Growing muscle has different sarcolemmal properties from adult muscle: A proposal with scientific and clinical implications

Reasons to reassess skeletal muscle molecular dynamics, cellular responses and suitability of experimental models of muscle disorders

Miranda D. Grounds and Thea Shavlakadze*

We hypothesise that *the sarcolemma of an actively growing myofibre has different properties to the sarcolemma of a mature adult myofibre*. Such fundamentally different properties have clinical consequences for the onset, and potential therapeutic targets, of various skeletal muscle diseases that first manifest either during childhood (e.g. Duchenne muscular dystrophy, DMD) or after cessation of the main growth phase (e.g. dysferlinopathies). These characteristics are also relevant to the selection of both tissue culture and in vivo models employed to study such myopathies and the molecular regulation of adult myofibres. During growth, multinucleated myofibres increase enormously in size and volume with dramatic increases in length (up to ~600 mm). This is in striking contrast with most mononucleated cells such as fibroblasts, that remain at a relatively small size (~10–20 μm diameter). The consequences of a dynamic, expanding sarcolemma during growth, compared with that of an adult myofibre of a fixed length, are discussed with respect to various aspects of muscle biology.

Keywords:
growth; muscular dystrophies; myofibre elongation; sarcolemma properties; skeletal muscle

Introduction

Multinucleated skeletal muscle cells (myofibres) increase enormously in volume and length, with corresponding great expansion of the area of the cell surface membrane (sarcolemma), during the growth phase of an animal until maturity when the myofibre reaches a relatively fixed length. In addition, an individual myofibre persists throughout life with minimal turnover of the original myonuclei, in the absence of disease or major damage. This paper will focus on the sarcolemma-associated properties of growing myofibres compared with adult myofibres in homeostasis. We propose that *the sarcolemma of an actively growing myofibre has different properties to the sarcolemma of a mature adult myofibre*. Surprisingly this issue has barely been formally considered.

This hypothesis has major implications for understanding the time of clinical onset of particular myopathies and for therapeutic interventions in growing compared with adult humans. The discussion will focus on two genetic forms of muscular dystrophy, the childhood disease Duchenne

DOI 10.1002/bies.201000136

School of Anatomy and Human Biology, The University of Western Australia, Western Australia, Australia

***Corresponding author:**
Miranda D. Grounds
E-mail: miranda.grounds@uwa.edu.au

Abbreviations:
DMD, Duchenne muscular dystrophy; **ECM,** extracellular matrix.

