exhibit a similar amount of calcium ATPase protein to the young rats (171). Partial uncoupling of ATP hydrolysis from calcium transport could explain the slower relaxation of slow-twitch muscle in aging. Resistance training in humans can partially reverse the reduced SR calcium uptake (172).

Age-related effects in contractile mechanisms beyond calcium release and uptake have not been described. The efficiency of the sliding mechanism, secondary alterations of myofibrillar proteins, or metabolic enzymes of energetic pathways of skeletal muscle cells could, theoretically, limit the contractile properties and/or fatigue resistance. Only very limited and isolated data are available. Nevertheless a basic aging mechanism for long-lived cells is alterations to the mitochondria that induce both energetic and free radicals secondary effects which could mediate all the previously cited changes.

7.3 Age-related mitochondrial changes

7.3.1. Mitochondrial changes and production of oxygen free radicals

The mitochondrial pool of skeletal muscle cells is subject to the same deteriorating trends of aging as the neuronal and other long-lived cells. The regulation of the mitochondrial population and their age-associated changes

are less known that the functional respiratory changes associated with muscular senescence. The electron transport chain is the major intracellular source of reactive oxygen free radicals species (ROS), which are generated as byproducts during the transfer of electrons from NADH or FADH, through the electron transport chain components to molecular oxygen, even under normal physiological conditions, where they may play a role in intracellular signalling (173).

The univalent reduction of oxygen that takes place in mammalian tissues produces superoxide radicals ($O_2^{\cdot -}$) at a rate of about 2% of the total oxygen uptake (174). Membranes isolated from mitochondria, endoplasmic reticulum and plasma membrane in many organs have been recognized as able to catalyse the univalent reduction of oxygen to $O_2^{\cdot -}$ (175). Hydrogen peroxide is generated both as the product of $O_2^{\cdot -}$ dismutation, as it is the case for mitochondrial $O_2^{\cdot -}$ and H_2O_2 generation, and as the product of two electrons transfer from flavin enzymes to the oxygen molecule. In mammalian cells, most of the H_2O_2-producing enzymes are located in the peroxisomes (175,176). These two products of the partial reduction of oxygen, $O_2^{\cdot -}$ and H_2O_2, are able in aerobic systems to co-react, to initiate a chain reaction of oxyradicals formation, including hydroxyl and peroxyl radicals, and singlet oxygen (175,177).

Regarding oxyradical production, it is convenient to first consider the mitochondrial production of $O_2^{\cdot -}$ and H_2O_2, since mitochondria in skeletal muscle cells appear as the quantitatively more important (and possibly unique) source of $O_2^{\cdot -}$ and H_2O_2 (178). The consideration of the physiological rate of the mitochondrial production of $O_2^{\cdot -}$ and H_2O_2 must be preceded by the estimation of the metabolic state of the mitochondrial population in the different types of skeletal muscle cells, under different physiological respiratory conditions. Each mitochondrial respiratory state has a typical rate of $O_2^{\cdot -}$ and H_2O_2 production (179,180). This evaluation is very difficult to achieve because of fibre type heterogeneity, aerobic dependence and the wide difference between basal and maximal rate of muscle oxygen consumption.

The resting mitochondrial state 4 has a relatively high rate of $O_2^{\cdot -}$ and H_2O_2 production, due to the highly-reduced state of the components of the respiratory chain, at level of flavin semiquinone of the NADH dehydrogenase and ubisemiquinone, that by autooxidation yield $O_2^{\cdot -}$. Ob the contrary, the active mitochondrial state 3 with a high rate of oxygen uptake shows a relatively slow rate of $O_2^{\cdot -}$ and H_2O_2 production, due to the highly oxidized states of the components of the mitochondrial respiratory chain. In the anoxic state 5, with no availability of oxygen, there is no respiration and hence no partial reduction of oxygen to $O_2^{\cdot -}$ or H_2O_2 (175,179,180).

In the liver and heart and in physiological conditions, most of the mitochondria are in the resting state 4 (178), and the active respiration yielding ATP is supported by a limited subset of the mitochondrial population of a cell. Mitochondrial respiratory functions decline with age in human liver and skeletal muscle cells. Both the respiratory control and oxidative phosphorylation efficiency of the mitochondria are decreased in an age-dependent manner (181,182).

