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Review

17β-Estradiol and testosterone in sarcopenia: Role of satellite cells

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ABSTRACT

The loss of muscle mass and strength with aging, referred to as sarcopenia, is a prevalent condition among the elderly. Although the molecular mechanisms underlying sarcopenia are unclear, evidence suggests that an age-related acceleration of myocyte loss via apoptosis might be responsible for muscle perfomance decline. Interestingly, sarcopenia has been associated to a deficit of sex hormones which decrease upon aging. The skeletal muscle ability to repair and regenerate itself would not be possible without satellite cells, a subpopulation of cells that remain quiescent throughout life. They are activated in response to stress, enabling them to guide skeletal muscle regeneration. Thus, these cells could be a key factor to overcome sarcopenia. Of importance, satellite cells are 17β-estradiol (E2) and testosterone (T) targets. In this review, we summarize potential mechanisms through which these hormones regulate satellite cells activation during skeletal muscle regeneration in the elderly. The advance in its understanding will help to the development of potential therapeutic agents to alleviate and treat sarcopenia and other related myophaties.

Contents

1. Introduction

Aging is an inevitable biological process characterized by the progressive deterioration of numerous tissues and their physiological functions (Young, 1997). Specifically, with regards to skeletal muscle, senescence implies a progressive loss of its performance (Evans, 1995) affecting the daily movements and independence in the elderly (Rantanen et al., 2002; Sinha-Hikim et al., 2003). One of the most striking effects of aging on muscle is the gradual loss of skeletal muscle mass and its associated loss of strength, also referred to as sarcopenia (Cruz-Jentoft et al., 2010). It is really a prime component of frailty syndrome, affecting radically the functional capacity, mobility and general health in adult people resulting in a poor quality of life and increased mortality (Fielding et al., 2011; Marzetti and Leeuwenburgh, 2006). Although there are several diagnostic criteria for sarcopenia, the general consensus indicates the evaluation of the presence of low muscle mass along with low muscle function (Cruz-Jentoft et al., 2010; Fielding et al., 2011; Morley et al., 2011; Muscaritoli et al., 2010).

Similar to other age-related conditions, sarcopenia is characterized by a multifactorial etiology, in which neuronal (Vandervoort, 2002) and hormonal (Szulc et al., 2004) alterations, high levels of catabolic cytokines (Visser et al., 2002), nutritional disorders (Dreyer and Volpi, 2005) and decreased physical activity (Szulc et al., 2004) are the key causal factors responsible of this pathology. However, the specific contribution of each of these factors and the molecular mechanisms triggered by those conditions that ultimately lead to fiber loss, are still largely unknown.

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In humans, skeletal muscle is one of the most abundant tissues in the body. It is composed of bundles of fibers (muscle cells) named as fascicles. The cell membrane surrounding the muscle fiber is the sarcolemma, and under this membrane lies the sarcoplasm, containing proteins, organelles, and myofibrils: the actin and myosin filaments. The arrangement of actin (the thin filaments) and myosin (the thick filaments) gives skeletal muscle its striated appearance (review in Scott et al., 2001). In addition, skeletal muscle is an extremely heterogeneous tissue, composed of a large variety of fiber types (McComas, 1996; Pette and Staron, 1997) that are classified based on histochemical, biochemical, morphological and physiological characteristics. However, classifications of muscle fibers by different techniques do not always agree (Staron, 1997). The composition of myosin heavy chain isoforms in the fiber is the determinant of muscle fiber type. Type I fibers have a predominance of myosin heavy chain 1 (MHC1). They are slow-twitch fatigue resistant fibers with greater oxidative capacity, higher mitochondrial content, and a greater capillary density. While type IIA fibers have a predominance of myosin heavy chain 2a (MHC2a), in type IIB fibers prevail myosin heavy chain 2x (MHC2x). The type II fibers are fast-twitch fibers with a high glycolytic capacity. The type IIA ones have intermediate oxidative and glycolytic capacity and are more fatigue resistant, whereas type IIB and IIC are more glycolytic. The differences in the mitochondrial content between fiber types could be responsible of the degree of susceptibility to aged deterioration of each skeletal muscle fiber type.

Skeletal muscle cross-sectional area decreases with normal aging, and its fiber distribution shifts to a slower profile. Endurance decrease can be due to a reduced number of mitochondria and a subsequent reduction in mitochondrial enzymes (Essen-Gustavsson and Borges, 1986). In agreement, Lexell et al. (1988) clearly showed that both, types I and II muscle fibers, are lost with aging. Nevertheless, others indicate that the impact of age on muscle is fiber type specific (Grimby, 1995; Hortobagyi et al., 1995; Klitgaard et al., 1990; Mc Kiernan et al., 2012; Singh et al., 1999). Accordingly, it has been shown an increased proportion of type I muscle fibers at advanced age, implying the predominant loss of type II fibers (Dreyer et al., 2006; Larsson et al., 1978). However, the most reliable findings are the decline in the total number of muscle fibers and the specific atrophy of the type II fibers, both contributing to sarcopenia (Grimby, 1995; Lexell et al., 1988; Nilwik et al., 2013). Furthermore, fiber type grouping (Lexell et al., 1988) and decreased capillarization have been observed in aged muscle tissue (Frontera et al., 2000). The knowledge of how the proportion of each specific fiber type is regulated in muscle during aging could be considered the primary step for the development of effective strategies for preventing or treating sarcopenia.

The failure of the regeneration of sarcopenic muscle is a major cause of physical incapacitation in the elderly. In addition, there is now a large body of evidence that both sarcopenia and frailty are closely related to the decline of sex hormones that occurs with aging (review in Morley and Malmstrom, 2013). The ability of skeletal muscle tissue to respond to physiological demands and injuries depends on a small population of skeletal muscle stem cells, named as satellite cells (Allen et al., 1999; Hawke and Garry, 2001). For this reason, this review focuses on the potential impact that the estrogen- and testosterone-regulation of satellite cell function has in aged skeletal muscle.