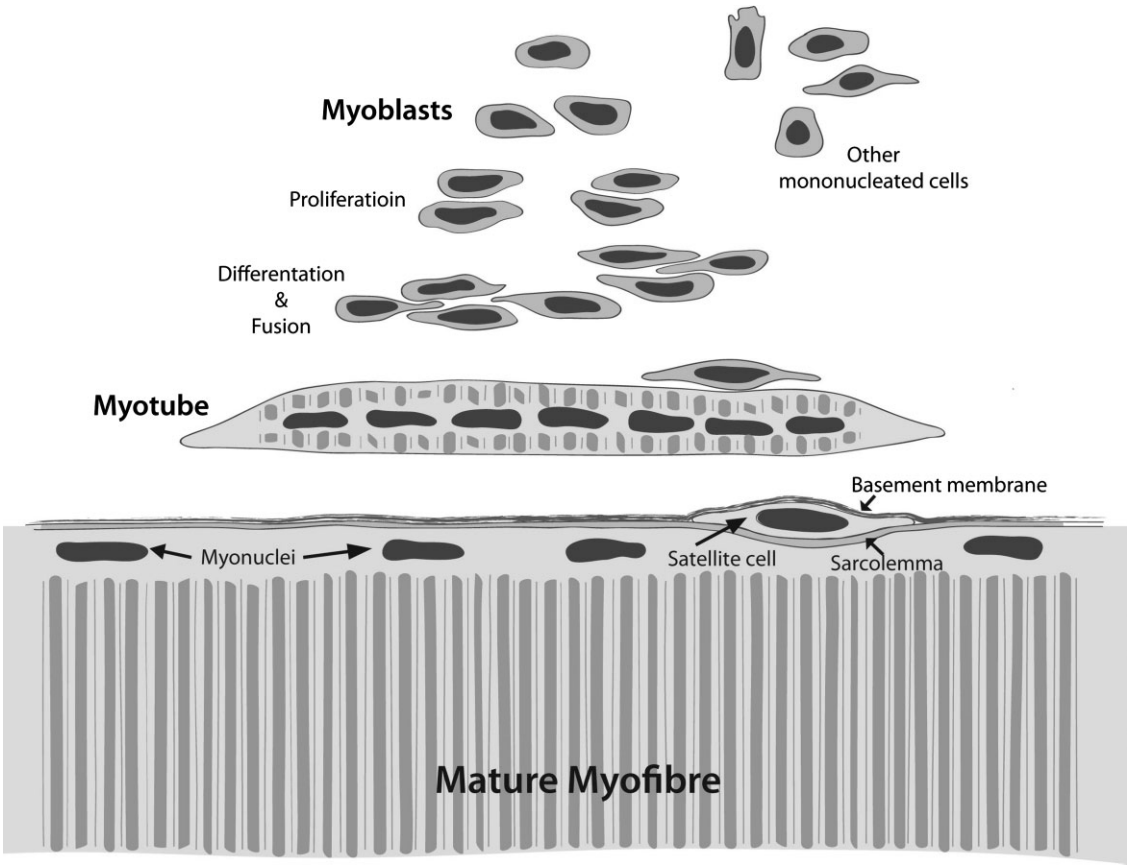


Figure 1. Diagram to illustrate myogenesis and the formation of skeletal myofibres, with comparison to cell sizes. Mononucleated muscle precursor cells (myoblasts) are similar in size to fibroblasts, macrophages and many other mononucleated cells (roughly 10–20 μm in diameter). Myoblasts proliferate and then differentiate and fuse to form thin multinucleated muscle cells (myotubes) that subsequently mature into myofibres. Some myoblasts persist as quiescent muscle precursor cells located on the surface of myotubes and myofibres, between the sarcolemma (muscle cell membrane) and the overlying basement membrane: these are called satellite cells and are responsible for generation of new myoblasts and muscle regeneration in response to necrosis of post-natal myofibres. The basement membrane is a light microscopy term that refers to the complex layer of specialised ECM molecules adjacent to the sarcolemma; it includes the basal lamina at the cell surface and others associated layers that are described by electron microscopy. The myotubes continue to differentiate and enlarge, become innervated (not shown) and expand dramatically in width (hypertrophy) and especially length during post-natal growth, until adult myofibres stabilise in length as bone growth ceases. This huge post-natal growth for almost 20 years in humans is associated with dynamic expansion of cell membranes, specifically sarcolemma. Only a portion of a mature myofibre is indicated, since the average adult human myofibre can reach about 60 μm in width and 40 mm or more in length. This dramatic difference between dimensions of mononucleated cells and a mature myofibre is correspondingly illustrated histologically in Fig. 2.

muscular dystrophy (DMD) and dysferlinopathies that manifest only after cessation of the main growth phase. The affected genes code for dystrophin and dysferlin proteins (respectively), both associated with the sarcolemma and other internal cell membranes of myofibres. The striking difference in age of manifestation of these two diseases, especially the post-growth on set of dysferlinopathies, implies that susceptibility to the specific genetic defect may relate to features of the growing and adult sarcolemma.

The key features of growing compared with adult (and ageing) muscle are discussed with respect to (i) general properties of skeletal muscles in humans, (ii) the signalling response to growth factors, (iii) the dynamic properties of membranes and the sarcolemma, (iv) the relative growth kinetics of animals and humans and their impact on two models of muscular dystrophy.

General properties of skeletal muscles in humans

Variations and similarities between skeletal muscles

Myofibres are very long multinucleated cells formed as a result of myogenesis as illustrated in Fig. 1. This discussion is not concerned with many well-described changes in gene expression during myogenesis and innervation but, instead, with properties of the fully formed myofibre as it expands in size during growth.

Humans have over 640 paired bilateral skeletal muscles of very different shapes and sizes. The muscles range in length from about 2 to 600 mm, and numbers of myofibres in different muscles vary from only a few hundred myofibres (e.g. tensor

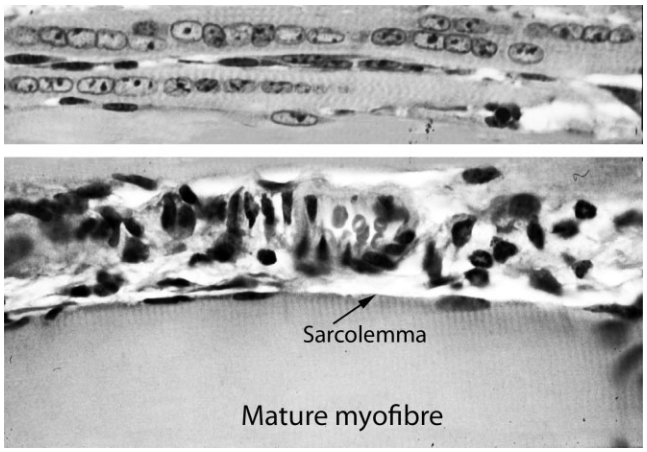


Figure 2. Histology demonstrating the relative size of mononucleated cells, myotubes and part of a myofibre shown in longitudinal section. Myotubes, in the top panel in regenerating mouse muscle after experimental injury, are long and thin and contain chains of centrally located myonuclei. The lower panel shows part of a mature myofibre (at the bottom) with the sarcolemma adjacent to interstitial connective tissue. Mononucleated cells in the interstitial connective tissue include fibroblasts, macrophages, lymphocyte and endothelial cells in a blood vessel; the dark stained nuclei are readily visible, and are ~5 μm in diameter. The myofibre is cut in longitudinal section and only a portion is shown to illustrate the relatively enormous size of a mature myofibre (see text in Fig. 1). Striated myofibrils of the sarcomeres are visible (especially near the sarcolemma). As a result of such light microscopy, it is not possible to distinguish between sub-sarcolemmal myonuclei, satellite cells and other nuclei of juxta sarcolemmal mononucleated cells. Mouse adult skeletal muscle, frozen longitudinal sections were stained with haematoxylin and eosin.

tympani in the ear) to about a million (gastrocnemius in the leg). Muscles of different anatomical origins also have different embryological origins (ranging from the myotome to pharyngeal arches) that may affect their properties. The speed of contraction varies from a predominantly slow pattern (e.g. postural muscles such as the soleus) to a very fast response (e.g. some extraocular muscle), while other muscles are constantly working (e.g. the diaphragm). Simplistically, the function of different muscles varies in terms of contraction speeds (influenced by the composition of fast and slow myofibres, motorneurone activity, muscle architecture and patterns of innervation [1]) and mechanical loading, that is further influenced by the size of the species and the force of gravity (e.g. small quadrupedal mouse compared with large biped human). The architecture of very long muscles and allometric effects of increasing fascicle length in humans and macaque primates show differences to many other species [2]. Muscle specific differences are reflected in their transcriptomes that are influenced by exercise, age, disease and many other factors [3].