7.3.2. Energetic effects of muscle mitochondria alterations in aging

The concept of a decreased capacity of energy production in aged organs or aged individuals is immediately associated with the decreased basal metabolic rate observed by Rubner in 1883 (183). The changes in body composition that occur with age and an about constant energy expenditure in vital organs and tissues in adult humans apparently account for a constant basal metabolic rate when taking in consideration organ energy expenditure (183,184). The contribution of muscle to basal metabolic rate is related to the percentage of blood flow derived through the tissue and the proportion of mitochondria in resting and controlled state 4 compared to the active state 3 at the maximal physiological rate of oxygen uptake and energy production (178). However, state 4 respiration has been reported to be increased in mitochondria isolated from aged mice, which would indicate a partial uncoupling in aged mitochondria (185). Uncoupling proteins UCP2, and UCP3 which is expressed only in skeletal muscle, could provide a mechanism for “mild” uncoupling that reduces ROS production by the respiratory chain (186), and their role in muscle aging remains to be explored. Muscle contraction would recruit mitochondria to state 3 until an adequate ATP production rate is established. A hypothesis is that aging could diminish the proportion of recruitment because of the elevated number of damaged and inefficient mitochondria. In fact, there is a decrease in maximal energy production, and a reduced proportion of mitochondria in active state 3 of respiration, in liver and heart mitochondria isolated from senescence-accelerated mice (187). This could represent a metabolic ceiling for energetic production also in skeletal muscle cells, even with an adequate supply of oxygen. The combination of decreased state 3 respiration and increased production of $O_2^{\cdot -}$ and H_2O_2 seems to be associated with aging for most organs studied (188).

7.3.3. Secondary effects of mitochondrial free radical production

A widely discussed theory of cellular aging, outlined by Szilard (189) and Orgel (190), considers the process in terms of the accumulation of molecular damage and informational errors at the DNA and RNA level. This damage has special traits in skeletal muscle cells because their high protein myofibrillar content, the turnover f which is possibly limited by architectural sarcomere restraints, and by the multinucleate character of skeletal muscle cells and incorporation of reserve satellite cells that, in addition to nuclear DNA repair mechanisms, could compensate genetic deficits from one nucleus. The damage may well be produced by some of the reactive oxygen species, such as $HO^{\cdot}$ and $ROO^{\cdot}$. These species are certainly able to abstract hydrogen from the purinic and pyrimidinic bases of nucleic acids, as is singlet molecular oxygen, which are also able to react with DNA and RNA bases yielding addition products. Although most of the damage produced by the reactive

oxygen species is dealt with by the specialized systems of DNA repair, it is accepted that random alterations may accumulate via the oxidative damage to mitochondrial and nuclear DNA which is known to occur in vivo (191,192). Mitochondrial aging (193,194) is influenced by the mitochondrial steady state production of $O_2^{\cdot -}$ and H_2O_2, the chemical species that produce cumulative and non repairable damage to mtDNA (195-197).

Reactive oxygen species modify most kinds of macromolecules, including unsaturated lipids, proteins and DNA (198-200). Antioxidant systems can eliminate many of these radicals, but this is not fully efficient. Greatest free radical damage is expected to occur near the sites of oxygen radical generation, since the most reactive radicals (particularly $OH^{\cdot}$) can not diffuse away, and react non specifically (in nanoseconds) with almost any nearby molecules (178,201). Thus skeletal muscle mitochondria are most affected by sustained free radical production, damaging enzymatic and electron transfer complexes and mtDNA. This creates an inability to be recruited to state 3, resulting in a more intense radical production creating a positive feed back loop of mitochondrial damage (201-203).

Based on the limited data, specific for aged skeletal muscle tissue, several biochemical indicators of oxidative stress have been found to be increased, although some results are controversial. Thus lipofuscine accumulates (204); lipid peroxidation is increased (205-207) or not modified (208,209); total glutathione (GSH) content is increased (210), oxidized glutathione (GSSG) levels are also increased (206), and mitochondrial GSH/GSSG ratio are elevated (211). Discrepancies in glutathione status can be explained if the timing of secondary adaptation of glutathione pool to oxidative stress is considered. Antioxidant enzymes activities are increased (212) as a cellular adaptive response to oxidative stress, the most significant being total superoxide dismutase activity (cytoplasmic Cu-Zn SOD and mitochondrial Mn-SOD) (208,209). However in aged human muscle, total superoxide dismutase (SOD) activity decreased with age, although mitochondrial superoxide dismutase activity is increased, perhaps as an adaptive response of mitochondria to oxidative stress, and coincident with a lower expression of cytoplasmic SOD. No alterations in glutathione peroxidase or catalase activities were reported (206) suggesting a secondary importance. State 3 respiration rate and electron transport system activities are very much lower in aged muscle cells (182,213-215). Multiple mtDNA deletions and mutations, and total content of modified bases (8-OH desoxiguanosine) have been described from the mtDNA fraction in aged skeletal muscle of animals and humans (216,217).