2. Role of satellite cells in sarcopenia

2.1. Satellite cells

As it was mentioned, adult skeletal muscle increases its size and shows a remarkable capacity to adapt to trauma and injury. However, myonuclei in skeletal muscle are postmitotic and cannot replicate. Therefore, any increase in myonuclear number required for growth or repair of damaged muscle depends on satellite cells, a pool of myogenic precursor cells. This distinct population of mononucleated cells (Campion, 1984; Grounds et al., 2002) was first described by Mauro (1961). They owe their name to their localization under the basement membrane but outside the plasma membrane of the muscle fiber. Their colocalization with blood vessels (Christov et al., 2007; Ryall et al., 2008) places satellite cells in an optimal position to respond to intrinsic signals from both the skeletal muscle fiber itself and from changes in the systemic environment. Satellite cells are activated in response to both physiological stimuli, such as exercise, and to pathological conditions, such as injury and degenerative diseases. During development and regeneration, quiescent satellite cells are activated and start to proliferate. At this stage, they are often referred to as myogenic precursor cells or myoblasts (Charge and Rudnicki, 2004).

Although satellite cells are the residential stem cells of skeletal muscle and regeneration/reparation mainly depends upon them (Allen et al., 1999; Hawke and Garry, 2001; Moss and Leblond, 1971), other cell types outside the basal lamina such as the bone marrow (BM), also have myogenic potency (Boppart et al., 2013; LaBarge and Blau, 2002; Torrente et al., 2004). An alternative route suggested for BM-derived cell contribution to skeletal muscle is via direct fusion to muscle fibers in response to a physiological stimulus such as injury (Ferrari et al., 1998; Gussoni et al., 1999). The availability of cell-autonomous, tissue-specific transgenic markers allowed the unequivocal demonstration of the existence of myogenic progenitors originating from tissues other than skeletal muscle (Cossu, 1997). Some examples of these unorthodox myogenic cells are mentioned here.

Neural stem cells are the only ectoderm-derived stem cells that have been shown to differentiate into skeletal muscle when cocultured with skeletal myoblasts or transplanted into regenerating skeletal muscle (Galli et al., 2000). Interestingly, cells expressing the myogenic regulatory factors Myf5 exist in the brain and spinal cord, suggesting a myogenic potency that becomes apparent in vitro (Tajbakhsh et al., 1994).

The CD45+ fraction of the BM has also been identified as a cell population with myogenic potential (McKinney-Freeman et al., 2002). Some approaches confirmed that hematopoietic cells have myogenic potential but disagreed on the stage at which myogenic differentiation would occur. Also it has been reported that the progeny of a single mouse hematopoietic progenitor cell can reconstitute the hematopoietic system and contribute, at low frequency, to muscle regeneration (Corbel et al., 2003), while others showed that in response to injury, CD45+ hematopoietic progenitors contribute to regenerating mouse skeletal muscle through fusion of mature myeloid cells rather than fusion of the hematopoietic stem cells (HSCs) (Camargo et al., 2003). A subpopulation of circulating cells expressing CD133 (a well-characterized marker of HSCs) also expresses early myogenic markers (Torrente et al., 2004). When injected into the circulation of dystrophic *scid/mdx* mice, CD133+ cells contribute to muscle repair, recovery of force, and replenishment of the satellite cell pool. Different subpopulations of hematopoietic cells, whose characterization is still incomplete, seem to have myogenic potency. Given the proximity of the satellite cells and fibers to blood vessels, HSCs could be a promising option in muscle repair. Unfortunately, none of these cells exhibit this property at high frequency. Other cells than hematopoietic, derived from mesoderm, such as mesenchymal stem cells (MSCs), multipotent adult progenitor cells (MAPCs), mesoangioblasts (MABs), endothelial progenitor cells (EPCs), and adipose-derived stem cells have also been shown to exhibit myogenic potential (Cossu and Sampaolesi, 2007; Péault et al., 2007). Human multipotent adipose-derived stem (hMADS) cells isolated from adipose tissue, that

differentiate into adipocytes, osteoblasts, and myoblasts (Meliga et al., 2007), have enhanced their myogenic and muscle repair capacities by transient expression of MyoD (Goudenege et al., 2009). The easy availability of their tissue source, their strong capacity for expansion and their multipotent differentiation, suggest that hMADS cells could be an important tool for cell-mediated therapy for skeletal muscle disorders.

Moreover, by the use of molecular techniques, we could increase the potential of these stem cells, making them an additional possibility for muscle repair when satellite cells fail or when, by physiological or pathological reasons, they are a minority. However until now, only satellite cells, and, to a very minor extent, CD133+ cells have been used in human clinical trials (Tedesco et al., 2010). Limitations for other cell types are the incomplete characterization and their overall minor myogenic potency. Thus, by studying more about satellite cells and other skeletal muscle progenitors, we can improve the use of them in clinical applications. In this review, we will focus mainly in satellite cells.

Satellite cell number is not constant throughout life and depends on age and muscle fiber type. These cells are most abundant during early development contributing to muscle growth, and decline in number thereafter. For example, in rats it has been shown that the absolute number of satellite cells was reduced by 22% in older animals compared with their younger counterparts (Nnodim, 2000). In humans, the number of satellite cells also decreases in the elderly (Kadi et al., 2004). Additionally, it has been shown that differences in satellite cell distribution between muscle groups are the result of the heterogeneity in satellite cell content between muscle fiber types (Hawke and Garry, 2001). Type I or slow-twitch oxidative fibers tend to have a five to six times greater satellite cell content than Type II (fast-twitch fibers), as a consequence of an increased blood and capillary supply (Hawke and Garry, 2001). This may be due to the fact that Type I muscle fibers are employed in a greater frequency, and thus, more satellite cells may be required for ongoing minor injuries to muscle. Type II muscle fiber atrophy in the elderly is accompanied by a type II muscle fiber specific decline in satellite cell content (Verney et al., 2008). Satellite cells are a heterogeneous population in terms of cell size and clonogenic potential (Zammit and Beauchamp, 2001). Indeed, several studies have raised the possibility that satellite cells are a heterogeneous mixture of stem cells and committed myogenic progenitors (Moss and Leblond, 1971; Schultz, 1996). It has been shown that 80% of satellite cells divide rapidly and are responsible mainly for providing myonuclei to growing fibers in rat skeletal muscle. The remaining 20% of the cells enter into the G0 phase regarding as reserve cells, thus dividing more slowly (Schultz, 1996). Of relevance, the 80% of satellite cells express the CD34, Myf5 and M-cadherin markers while the other 20% of cells do not express these genes (Beauchamp et al., 2000). Really, satellite cells express specific markers during the different stages of muscle regeneration (Table 1) (review in Carosio et al., 2011). Thus, although this evidence shows distinct subpopulations of satellite cells, it is not clear the specific role of each of them. The characterization of the molecular mechanisms that govern satellite cells death may clarify their role on muscle homeostasis and regeneration, and define whether pathological conditions differently affect the behavior of distinct satellite cell populations. Moreover, this information could be useful for the implement of successful therapies using satellite cells.