What myofibres have in common is that during growth they increase in volume enormously, for both cross-sectional area (CSA) and length, and thus the sarcolemma must be dynamic and constantly expanding throughout this process (Fig. 1). The CSA of mature myofibres is variable but a CSA of around 3,000 μm^2 or more is not uncommon [4]. This huge size is in marked contrast to the situation for most mononucleated cells (e.g. fibroblasts, macrophages) which remain at roughly the same size (Figs. 1 and 2). This demonstrates a general principle, although it is clearly difficult to generalise since even mononucleated cells have very different shapes and functions. Extreme examples are human oocytes with a diameter of about 100 μm, about 5–10 times larger than the average mononucleated cell (roughly 10–20 μm) and axons of neurons that innervate muscles which elongate during growth (e.g. the sciatic nerve that innervates the lower limb).

The increase in length of myofibres is far more dramatic than the CSA. Even the smallest human muscle, the stapedius in the ear at 2 mm in length (2,000 μm), is 100-fold longer than the average mononucleated cell (~10–20 μm). There is a great range in muscle size and architecture. In human muscles, many myofibres can extend from tendon to tendon [2] and the average myofibre is around 20–30 mm in length (i.e. 1,000-fold longer than a mononucleated cell). The longest muscle is the sartorius in the thigh which can reach about 600 mm in length for a tall human [1]: since some myofibres appear to extend the entire length of this muscle [1] these myofibres are about 25,000 times longer than the average mononucleated cell, emphasising the huge expansion of sarcolemma area that must occur in growing myofibres.

Human muscle elongation during growth varies greatly between different anatomical locations

One can also consider the relative amount of elongation and change in diameter that different muscles are subjected to during human growth. Humans undergo roughly a 25-fold increase in weight from a baby (3–4 kg) to an adult (60–80 kg), a 40-fold increase in height (from about 40 cm to 1.7 m) while the head is disproportionately much larger at birth with relatively little post-natal increase in size [5]. Muscles are attached to bones and, accordingly, differential elongation is very pronounced for muscles associated with long bones in the limbs. Thus the associated post-natal increase in muscle size varies across the body, with striking differences between the head and limbs as outlined below (Fig. 3).

While many studies of muscle growth use animal models there are relatively few studies on in utero and post-natal muscle growth in humans, due to limited tissue availability. A study of human foetal sartorius muscle [6] showed rapid linear elongation throughout gestation (relative to crown-rump length) reaching about 80 mm prior to birth. It is generally believed that there is no change in human myofibre number after birth, although evidence for this appears scanty [6].

Comprehensive documentation of biometrics for growth of many muscles was provided in 1971 by Moore et al. [7] and differences between growth kinetics of myofibre diameter for some muscles are indicated in Fig. 3. In brief, the post-natal increase in myofibre width ceased by about 20 years. The mean narrow diameter for adult myofibres from the muscles of the neck, shoulder, arm and leg ranged from about 30 to 60 μm whereas, in striking contrast, the superior rectus muscle of the eye barely changed in width post-natally, remaining at 10–15 μm (Fig. 3). The variable impact of growth throughout the body is reflected by a pronounced difference in the susceptibility of limb and extraocular muscles to damage in DMD boys (discussed below in 'Growth kinetics and onset of dystropathology in humans and animal models').