7.4. Nitric oxide, peroxynitrite and aging

A second pathway that could mediate some of the biochemical changes in mitochondria and skeletal muscle cells during aging is the formation of peroxynitrite derived from the interaction of nitric oxide (NO) and superoxide radicals (218). Nitric oxide has low direct toxicity; the major mechanism of injury associated with nitric oxide in vivo most likely results from the diffusion-limited reaction with superoxide to form peroxynitrite (219). Nitric oxide also reacts with ubiquinol in a redox reaction (220,221), with cytochrome oxidase in competition for oxygen (222), and oxymyoglobin and oxyhemoglobin (223), displacing oxygen (224). Since peroxynitrite does not contain unpaired electron it is not a free radical. The half-life of peroxynitrite is less than 1 sec., which enables it to diffuse over considerable distances and exert biological effects (225). The production of NO in skeletal muscle cells is mediated by the so called neural isoform of nitric oxide synthase (nNOS), which is expressed in both slow and fast muscle fibres at birth, but rapidly increases in fast muscle due to motor activity (226). The sarcolemmal localization of nNOS at force transmitting sites, associated to dystrophin and dependent on it for expression (227), suggests a role in mechanotransduction signalling elicited by muscular tension with the expression upregulated by mechanical loading (228). The age-related increased levels indicate perhaps a secondary response to altered mechanotransduction pathways in aging (227). Other reports indicate that nNOS activity and the percentage of nNOS positive fibres are reduced in all muscle types with age (229).

Mitochondrial production of nitric oxide by a mitochondrial isoform of nitric-oxide synthase (mtNOS) isolated from rat liver (230) and also present in muscle mitochondria (A Boveris, personal communication) seems to have local mitochondrial effects on Fe-S centers of metalloproteins (231), particularly cytochrome oxidase which is competitively inhibited by NO (223). The NO production rate is dependent on the metabolic state of mitochondria and their NADPH levels (223) one of their effects on oxidative phosphorylation being to increase free radical leakage from respiratory complexes (223), and also the final ATP synthesis rate. In spite of these primary effects and their influence on contractility, blood flow and glucose metabolism, NO decays by ubiquinol oxidation and peroxynitrite formation (221). The peroxynitrite production rate competes with superoxide dismutase for superoxide anion (224). Short-time range toxicity effects of peroxynitrite leads to release of creatine kinase, a marker of muscle injury, and this effect is more intense in old animals that show an increased susceptibility to NO toxicity (232). Both basic age-related production of mitochondrial derived superoxide and peroxynitrite constitute a primary event that could explain the widespread metabolic, signalling and even regulatory genetic changes described during skeletal muscle cell aging.

8. SKELETAL MUSCLE AGING MODULATION BY CALORIC RESTRICTION AND EXERCISE

In monkeys, preliminary data suggest that the physiological changes in response to caloric restriction are similar to those in rodents (233,234). Rubner (235) postulated an inverse relation between metabolic rate (usually expressed as oxygen consumption per unit of lean body mass) and life span. Some experimental studies confirmed this postulated relation, and these findings formed the basis of Pearl's “rate of living” theory of aging

(236). More recently, metabolic rate has been linked to the rate of production of partially reduced oxygen species, (237,238) which are normal byproducts of oxygen metabolism (175). Caloric restriction attenuates oxidative damage and the associated decline in mitochondrial function (238), with reduced steady-state concentrations of the products of oxidative damage to proteins, DNA, and lipids (185,239). In mice, most of the age-associated increase in oxidative damage to DNA occurs in postmitotic tissues such as skeletal muscle, with most of the attenuation of oxidative damage by caloric restriction also occurring in these tissues (239).