As it has been mentioned, in adult skeletal muscle, satellite cells or skeletal muscle stem cells, normally reside in a quiescent state. However, in response to a stimulus, they are activated, enter to the cell cycle, proliferate and migrate to the site of injury (Filippin et al., 2011; Hawke and Garry, 2001; Morgan and Partridge, 2003).

Table 1
Satellite cells express specific markers during the different stages of muscle regeneration. ■ Regulated by E2, □ Regulated by T.

Satellite cells stages	Quiescent	Proliferating	Differentiating
Markers	C-Met M-cadherin FoxK1 Pax-7 □ CD34 NCAM Syndecam3/4 VCAM-1 Pax-3 Caveolin-1	FoxK1 Pax-7 ■ CD34 Myf-5 MyoD ■ Desmin	Atp2a2 Tnnt1 Igf2 ■ Chrna1 Fgfr4 Myogenin □ ■ Tncc

Source: review in Carosio et al. (2011).

Depending on the severity of the trauma, they either differentiate to form new muscle to repair damaged myofibers and/or to increase hypertrophy of the muscle fibers (Yamada et al., 2008), or return to quiescence replenishing the resident satellite cell pool through self-renewal (Dhawan and Rando, 2005; Zammit et al., 2004). Despite there is abundant evidence indicating the role of these cells in hypertrophic responses, there are controversies in regards to this, since it has been shown in a mouse strain (Pax7-DTA) with ablation of >90% of satellite cells that muscle fibers are capable of mounting a robust hypertrophic response to mechanical overload (McCarthy et al., 2011). In view of this interesting observation, exercise protocols designed to promote muscle growth could be particularly important for populations, such as the elderly, in which satellite cell activity is compromised.

Possibly, the decision of satellite cells to repair muscle or to return to quiescence, could be regulated by their specific microenvironment or niche. The niche is a unique combination of cellular, biophysical and biochemical components that maintain satellite cells in a quiescent state until they are activated. It is enriched in proteoglycans that bind and sequester growth factors. In addition, a great diversity of stimulatory and inhibitory growth factors such as IGF-1 and members of the transforming growth factor β (TGF-β) pathway regulate satellite cells activity (Bhatnagar et al., 2010; Broek et al., 2010; Carlson et al., 2008). Other factors that have received increasing attention as they can influence satellite cells behavior and skeletal muscle mass/strength are myostatin, another member of the transforming growth factor β family, and follistatin, a secreted glycoprotein, which antagonises myostatin (Cassano et al., 2009; Lee and McPherron, 2001; Matzuk et al., 1995; McPherron et al., 1997).

Of relevance, it has been shown that during skeletal muscle regeneration, canonical Wnt/β-catenin signalling is activated, which induces follistatin and myogenin expression in myoblasts, promoting their fusion and myogenic differentiation to repair the injured muscle (Jones et al., 2015). Follistatin, a secreted glycoprotein that antagonizes members of the TGF-β superfamily (Hemmati-Brivanlou et al., 1994), has received special attention since it exerts an important function in the regulation of skeletal muscle mass. Indeed, transgenic mice overexpressing follistatin have an increment of their muscle mass (Lee and McPherron, 2001), while knockout mice for this glycoprotein have skeletal abnormalities and an impaired muscle development that provoke their death (Matzuk et al., 1995). Moreover, it has been proven that the regenerative potential of satellite cells is impaired in the old muscle as a result of a decrease in Notch activation and the production of excessive TGF-β, that leads to an increase of p-SMAD3 in satellite cells, impairing its regenerative capacity (Carlson et al., 2009; Conboy et al., 2003). In accordance with this, it has been observed that Wnt/β-catenin signaling can antagonize the effects of Notch activation, promoting the myogenic differen-

tiation. However, there exist contradictory roles for the canonical Wnt/β-catenin pathway during adult skeletal muscle regeneration. Others have reported that the activation of this signaling induces the proliferation and self-renewal of satellite cells that in turn prevent myogenic differentiation to form new muscle (Otto et al., 2008).

Thus, satellite cells are affected by a variety of factors among which the influence of mechanical stimulation, aging and hormones are of special interest (Dhawan and Rando, 2005; Scime and Rudnicki, 2004; Wozniak et al., 2005). Given the important role of satellite cells in the regulation of skeletal muscle mass, those factors might be involved in the mechanisms underlying muscle loss. Although the interest in satellite cells related to sarcopenia has increased rapidly, it is difficult to determine if the overall number of satellite cells in aged muscle is a causal factor of a reduced repair, since there are confounding proofs respect to the diminution in satellite cell number with age. However, there is a preponderance of evidence supporting the concept of decreased satellite cell number during aging. Carlson et al. (2009) reported a decline in satellite cells in resting aged muscle relative to young human muscle using Pax 7 (a paired-box transcription factor which is specifically expressed in quiescent and newly activated satellite cells), neural cell adhesion marker (NCAM) and M-cadherin satellite cell markers. In a mouse model, it has been shown a 60% decrease in Pax 7 positive cells in aged respect to young extensor digitorum longus muscle (Shefer et al., 2006). An explanation for this perceived decrease of resident satellite cell number with age is that these cells may have reached the maximum threshold of cell divisions. Moreover, Fulle et al. (2013) showed an age-related susceptibility of human satellite cells to spontaneous apoptosis, being the aged satellite cells more prone to apoptosis compared to the young satellite cells. Nevertheless, observations of our group demonstrated resistance to apoptosis in aged C2C12 cells; probably due to the loss of their mitochondrial genetic material (Pronsato et al., 2013). However, by immunohistochemistry assays and techniques for quantifying satellite cells, Brooks et al. did not observe any significant difference in satellite cell number between young (5 mo) and aged (24 mo) rat muscles (Brooks et al., 2009). In agreement, it has been postulated that satellite cell number does not reflect a change in the regenerative ability of the muscle. In addition to the reports stating no decrease in satellite cell number with age, others indicate an increase in the number of resident satellite cells in aged skeletal muscle (Beccafico et al., 2007; Brooks et al., 2009; Carlson et al., 2009; Conboy et al., 2003; Conboy and Rando, 2005; Day et al., 2010; Dreyer et al., 2006; Nnodim, 2000; Shefer et al., 2006). As mentioned above, in accordance with this last suggestion, evidence from in vitro assays shows that aged cultures of murine myoblasts are resistant to death by apoptosis, probably due to the loss of mitochondrial DNA (mtDNA) by high passage numbers (Pronsato et al., 2013). The mechanism responsible for the loss of genetic material from the organelle could be through the secretion of extracellular vesicles such as exosomes (<100 nm) and microvesicles, ectosomes, or shed vesicles (>100 nm) (Witwer et al., 2013). There is plenty of quality evidence about extracellular vesicles indicating that they are secreted by every cell type that has been examined, including tumor cells, reticulocytes, epithelial cells, neurons, muscle cells and Schwann cells (Guescini et al., 2010; Lopez-Verrilli and Court, 2012; Smalheiser, 2007), and that they are found in most biological fluids (Simpson et al., 2008). Remarkable, these vesicles contain proteins involved in signal transduction. Thus, they could represent a novel mechanism for long distance intercellular signalling. Experimental data showed that C2C12 myoblast cells release exosomes containing some functionally relevant proteins and mtDNA (Guescini et al., 2010). The C2C12 cell line are murine myoblasts derived from satellite cells, whose behavior corresponds to that of progenitor lineage. This cell line is a subclone of C2 myoblasts (Yaffe and Saxel, 1977) which spontaneously proliferate, differentiate and synthesize characteristic muscle proteins in culture (Blau et al., 1983; Patz et al., 2005). Since C2C12 cells are comparable to satellite cells in muscle fibers (Yoshida et al., 1998), they represent an appropriate experimental model of them.