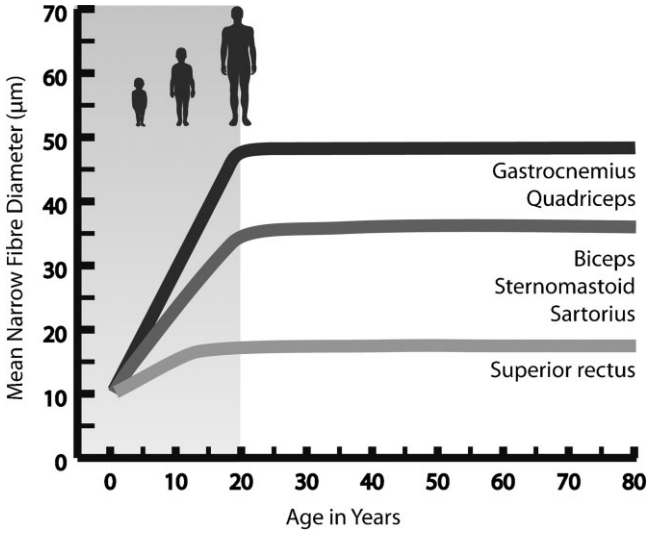


Figure 3. Biometrics of human muscles, showing mean narrow diameter of myofibres during post-natal growth and adulthood (adapted from [7]). These simplified graphs are based on human data published in 1971 [7]. Myofibre widths (μm) were measured for biopsies from more than 50 normal post-mortem human skeletal muscles of men and women, with ages ranging from foetal (4 and 5 months), neonatal (19 and 36 hours, 3–10 days, 7 weeks) and young to very old (3 and 10 months, 4–86 years). This study did not measure myofibre length since this is not possible from such biopsies. Most adult myofibres increased markedly in size during growth, to reach adult widths (narrow diameter) of between about 60 and 30 μm. The scale of the human figures indicate the differential growth between various parts of the body, e.g. limbs and head (from Fig. 4.1 in [5]). A broad outline of the pattern of increase in width during growth is indicated for several muscles: large muscles around 50 μm diameter include the gastrocnemius (lower leg) and quadriceps (thigh); around 40–30 μm includes the biceps (upper arm), sterrnomastoid (neck) and sartorius (thin strap-like muscle in the thigh). In striking contrast, the superior rectus of the eye (extraocular muscle) is much smaller, 10–15 μm, in diameter and barely changes in size post-natally. Once muscles cease growing, they remain at a relatively constant size (cross-sectional area) unless increased by hypertrophic exercise, until later in life when there is an age-related decrease in muscle mass and function known as sarcopenia; this is evident from about 65 years of age [73, 74]. Many factors contribute to sarcopenia and studies in mice show that loss of function can occur before muscle atrophy [75]. The peak muscle mass and strength attained in early adulthood influences this age-related decline in human muscle function [76, 77]. The fact that the original data [7] did not show any striking decrease in the size of myofibres in very old muscles might reflect the measurement of 'mean narrow diameter of myofibres', since changes in 'cross-sectional area' of myofibres or of whole muscles are more generally used, as are 'weights of whole muscles' to demonstrate sarcopenia in different species. In humans, functional measurements are also employed. Furthermore, variations between measurements from individual humans [7] may obscure any age-related changes.

How stable are myofibres and what is the extent of turnover of myonuclei throughout life?

In mice, the proliferation of satellite cells and their incorporation into growing myofibres as new myonuclei (hyperplasia) decreases rapidly after birth and almost ceases by three weeks. This is indicated by rapid downregulation of MyoD transcription, a marker of activated satellite cells, by around three weeks in muscles of normal mice [8] and strongly supported by studies using LacZ marked muscle nuclei that demonstrate minimal addition of myonuclei into growing myofibres after day 21, despite the fact that myofibre volume (and weight) continues to increase after this time [9]. While other studies of post-natal muscle growth in mice show increasing DNA content of growing muscles even after five weeks, this DNA might largely reflect increasing numbers of nuclei in cells of adjacent connective tissue associated with increasing strength of growing muscles, rather than additional myonuclei within myofibres [10]. For humans, we are not aware of information regarding the extent of addition of new myonuclei to growing myofibres that are expanding in volume and elongating over many years.

Individual myofibres appear to be stable and to persist throughout the life of an adult, in the absence of gross injury or disease that results in necrosis and new muscle formation. Thus individual myofibres 'age' in contrast with the high turnover of mononucleated cells in many tissues. Most neurons that innervate muscles also persist throughout life and must similarly 'age'. While many cell components, e.g. organelles and membranes, may turn over quite rapidly, the myonuclei probably do not. This myofibre longevity is based on evidence of minimal turnover of myonuclei in specific muscles after about 20 years of age in humans (see below).

While many discussions focus on the regenerative capacity of normal and ageing mature skeletal muscle, there actually may be very little or no necrosis of normal sedentary adult human skeletal muscles. Thus regeneration/myogenesis per se may be of minimal importance post-natally, in the absence of myopathy or repeated major damage. Since there appears to be little or no turnover of myonuclei throughout the life of most normal adult myofibres, myogenic precursor (e.g. satellite) cells may contribute relatively little to homeostasis of such myofibres; although such 'reserve' cells are clearly crucial if damage does indeed occur. Myonuclei are considered to be post-mitotic although data addressing myonuclear turnover in adult muscles are scant. There appears to be remarkably little turnover of myonuclei for limb and masseter muscles throughout life, as demonstrated by historic C14 cell dating in humans [11] and measurements of relatively stable telomere lengths (reviewed in [12]); although abnormal shortening of telomeres can occur with excessive exercise/overtraining [13]. In marked contrast, ongoing satellite cell activation/proliferation is reported for both extraocular and laryngeal muscles with craniofacial muscles having many different properties to limb muscles [14].

The consequences of different functions and mechanical activity on muscles over time, with reference to extracellular matrix (ECM) changes

A selective involvement of specific muscles is a feature of many muscular dystrophies [15] and other gene effects such as hypertrophy of buttock muscles in Callipyge sheep [16], with these phenotypes often being manifested post-natally. However, the reasons for such striking susceptibility of different muscles are unknown. During embryogenesis, clearly an

extraordinarily strict regulation of molecular and cellular events in a very precise sequence is required to correctly construct the complex tissues of developing animals. Yet once this is achieved and skeletal muscles start to function it seems likely that this unique 'handbook of development' is of lesser significance. Instead, very different mechanical activity that is especially important post-natally when muscles become loaded, influences a range of properties including molecular, metabolic and biochemical parameters of myofibres [17], capillary density [18] and composition of extracellular matrix (ECM) [19].

The ECM of skeletal muscle is comprised of the specialised basement membrane in intimate contact with both the sarcolemma [20] and interstitial connective tissue (reviewed in [21, 22]). The basement membrane is crucial for transmission of contractile force generated by the sarcomeres within the myofibres to the ECM where interactions with many molecules, initially laminin in the basement membrane and ultimately collagen 1, mediate movement of the skeleton [19, 23]. This force transmission involves a complexity of linkages between nuclear-lamins, cytoskeletal proteins and trans-sarcolemma proteins such as the dystrophin glycoprotein complex [24] and integrins [25]. All of these also play important roles in signalling and mechanosensing and defects result in various muscular dystrophies [26–28].