Growing evidence indicates that free radicals play an important role in exercise-related damage in skeletal muscle (211,240-247). However, there are fewer data on exercise-related free radical production in aged skeletal muscle, and the results are discrepant (59,208,209,248). It is important to consider that the data relating to free radical production and antioxidant defences in aged skeletal muscle after exercise, are dependent on the type, intensity and duration of exercise protocol (208). In general, however, such data seem to support the theory of an age threshold in exercise, as proposed by Reznick (249).

9. PERSPECTIVE

When considering the structural and functional changes that define muscle senescence phenotype, it is important to consider the chronological and hierarchical organization of events and processes that lead to the phenotypic modulation and reduced efficiency of senescent skeletal muscle cells. One approach is to extrapolate the consequences of the most basic and well known aging mechanisms common to all cells. Such a basic mechanism for the deterioration of long-lived somatic cells is the cumulative, non repaired damage resulting from free radical production which elicit adaptive responses of the antioxidant systems. The progressive imbalance leads to an internal cell state defined as oxidative stress. A first level of impact of this basic endogenous and cumulative mechanism of molecular damage is the mitochondrion itself, whose lipid peroxidation levels are increased, with presumed effects on membrane fluidity and functional properties. Effects on mitochondrial matrix metabolic pathways may diminish the reductive capacity, that in conjunction with the internal membrane alterations and selective inhibition of key points in oxidative phosphorylation chain leads to a reduced rate of ATP synthesis. The energetic crisis of skeletal muscle under the demands of exercise has a lower threshold, not only by the reduced efficacy of mitochondria, but also by the decreased number of recruited mitochondria in the active state 3. Mitochondrial DNA damage leads to a mosaicism in mitochondrial population in many tissues, and increases the risk of activation of programmed cell death by apoptosis. This is not the case in skeletal muscle cells where the multinucleate character does not permit the typical morphological changes of apoptosis of mononuclear cells, where the increased probability of entering apoptosis secondary to mitochondrial alterations is easily detected.

The proposed mechanism for age-associated changes in cellular metabolic pathways based on the direct effect of free radical species derived from mitochondrial superoxide, and perhaps other internal sources (that in skeletal muscle cells could include aconitase or xantine oxidase) during aging, or reduced antioxidant activities, in aged cells. This second level corresponds to a “metabolic stress” that the cell could meet through protein repair, protein degradation mechanisms or upregulating deficitary enzymes of metabolic pathways.

It would be useful to consider specially the possible effects of this basic aging process on myofibrillar or sarcomere proteins (or their turnover) and hence the sliding efficiency mechanism, over excitation-contraction coupling mechanisms, or even on the integration network of signalling pathways from the surface membrane in response to synaptic, paracrine or hormonal signals. Many of the stoichometric changes in the components of the signal-transduction and signal-integration systems could be considered adaptive responses from the gene regulation level, to maintain the functional effects of these in response to hormonal local signals, even if they were not altered in the aged organisms.

There are some data that indicate a direct effect on basic translational machinery at the ribosomal level, and it is suspected that there may be interference with the mixture of the pool of transcription factors that maintains the pattern of genetic expression for phenotype maintenance in skeletal muscle cells. Techniques that will permit monitoring of the global pattern of gene expression inside a single cell will confirm the above.

This scenario at the cell level can then be related to the effects on the ability of muscle cells to adequately transduce the signals that normally permit the hypertrophy response, which may be less altered in aged muscle than expected. At the tissue level, the senescent muscle cells could adopt an altered pattern of intercellular biochemical communication, by reduced signal production, or inadequate transduction or effector response, and their effect on tissue architectural changes could be explored.

Until such a basic and well established biochemical mechanism of aging at the cellular level as free radical induce cumulative damage, is fully explored in terms of direct molecular consequences *versus* the compensatory homeostatic intracellular mechanisms, it is not necessary to postulate other primary events which may underlie the hierarchy of cascade events accumulated in long-lived senescent cells

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Send correspondence to: Professor Dra. Ana Navarro, Departamento de Bioquímica y Biología Molecular. Facultad de Medicina, Universidad de Cádiz, Plaza nº 9, 11003-Cádiz, Spain, Tel: +34-956015244, Fax: +34-956015230, E-mail: ana.navarro@uca.es