Evidence discussed here suggests that the cause of the poor skeletal muscle functionality during aging is mainly intrinsic to satellite cells, given little importance to the influence of their microenvironment. However, the loss of muscle mass and strength in the elderly possibly reflects changes of the microenvironment, which affect the proper activation and differentiation of satellite cells (Charge et al., 2002; Renault et al., 2000; Seale et al., 2001). In accordance, it was observed that growth factors and cytokines of the connective tissue environment of satellite cells could be involved in the decrease of its ability to repair and regenerate the aged muscle (review in Hikida, 2011). Interestingly, the mammary stem cell niche responds readily to hormonal stimuli (Joshi et al., 2012). Moreover, novel findings point to a critical role of testosterone in restoring the systemic environment that favors muscle growth in aging (Sinha et al., 2014). Then, the identification of the testosterone-regulated rejuvenating factors in the aged niche may reveal novel therapeutic targets for regenerative medicine approaches. The concept that stem cells are controlled by their own niches has been widely invoked. However, niches have remained largely a theoretical construct because of the difficulty to identify and manipulate individual stem cells and their surroundings. Scientific advances now make it possible to characterize small zones that maintain and control stem cell activity in several tissues (Spradling et al., 2001). These studies are beginning to clarify the understanding of stem cell regulation, and promise to advance efforts to use myoblastic cells therapeutically.

2.2. Role of 17β-estradiol and testosterone on satellite cells

As it was described in Section 1, satellite cells are the myogenic precursors of postnatal skeletal muscle, which in response to myofiber injury and hypertrophic signals, they provide the necessary precursors for skeletal muscle growth and repair (Seale et al., 2001). The understanding of the influence of estrogens and androgens on satellite cells has significantly grown over the last decade. Of relevance, it has been shown that there are gender differences in those responses (Amelink and Bar, 1986; Amelink et al., 1990; Bar et al., 1995; Bar and Amelink, 1997; Reisz-Porszasz et al., 2003), suggesting that satellite cells could be regulated by sex steroids. In fact, several muscle genes detailed in Table 1 are affected by these hormones; e.g., Pax-7, MyoD, myogenin, among others (Braga et al., 2012; Cleveland and Weber, 2015; McFarland et al., 2013; Rana et al., 2014).

During the inflammatory response after muscle-damaging exercise (Tiidus et al., 2001) or ischemia/reperfusion injury (Stupka and Tiidus, 2001), chemical factors released from leukocytes, in particular macrophages, activate and induce proliferation of muscle satellite cells, a process that is critical to muscle repair (Hawke and Garry, 2001). Of interest, it was proven that 17β-estradiol (E2) attenuates this inflammatory response. The post-damage muscle inflammatory response can also be responsible for further damage to healthy tissue (Pizza, 2008; Tiidus, 1998), thus, its limitation may reduce further post-injury deterioration without attenuating the repair. To counteract these injuries, E2 decreases the secretion of proinflammatory cytokines released by leukocytes and inhibits the production of the inflammatory mediator nitric oxide (NO) elicited by NO synthase activity (Viña et al., 2013). However, this reduction in the inflammatory response mediated by E2 could be related to an effort of the hormone to counteract the oxidative stress established after ischemia/reperfusion injury. In this regard, it has been shown that changes in the expression of particular microRNAs (miRs), such

as the muscle specific miR-1, occur in response of oxidative stress. Indeed, miR-1 was downregulated by H_2O_2 in C2C12 cells (Magenta et al., 2011), suggesting that E2 can modulate its expression to limit the tissue injury. Additionally, E2 can upregulate antioxidant genes to prevent oxidative stress (La Colla et al., 2013; Viña et al., 2013). Despite its negative effect on post-damage inflammatory response, evidence has been provided supporting that estrogen may promote activation and proliferation of satellite cells via mechanisms involving estrogen receptor α and β (ER α/β) (Thomas et al., 2010; Velders et al., 2012) that in turn, promotes muscle repair. Others reported that the blocking of estrogen receptors eliminates post-exercise muscle satellite cell activation (Enns et al., 2008). Moreover, it has been shown that ovariectomised (OVX) rats failed to fully recover skeletal muscle mass after a period of hind limb unloading followed by a subsequent period of reloading, compared to OVX rats being administered with E2 (McClung et al., 2006; Sitnick et al., 2006; Sugiura et al., 2006), indicating that E2 attenuates the rate of disuse atrophy. Additionally, estrogen supplementation to OVX rats prior to exercise improves satellite cell number and performance, playing the ERα a key role (Thomas et al., 2010).