The ECM also regulates myofibre properties by a multitude of molecules such as glycosaminoglycans and metalloproteinases that modulate the availability and response to growth factors and other signalling molecules that interact with the sarcolemma. Even the molecular composition of basement membranes might differ between muscles, similar to the variation reported for endothelial cells in different vascular beds and pathophysiological states [29].

Function-based variation in molecular composition of ECM with age (along with density of capillary beds and influence of ECM on vasculature and innervation) driven by mechanical demands, will have a major impact on the post-natal environment of different muscles and may well contribute to the very different susceptibility of muscles to specific gene defects over time. The influence of age even on early events is emphasised by striking differences between embryonic compared with neo-natal fibroblasts with respect to ECM production and associated fibroblast proliferation and survival. This has a major impact on the post-natal onset of progeria, a laminopathy characterised by premature ageing [30]; other laminopathies manifest as muscular dystrophies. The ECM composition is further modified during ageing, through glycation and fibrosis, and damage that causes inflammation associated with increased fibrosis [21, 31]. A complexity of enzymes, e.g. proteases and sulphatases, modify many proteins and sugars in the ECM and the fine balance of metalloproteinases and their inhibitors controls dynamic remodelling of the matrix and intracellular proteins [32]. Such molecular variation in ECM composition both post-natally and during ageing has barely been considered with respect to the disparate function of the plethora of skeletal muscles.

We propose that manifestation of genetic disorders in some skeletal muscles (whereas others are spared) may reflect in large part the pattern of mechanical use (and damage) of these muscles post-natally that impacts on the ECM/sarcolemma interface, especially after the main growth phase has ceased.

Evidence for molecular differences between growing and adult sarcolemma

The nutrient and metabolic demands of growing children and animals are high and this is a major focus of animal nutrition research. Studies in livestock animals show that 'catchup growth' by additional booster feeding, e.g. with the amino acids leucine and arginine, is pronounced in very early post-natal stages and significantly reduced within 2 weeks in pigs [33, 34]. Such studies have major implications for 'windows of opportunity' to try and correct for adverse effects of low birth weight babies in humans, yet little is known about why neonatal muscles rapidly become unresponsive to booster growth stimuli. We suggest that, at least in part, this may reflect changing dynamics of sarcolemmal properties. This topic is also of central interest to the meat and livestock industry that is focussed on optimal increase in muscle mass during the growth phase.

One key growth factor that increases the size of myofibres in vivo and myotubes in culture is insulin like growth factor (IGF-1). It is well documented, especially using tissue cultured myotubes of the mouse C2C12 cell line, that activation of the signalling pathways downstream of the IGF-1 receptor that involve Akt and mTOR can enhance net protein synthesis and result in myotube hypertrophy [35, 36]. However, it has recently been demonstrated that while signalling related to protein synthesis is readily induced by elevated transgenic IGF-1 in young growing muscle, such response is blunted in adult muscle in vivo [37]. This heightened sensitivity to IGF-1 of growing myofibres applies during embryogenesis and in the early post-natal developmental phase, when muscle growth is the most intense [9, 10]. Few factors cause hypertrophy of adult myofibres in the absence of resistance exercise [38], although JunB has now been identified as such a hypertrophic factor and does so without activating the Akt/mTOR pathway [39]. Studies in young pigs also showed that growing muscle has a heightened capacity to activate the signalling cascades that promote protein synthesis required for muscle growth, and that this signalling is attenuated with maturation [33]. Another in vivo situation of growth where muscles are responsive to IGF-1 is new myotubes and myofibres formed as a result of necrosis and regeneration in adult muscle [37]. Myotubes in vitro are widely used as a convenient model to study many aspects of skeletal muscle, yet we emphasise that such cultured myotubes do not represent the response of mature myofibres; instead they resemble growing immature myofibres in the absence of innervation and of mechanical loading.

This impact of myofibre maturation has many implications that have not been widely appreciated. For example, where the aim of the study is to actually test what happens in a mature adult muscle, the growing myotube is sometimes not an accurate model. While in vivo studies remain ideal, it appears that isolated post-natal or adult myofibres represent a more suitable ex vivo model (compared with myotubes) for investigations related to mature muscle [40, 41], although they

are disconnected from innervation, the ECM and mechanical loading. The impact of growth and maturation in muscle is illustrated by a study investigating the intracellular localisation of Ca^{2+}, a key regulator of many cellular and signalling events. The near-membrane indicator dye FFP-18 was used to visualise intracellular Ca^{2+}, with the expected subsarcolemmal localisation clearly present in myofibres isolated from young adult mice whereas the dye was mainly cytoplasmic in C2C12 myotubes in culture [42].

Many other examples of unexpected differences between results for mature adult myofibres and cultured myotubes might also be explained by the proposed differences in 'growth related properties of the sarcolemma' that have not been considered previously. For certain parameters, myotubes are not an accurate model for comparison and extrapolation to a mature myofibre and can therefore represent erroneous models: this point was recently emphasised with respect to major differences in contractile function, metabolic responses and cellular Ca^{2+} handling [17].

Dynamic properties of the sarcolemma

Structure and composition of the sarcolemma

The sarcolemma (plasma membrane) is composed of a fluid phospholipid bilayer with highly mobile phospholipids, that contains many integral membrane proteins with more restricted mobility. The sarcolemma is a highly dynamic yet ordered structure involved in many cellular processes such as adhesion, ion conductivity and signalling, with attachment to the ECM and the intracellular cytoskeleton being essential for muscle contraction that requires flexibility in combination with force resistance: clearly a multitude of sarcolemma-associated proteins are involved with these diverse processes [20–28].