Likewise, there have also been described effects of androgens on satellite cells (Sinha-Hikim et al., 2002). Testosterone induces a dose dependent increase in satellite cell number, myofiber cross-sectional area and myofiber nuclei in young and old men (Kadi 2008; Sinha-Hikim et al., 2003, 2006) promoting thus, muscle fiber hypertrophy. Also, T supplementation conduces to higher mitochondrial areas and lower nuclear-to-cytoplasmic ratio in satellite cells. Therefore, this supplementation in muscle fibers causes hypertrophy associated with an increase in satellite cell number, a proportionate increase in myonuclear number and changes in satellite cell ultrastructure. In addition, androgens have long been regarded as having regulatory effects on muscle size and strength. They have been used to increase muscle bulk in hypogonadal (Bhasin et al., 1997), HIV-infected (Bhasin et al., 2000) and healthy men (Bhasin et al., 1996, 2001).

With respect to the influence of myostatin over satellite cells and its relationship with E2 and T, there are controversies. As mentioned before, myostatin is a member of the TGF-β superfamily that is expressed in skeletal muscle and believed to suppress muscle growth. Some studies reported that myostatin inhibition positively regulates satellite cells activation which leads to a faster regeneration of the injured muscle, whilst others supported that these conclusions were erroneous as satellite cells were not involved in muscle hypertrophy caused by myostatin blockade (Cassano et al., 2009). Whether the age-related decline in steroid hormones leads to a progressive augment in myostatin expression that can contribute to sarcopenia is not completely understood. However, it has been shown that the overexpression of myostatin mRNA and protein in skeletal muscle resulted in a significant decrease of muscle mass and muscle fiber size of male but not female mice, showing a gender-dependent effect of its expression (Reisz-Porszasz et al., 2003). Androgens might regulate myostatin expression also indirectly. For example, the inhibitor of myostatin, follistatin, is upregulated after T treatment in satellite cells, which in turn inhibits various members of TGF-β signalling pathway, contributing to the hypertrophic effect of this steroid (Braga et al., 2012).

In agreement with these observations it has been demonstrated that satellite cells express the androgen receptor (AR) (Doumit et al., 1996; Sinha-Hikim et al., 2004) as well as the estrogen receptors (ERs) (Kalbe et al., 2007). In the C2C12 skeletal myoblast cell line, biochemical, immunological and molecular data support the mitochondrial-microsomal localization of ERα. Specific and saturable [^{3}H]-E2 binding sites of high affinity were detected in mitochondrial fractions of these cells in culture (Milanesi et al., 2008). Immunocytological, Western and ligand blot studies corroborated this non-classical localization of estrogen receptors, results that were confirmed using a fluorescent 17β-estradiol-BSA conjugate (Milanesi et al., 2008).

Expression and subcellular distribution of ERα proteins were confirmed in C2C12 cells transfected with ERα siRNA and by RT-PCR employing specific primers (Milanesi et al., 2008). In another set of experiments, ERβ was detected mainly in mitochondria and in lower amounts in the cytosolic fraction by immunoblotting using specific antibodies and ligand blot analysis after subcellular fractionation, results that were confirmed using conventional and confocal microscopy, and corroborated after transient transfection with specific ERβ siRNAs (Milanesi et al., 2009). The expression of ERβ was also demonstrated by RT-PCR. These results show that ERβ localizes differently to ERα in C2C12 skeletal myoblast cells (Milanesi et al., 2009; review in Vasconsuelo et al., 2011).

AR was characterized in several cell types and tissues, including skeletal muscle and satellite cells (Alexaki et al., 2006; Benten et al., 1999; Doumit et al., 1996; Gatson et al., 2006; Gu et al., 2009; Guo and Qiu, 2011; Hatzoglou et al., 2005; Kallergi et al., 2007; Kampa et al., 2002; Krieg, 1976; Michel and Baulieu, 1980; Papadopoulou et al., 2008; Papadopoulou et al., 2009; Saartok et al., 1984; Sinha-Hikim et al., 2004; Solakidi et al., 2005; Villavicencio et al., 2006; Wang et al., 2008; Watson et al., 2010). In the C2C12 cell line, biochemical and immunological data demonstrated mitochondrial and microsomal localization of AR (Pronsato et al., 2013). As a first approach, AR was detected by immunoblotting, using specific antibodies after subcellular fractionation, not only in nucleus and cytosol, but also in mitochondria and microsomes (Pronsato et al., 2013). When [^{3}H] testosterone binding characteristics in total homogenates and subcellular fractions were investigated, specific and saturable binding sites were detected in mitochondria and microsomes. Immunolocalization of the non-classical AR was also confirmed using confocal microscopy. Sucrose gradient fractionation demonstrated the presence of the AR in lipid rafts and caveolae in these cells (Pronsato et al., 2013). Besides, the AR was able to interact physically with caveolin-1, association that is lost after testosterone treatment. Accordingly, Western blot analysis revealed a decrease of AR expression in the microsomal fraction after testosterone treatment, suggesting translocation of the membrane AR to another subcellular compartment. This non-classical distribution of native pools of AR in skeletal muscle cells suggests an alternative mode of AR localization/function (Pronsato et al., 2013).

Satellite cell cultures from human skeletal muscle were also tested for AR expression (Sinha-Hikim et al., 2004). AR protein was expressed predominantly in satellite cells, identified by their location outside sarcolemma and inside basal lamina. Many myonuclei in muscle fibers also demonstrated AR immunostaining. Also, stem cells in the interstitium, fibroblasts, and mast cells showed AR immunoreactivity, as well as in vascular endothelial and smooth muscle cells (Sinha-Hikim et al., 2004). Immunoelectron microscopy revealed aggregation of immunogold particles in nucleoli of satellite cells and myonuclei; testosterone treatment increased nucleolar AR density. AR mRNA and protein expression in satellite cell cultures was confirmed by RT-PCR with sequencing of products, real-time PCR, and Western blot analysis. Incubation of satellite cell cultures with supraphysiological testosterone and dihydrotestosterone concentrations modestly increased AR protein levels. So, in this work, it was shown that even though the AR is expressed in several cell types in human skeletal muscle, including satellite cells, fibroblasts, vascular endothelial, smooth muscle cells, and mast cells, satellite cells are the predominant site of AR expression. These observations could support the hypothesis that androgens increase muscle mass in part, by acting on several cell types to regulate the differentiation of satellite cells in the skeletal muscle (Sinha-Hikim et al., 2004). In addition, AR was classically localized in the nucleus of satellite porcine cells and myotubes. Testosterone treatment increased AR levels not only in

satellite cells, but also in myotubes and muscle derived fibroblasts. It was also observed, after testosterone treatment, a reduced differentiation of satellite cells in vitro. These data provide further information demonstrating that satellite cells are targets for androgen action (Doumit et al., 1996).

Since the non-classical localization of ERs and AR in mitochondria of satellite cells has been demonstrated, investigations in the last years have been focused on the evaluation of the role of E2, T and their receptors on mitochondria. Due to the close relation of this organelle with apoptosis, arises the concept of the regulation of programmed cell death and thus of sarcopenia, by these steroids (review in Vasconsuelo et al., 2011). However, the underlying molecular mechanisms by which androgen and estrogen regulate satellite cells physiology are still not clear.

Alternatively, although in sarcopenia the ubiquitin-proteasome system (UPS) seems not to be the major pathway responsible for muscle loss, several authors described an up-regulation of components of the UPS in the muscle wasting (Clavel et al., 2006; Ogawa et al., 2011; Raue et al., 2007), whereas others found a down-regulation or no changes (Bossola et al., 2008; DeRuisseau et al., 2005). Of interest, it has been shown that the UPS is also suitable for estrogen regulation, through a mechanism that involves MAPK-ERK1/2-dependent phosphorylation of nuclear p27 on T187 (Huang et al., 2012). Moreover, it has been shown that testosterone maintains muscle mass by repressing atrogin-1 and Murf-1 ubiquitin ligases in rat skeletal muscle, indicating that inhibition of ubiquitin-proteasome catabolic system is critical for trophic action of androgens in this tissue (Pires-Oliveira et al., 2010).

2.3. Protective effects of 17β-estradiol and testosterone on satellite cells

The implications of the estrogenic and androgenic effects on satellite cells are particularly important for aged individuals, where circulating estrogen and testosterone levels decline and in consequence, their positive influences on skeletal muscle may diminish. The best studied signaling molecules involved in the regulation of satellite cells function and the effects of E2 and T over them are summarized in Table 2. Although the molecular mechanisms responsible of sarcopenia have not been completely clarified, oxidative stress, mitochondrial dysfunction, hormonal deregulation and apoptosis have shown to play important roles in age-dependent muscle atrophy (Hiona et al., 2010; Meng and Yu, 2010; Siu et al., 2008 review in Vasconsuelo et al., 2013). Oxidative damage in DNA, lipids, and proteins of aged human muscle, may contribute to age-dependent losses of muscle strength and resistance (review in Vasconsuelo et al., 2013). The measurement of markers of oxidative damage to DNA, lipids and proteins, some antioxidant enzyme activities as well Ca^{2+} transport in sarcoplasmic reticulum membranes subjected in muscle biopsies from vastus lateralis of young and elderly healthy subjects of both sexes, showed the presence of age- and sex-related differences (Fanò et al., 2001; Mecocci et al., 1999). A significant increase in oxidation of DNA and lipids in the elderly and a reduction in catalase and glutathione transferase activities were detected, more evident in males. An abnormal functional response of aged muscle after exposure to caffeine, which increases the opening of Ca^{2+} channels, as well as a reduced activity of the Ca^{2+} pump were detected in elderly males (Fanò et al., 2001; Mecocci et al., 1999). Thus, it is possible to assign to oxidative stress a significant role in muscle aging, and because of the fact that oxidative damage is much more evident in elderly males, a gender difference, probably related to hormonal factors, is suggested. A wealth of experimental data indicates that apoptosis is activated in the aged skeletal muscle, likely contributing to the pathogenesis of sarcopenia (Marzetti et al., 2008). However, it is difficult to determine a single causal mechanism, since the listed factors do not act independently, but often closely related. Moreover, it is known that there exists a relation between aging, mitochondrial dysfunction and apoptosis (review in Vasconsuelo et al., 2013). It has been demonstrated that deleterious changes in the mitochondria of human skeletal muscle begin at 40–50 years of age (Pesce et al., 2001) matching with changes in the sex hormonal state of individuals (Lamberts et al., 1997), suggesting a relationship between sex hormone levels and mitochondrial status. In accordance with these observations, it has been revealed that human skeletal muscle contains both estrogen (ERs) and androgen receptors (AR) (Kadi et al., 2000; Lemoine et al., 2003; Wiik et al., 2005). Remarkable, as it was mentioned before, both receptors are expressed in satellite cells (Doumit et al., 1996; Kalbe et al., 2007; Sinha-Hikim et al., 2004), suggesting that both steroids can act directly on AR and ERs—expressing satellite cells. Although little is known about the effects of estrogen or androgen on the molecular mechanism of apoptosis in skeletal muscle, the available evidence indicates that both steroids are associated with survival/beneficial actions in this tissue (Bhasin 2003; Deasy et al., 2007). Experimental data indicate that androgens and estrogens regulate apoptosis via different cell signaling pathways, depending on cell type, apoptosis inducer, hormone concentration and cellular environment (review in Vasconsuelo et al., 2011).

In the C2C12 murine skeletal muscle cell line, T and E2 protect against H_2O_2-induced apoptosis (Pronsato et al., 2010; Vasconsuelo et al., 2008). This response of C2C12 cells exposed to the apoptotic inductor hydrogen peroxide (H_2O_2) in presence of E2 or T suggest that the decline in sex hormones make these cells more susceptible to oxidative stress.

Typical changes of apoptosis such as nuclear fragmentation, cytoskeleton disorganization, mitochondrial reorganization/dysfunction and cytochrome c release induced by H_2O_2 are abolished when C2C12 cells or primary culture of mouse skeletal muscle cells, are previously exposed to androgen or estrogen (review in Vasconsuelo et al., 2011). A number of molecular events that occur during the antiapoptotic action of T or E2 on those experimental systems have also been identified. For instance, at short times of exposure to H_2O_2, cells exhibit a defense response showing ERK2, Akt and Bad phosphorylation and an increase of HSP70 levels. However, at longer treatment times with the apoptotic agent, dephosphorylation of these proteins, loss of the mitochondrial membrane potential, cytochrome c release, PARP cleavage and DNA fragmentation occur. When cells are treated with T prior to H_2O_2, Bad inactivation, increase in actin levels, translocation of HSP90 to mitochondria, prevention of the loss of the mitochondrial membrane potential, and a reduction in PARP cleavage and Bax levels, are observed (Pronsato et al., 2012; Pronsato, 2014). This protective action of the male hormone involves the AR (Pronsato et al., 2013), indicating a specific survival action of the steroid which results in the activation of different cellular signaling pathways. These findings reveal that at least the intrinsic pathway, is regulated by testosterone. Likewise, E2 inhibits apoptosis in murine myoblasts through ERs involving MAPKs, and the survival PI3K/Akt pathway which phosphorylates proapoptotic members of the Bcl-2 family inactivating them. Comparable to T, in the protective effects of estrogen on mitochondria is implicated the PI3K/Akt pathway. But unlike T, E2 antiapoptotic action involves HSP27 and not HSP90 (review in Vasconsuelo et al., 2011), indicating specificity of signalling targets for each hormone in myoblasts. Probably these differences in the chaperones involved are driven by specific domains in estrogen or androgen receptors. In view of that, these HSPs are able to interact with ERs or AR stabilizating them (Reebye et al., 2012; Zoubeidi et al., 2007).