The structure and biophysics of the sarcolemma are highly complex and only a very brief overview is possible here; for a comprehensive review especially of earlier studies see [43]. The initial fluid-mosaic model of membrane structure has now evolved to include the concept of lipid rafts and lateral organisation where the lipid bilayer is segregated into raft and non-raft microdomains of distinct lipid composition: the lipid composition is extremely complex with up to 500 different kinds of lipids [44]. Simplistically, the rafts are formed by assemblies of cholesterol and glycosphingolipids, interspersed with glycerophospholipid enriched non-raft regions. Alterations to levels of cholesterol and sphingolipid can perturb membrane fluidity and different aspects of cell physiology. A study using a variety of developing and adult human and mouse skeletal muscles in vivo and in vitro [45] showed that rafts are characterised by marker proteins of the annexin family (associated with Ca^{2+} regulation) which localise to the inner membrane leaflet, with glycosyl-phosphatidylinositol (GPI) anchored enzymes attached to the outer leaflet: this study identified profound structural changes within the sarcolemma during differentiation and myofibre maturation [45]. Other studies using membranes isolated from normal and laminin-deficient dystrophic mice show that the lipid rafts provide a pathway for targeting proteins like caveolin and AChE to specialised domains of the sarcolemma [46]. Some rafts are large and stable and associated with calveolar regions and receptors for certain growth factors. Another class of rafts that are small and more dynamic are associated with Ca^{2+} regulation and annexin, and may be involved in rapid regulation of excitation contraction coupling (discussed in [45]). Raft organisation is highly dynamic and rafts containing specific types of proteins can self-associate to form higher-order structures or hubs of signalling activity [44].

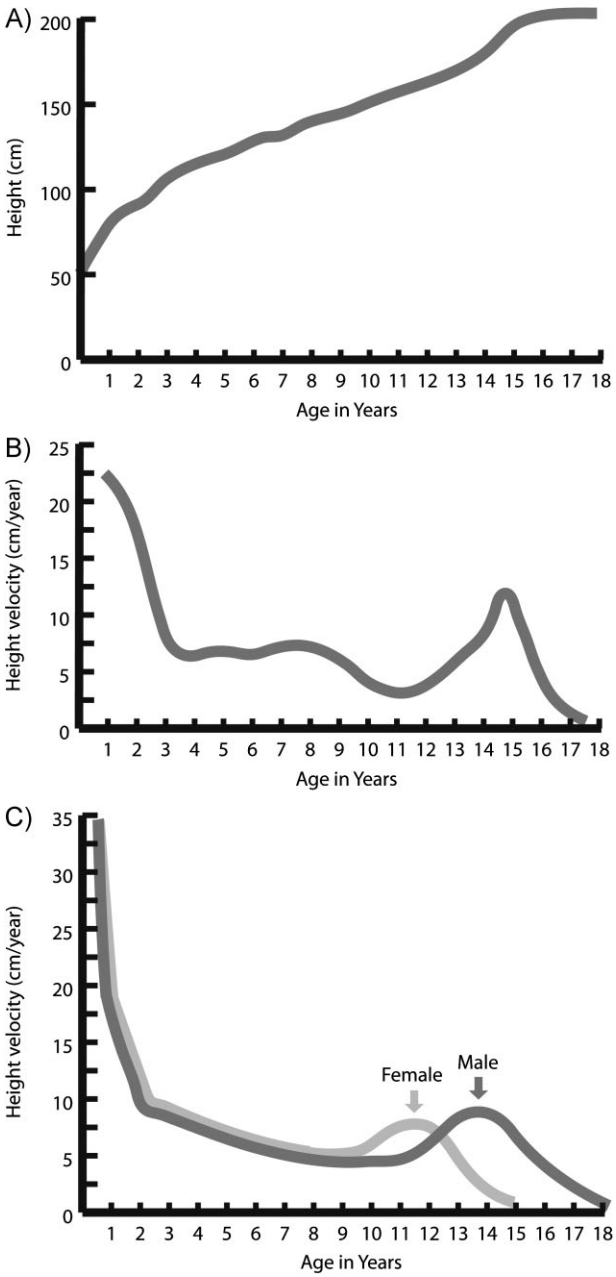
Many striking changes occur in the cell and transverse tubular membranes during myoblast differentiation and fusion as well as myotube and myofibre maturation, even in the neonatal period [47] and studies are now identifying proteins localised to lipid rafts in developing skeletal muscles in tissue culture and in vivo. While such observations are of considerable interest to myogenesis per se, none seem to yet address the question of the fluidity and properties of sarcolemma of fully formed myofibres subjected to the extraordinary expansion in volume and length during the post-natal growth phase, the central focus of the present hypothesis. Such studies have been difficult in the past due to technical limitations.

The power of emerging technologies in ultra-sensitive microscopy, fluorescent probes and live cell imaging in combination with computational simulations [48] present new possibilities to describe such dynamic aspects of lipid composition, fluidity, membrane activity and sarcolemma nanostructure. Super-resolution microscopy techniques, such as photoactivation localisation microscopy (PALM) and stochastic optical reconstruction microscopy (STORM), allow very fine resolution of dynamic nanoscale events within cell membranes [49]. For example, nanoscopic raft like platforms have been described diffusing in the plasma membrane of living cells [50].The first imaging of membrane lipid order in a whole, live vertebrate, namely the transparent zebra fish embryo was recently achieved with the phase-sensitive, membrane-partitioning fluorescent probe Laurdan and multi-photon microscopy [51]. What is needed to test our hypothesis is information about the composition of lipids and proteins in rafts on the inner and outer layers of the sarcolemma in living myofibres, at different spatio-temporal scales, ideally in situ for myofibres in growing and adult mammalian muscles, and their modulation by different signalling stimuli. This is challenging.