Table 2
Effects of E2 and T on various regulatory factors involved in satellite cell function.

Signal/molecule	Downstream target	Function on satellite cell	Effect of hormone
Notch ligand	Notch signaling	Maintaining of SC number and prevention of SC premature differentiation	T: ↑[a]
Wnt	Inhibition of Notch signaling; induction of Fst and myogenin expression	Regulation of SC fate	T: ↓[b]
Myostatin	Inhibition of MyoD and Pax7 expression	Inhibition of SC activation and self-renewal	T: ↓[c]
Follistatin	Inhibition of myostatin expression and TGF-β induced SMAD 2/3 phosphorylation	Promotion of SC activation	T: ↑[b]
Oxidative stress	Downregulation of antioxidant genes and upregulation of apoptotic pathways	Reduction of SC number	E2: ↓[d,e] T: ↓[f]

↑: Positive regulation of the signal/molecule by the hormone, ↓: negative regulation of the signal/molecule by the hormone.
[a] Sinha-Hikim et al. (2006).
[b] Braga et al. (2012).
[c] Mendler et al. (2007).
[d] Viña et al. (2013).
[e] La Colla et al. (2013).
[f] Pronsato et al. (2013).

Since both ER subtypes have been shown to be expressed in murine and human satellite cells (Matthews and Gustafsson, 2003; Milanesi et al., 2008; Wiik et al., 2003, 2005), it is important to evaluate the contribution of each ER in muscle regeneration. As regards to this, it has been shown that in ERβ knockout mice, the absence of this ER leads to a diminished Pax7 mRNA expression in the injured muscle (Velders et al., 2012). The importance of this paired-box transcription factor, which is expressed in quiescent as well as in activated satellite cells, in muscle regeneration was established in Pax7 knockout mice, which do not have satellite cells and in which exist an impaired capacity of muscle regeneration (Kuang et al., 2006). On the contrary, this reduced regeneration after injury, associated to Pax7 expression, was not evidenced in wild type and ERα knockout mice (Velders et al., 2012). Additionally, ERβ was involved in the expression of MyoD, a marker of satellite cell activation, and embryonic myosin heavy chain (MHC), a marker of de novo myofiber regeneration, which have been shown to be induced by E2 (Velders et al., 2012). Indeed, their mRNA expressions were significantly reduced in ERβ knockout mice in comparison to wild type and ERα knockout mice. The use of ERβ agonists after injury provides further evidence to support the notion that this isoform is involved in muscle regeneration via satellite cells activation (Velders et al., 2012). On the other hand, it has also been reported that E2 can inhibit myogenesis as a result of an increased ubiquitin-specific peptidase 19 (USP19) expression through ERα (Ogawa et al., 2011). Along with the augmented USP19 expression, E2 decreased the levels of MHC and tropomyosin, which are markers of differentiated muscle. These results appear to contradict the above reported E2 protective effects. This discrepancy can be assigned to the opposite roles that ERs play during skeletal muscle formation or to the different experimental conditions employed in both studies. Indeed, the capacity of muscle regeneration via satellite cells activation was evaluated after an injury in Velders et al. (2012) while this was not done in Ogawa et al. (2011). Additionally, it has been shown that ERβ inhibits ERα regulated gene transcription, reducing USP19 expression and repressing the negative effect of ERα action over myogenesis. It could be hypothesized that the balance between the expressions of both isoforms regulates USP19 expression that in turns influences myogenesis and muscle regeneration. These data reinforce the hypothesis that sex hormones play an important role in regulating muscle physiology.

Among the protective actions of both steroids at mitochondrial level, it has been demonstrated an increased mitochondrial manganese superoxide dismutase protein expression and activity, and protection of mitochondrial membrane potential abrogating the mitochondrial permeability transitory pore (MPTP) opening induced by H_2O_2 (La Colla et al., 2013; Pronsato et al., 2013). In accordance with these events, the steroids inhibit the translocation of Bax to mitochondria, induced by hydrogen peroxide (La Colla et al., 2013; Pronsato, 2014). The defensive effects of androgens apparently are specific of skeletal muscle cells since in cardiomyocytes, testosterone therapy has no protective effect in acute muscle injury associated with increased muscle cell death after cardiotoxin treatment (Sinha-Hikim et al., 2007).

These data are relevant since, as it was mentioned above, growing evidence indicates that an age-related deregulation of apoptosis may contribute to the onset and progression of sarcopenia due to an increase in the rate of cellular death. Indeed, an accelerated death of satellite cells in aged muscle could conduce to an impaired muscle regenerative process in the elderly. In addition, the data suggest that altered sex hormone levels could affect the normal response of the cells that maintain and repair skeletal muscle during sarcopenia or other deleterious effects of aging. The fact that the results described above were performed in satellite cells treated with hydrogen peroxide, strengthen this concept. Indeed, aging can be induced prematurely by oxidative stress, through the accumulation of reactive oxygen species (ROS) (Renault et al., 2002). ROS, such as hydrogen peroxide, may attack several types of tissue, including skeletal muscle. This tissue is prone to oxidative stress-induced aging as the myofibers are highly oxygen-consuming structures, and the level of ROS produced in skeletal muscle is higher than in other tissues (Renault et al., 2002; Kayo et al., 2001). Certainly, aging is associated with excessive ROS levels, which increase mitochondrial damage (review in Vasconsuelo et al., 2013). Mitochondria might produce higher ROS levels in the aged muscle, inactivating satellite cells and then, contributing to the impairment of its restorative function. Thus, C2C12 cells exposed to H_2O_2 resemble an aging-like phenotype similar to aged skeletal muscle (Lim et al., 2013) and represent a useful tool to understand the molecular mechanisms implicated in sarcopenia. This knowledge will likely help to the design of more effective therapeutic strategies to preserve muscle mass in the elderly, thus encouraging the independence of aged population and reducing the socioeconomic burden linked to sarcopenia (Marzetti et al., 2010).