Sarcolemma damage and mechanisms for membrane resealing

Myofibres require an efficient membrane repair system to overcome the rigours of frequent skeletal muscle contractions that can deform the myofibre surface and result in minute lesions in the sarcolemma. It appears that such sarcolemmal damage in normal muscles is rapidly resealed to avoid the massive influx of Ca^{2+} and other cellular changes (e.g. elevated oxidative stress) that can lead to myofibre necrosis. Membrane resealing is a function conserved by most cells. Resealing via membrane vesicles is mediated by a mechanism resembling Ca^{2+}-dependent exocytosis, which involves membrane fusion and key membrane proteins such as synaptotagmins and members of the ferlin family, with dysferlin being predominantly expressed in skeletal muscle [52–54].

Associated proteins involved in skeletal muscle membrane resealing, membrane trafficking, exocytosis and endocytosis are members of the soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) family such as syntaxin-4, Ca^{2+}-activated proteins such as calpains and annexins, caveolin-3 and mitsugumin 53 (MG53). Defects in many of these molecules are now also known to cause muscular dystrophies [52, 54–56]. Vesicle-mediated membrane resealing is clearly a dynamic process that involves both dysferlin and a complexity of molecules in order to patch the lesion. This mechanism seems important in mature adult myofibres, although the situation is less clear for the sarcolemma of growing myofibres. Alternatively, in some situations lipids may automatically flow from the membrane at edges of the lesion across the damaged site to reseal the membrane and this is influenced by membrane tension and fluidity [52].



Growth kinetics and onset of dystropathology in humans and animal models

Patterns of post-natal human growth

Height increases steadily during human growth and usually ceases by about 18 years of age (Fig. 4A). However, the growth rate is not constant during this time (Fig. 4B and C). In humans, the post-natal growth rate declines sharply during the first two years, followed by a fairly steady growth rate for several years and culminating in a burst of increased growth velocity associated with puberty and adolescence. Women usually reach their greatest height at a younger age than men, because puberty occurs earlier in women (Fig. 4C). The peak of this adolescent growth spurt is around 11–12 years for females and 13–14 years for males, the timing and extent varies throughout the body with the average velocity in growth being +7.0 cm/year for girls and 9.0 cm/year for boys [5]. This is followed by a steady decline of growth to the adult state of 0, that is about three years earlier in females compared with males (15 compared with 18 years, respectively).

Figure 4. Human post-natal growth rates for 'normal' males and females. **A:** Height increase during the growth phase from birth to 18 years shown for an individual male and (**B**) demonstration of very variable rate of growth (in terms of height) during this time for the same individual. Both measurements were made about 250 years ago by Count Montbeillard who measured the height of his son every 6 months from 1759 to 1777 (based on Fig. 1.4 cited in [78]). **C:** Average (50 percentile) growth curves for men and women aged 0–18 years (based on http://en.wikipedia.org/wiki/Human_height): note the overall similarity of these population data to the pattern for the individual male shown in (B). (Similar data are shown for males aged 6–18 years in Fig. 3.14 [5].) Immediately after birth there is a sharp drop in the rate of growth (velocity) in terms of height (cm/year) until about 2 years, followed by a period of steady growth (increase in height) until the marked growth spurt associated with puberty from around 10 years of age in girls and 12 years in boys (C). The height of boys is generally greater than for girls and the actual height varies between populations and is affected by nutrition and other factors. The steep post-natal drop in human growth rate may be associated with a rapid decline in proliferation of myogenic precursor cells (myoblasts) to reach a relatively low level of proliferation corresponding with more stable growth. This suggestion is based on observations in mice where myogenic cell proliferation declines rapidly post-natally to almost cease by 2–3 weeks (discussed in Section on Myofibre Stability [8, 9]). It is tentatively suggested that the sarcolemma of neonatal myofibres where growth involves fusion of many myoblasts (hyperplasia) may have different properties to the sarcolemma of myofibres where growth is due mainly to hypertrophy. Such differences might contribute to the acute manifestation of dystropathology around 3 weeks of age in the mdx mouse model of DMD (Duchenne muscular dystrophy) a human paediatric disease that is clinically evident from around 2 years of age, when the growth rate (B,C) starts to stabilise (see caption for Fig. 5).