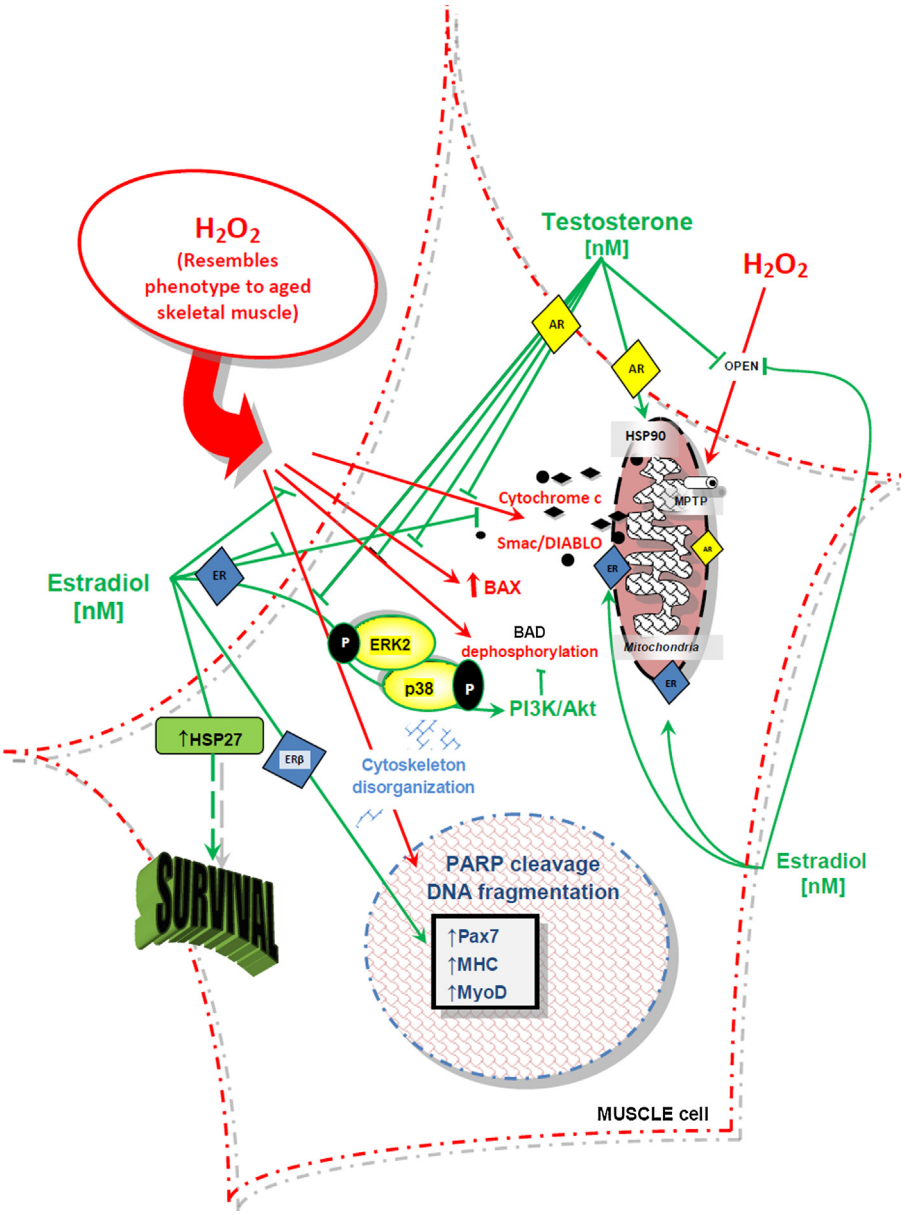

Fig. 1. Schematic diagram showing events involved in the effects of Ttestosterone and 17b-estradiol in aging-like phenotype of C2C12 cells. The diagram depicts both steroid hormones activating diverse intracellular signaling pathways to inhibit H_2O_2-induced apoptosis in skeletal muscle cells. E2 can interact with estradiol binding proteins/receptors (ER) localized in cell membrane and mitochondria promoting activation of ERK, p38 MAPK and the PI3K/Akt/p-Bad cascade, involving HSP27 and resulting in satellite cells survival. Thus, E2 abrogates mitochondrial membrane damage, and consequently Smac/DIABLO and cytochrome c release, induced by hydrogen peroxide. Furthermore, similar to estrogen, T is able to act at the mitochondrial level, probably involving the AR and HSP90. T abrogates the H_2O_2-induced Bax expression. Both hormones prevent DNA fragmentation and cytoskeleton disorganization.

3. Conclusion

Overall, survival radically increased as a result of advances in medicine. Consequently, we are looking at a rising percentage of an aging population. It is well known that sarcopenia increases linearly with aging. Since people are living longer, understanding the molecular mechanisms underlying sarcopenia is critical to the development of therapeutic and preventive strategies to decrease sarcopenia associated poor life quality in the elderly. Since the discovery of skeletal muscle satellite cells in 1961, a substantial body of scientific research has been dedicated to the properties and functions of these cells. The results of these studies have encouraged Zammit et al. (2006) to state that the satellite cell has earned its place at the very centre of adult muscle physiology. However, there is still much to know about this unique muscle stem cell in aging. Since satellite cells play a relevant role in the development of sarcopenia, the factors that modulate/affect the physiology of this kind of cells are also involved in the etiology of this pathology.

Clearly, deregulation of apoptosis affecting satellite cells due to sex steroids misbalances in the elderly, could trigger sarcopenia. Pharmacologic inhibitors of critical second messenger systems have been developed to block apoptosis. It is imaginable that, in the future, therapeutic modalities inhibiting apoptotic processes may have a role in minimizing the loss of satellite cells. However, several critical issues are still unsolved. The relative contribution of the different apoptotic pathways to sarcopenia is still unknown. Likewise, it is unclear whether specific signaling transduction cascades modulated by E2 or T, are selectively activated during the progression of muscle wasting affecting the apoptotic pathways (Fig. 1).

Future investigations will help us to unravel the role of sex steroids to ameliorate satellite cell death in skeletal muscle during elderly.

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