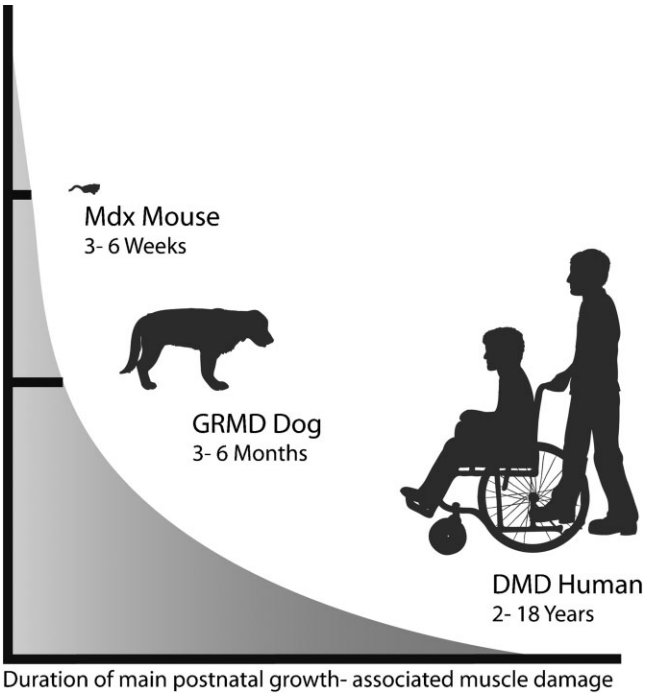


Figure 5. Duration of the main post-natal growth phase in three species. This comparison emphasises the vastly different length of the main growth phase (weeks, months, years) in mice, dogs and humans (this is not drawn to scale due to the huge differences in parameters). The very different body size and weight of these species and consequences on muscle loading also need to be considered. The duration and pattern of the growth phase in these three species is important with respect to dystrophin deficiency, since it is proposed that necrosis of dystrophic myofibres is exacerbated by growth [58]. The dystropathology in mdx mice is very mild whereas the severity of the disease in dog models of DMD more closely resembles the human condition. In mice, the growth rate (measured by body weight mg/day) is maximal at 3 weeks and then drops dramatically to a low level by 6 weeks, followed by a very slow phase up to 10 weeks where growth almost ceases (adult phase). The proposal that maximum necrosis occurs during growth is supported by the high level of acute necrosis in limb muscles of the dystrophic mdx mouse between 3 and 4 weeks of post-natal age that abates after the main growth phase to a relatively mild, albeit ongoing, dystropathology in adult mice. Pronounced fibrosis is only manifested in mdx limb muscle very late in life, although it is a feature of adult mdx diaphragm muscle probably due to the repeated contraction of this muscle [79]. Contraction resulting from exercise also exacerbates the mild dystropathology of limb muscles in adult mdx mice [79]. For dogs, the most widely studied is the golden retriever muscular dystrophy (GRMD) dog model of DMD where the main growth phase is around 3–6 months of age and the phenotype can be severe, although is highly variable [80]: dystrophin-defective dogs have been described for over 15 breeds [81]. The dogs manifest dystropathology at birth and even within a short time, e.g. months in the GRMD dogs, the sustained and increasing fibrosis associated with repeated cycles of necrosis progressively impairs myogenesis, so that damaged muscle is instead replaced by fat and fibrosis connective tissue during this time. Similarly, in humans this accumulating fibrosis from ongoing damage associated with growth over a few years in children results in pronounced dystropathology with major loss of skeletal muscle mass and function, even in very young boys.

The elongation of bones that results in a height increase is also not continuous and varies throughout the day: implantation of tiny sensors into the tibia of lambs showed that at least 90% of bone elongation occurred when the animals were sleeping or at rest [57]. It was suggested that pressure on the growth plates of long bones, such as the tibia, was eased when the animal was at rest, permitting the bones to elongate. It seems likely that this is also the case in humans and that the sudden leg pains experienced by children, frequently at night, are truly growing pains, especially since these occur predominantly in the weight-bearing lower extremities. Clearly such bone elongation has consequences for the attached muscles.

Does growth exacerbate myofibre necrosis in DMD?

The importance of growth (Fig. 4) is emphasised for the X-linked paediatric muscle disease DMD that affects mainly boys, where it is proposed that growing skeletal muscle is more susceptible to necrosis than mature muscle [58]. The disease manifests clinically around 2–3 years of post-natal age. This time of onset may relate to walking and greatly increased bipedal activity and muscle loading. Interestingly, onset occurs after the pronounced neonatal drop in growth rate and corresponds with transition to the steady growth rate of childhood (Fig. 4B and C). This might reflect transition from hyperplasia to hypertrophy with further differences in sarcolemmal properties (discussed in Fig. 4 caption). The disease progresses with increasing muscle weakness and loss of muscle mass, leading to death by about 30 years due to respiratory and cardiac failure: lifespan can be extended by assisted ventilation and drugs such as corticosteroids [59, 60]. While corticosteroids can reduce the severity of dystropathology, they are commonly associated with adverse side effects including vertebral bone fracture [60], growth retardation and restricted height. For example, DMD boys treated with deflazacort were about 20 cm shorter at 15 years of age [61] and it has been proposed that such stunted growth may actually be beneficial and contribute to the reduced severity of muscle damage and dystropathology. Strong evidence to support this is provided by studies of pituitary dwarfism associated with inherited growth hormone deficiency in DMD boys and animal models of dystrophy [62, 63]. Although caution should be exercised because of the small sample size, further support for the proposal that growth exacerbates myofibre necrosis in DMD is provided by a one-year controlled, double-blind therapeutic trial with the drug mazindol, an inhibitor of growth hormone, administered to one sibling of a pair of monozygotic twins aged 7.5 years with DMD: after one year the placebo treated twin was strikingly worse than his brother, whose condition was almost arrested [64].

A striking feature of DMD is that specific muscles, such as extraocular muscles, are relatively spared in DMD boys (and models of this dystrophy) in marked contrast with limb and other muscles [65]. Many factors have been investigated to further understand the basis for these marked differences in susceptibility to myofibre necrosis [66]. Since the onset of dystropathology occurs during the growth phase, it seems pertinent to consider the contribution to the severity of dystropathology of the vastly different post-natal growth kinetics for these muscles in vivo, contrasting the dramatic length

increase of limb muscles with minimal growth of extraocular muscles (discussed above and in Fig. 3).

The impact of the length of the growth phase associated with increased myofibre necrosis also needs to be considered with respect to mouse, dog and other mammalian models used to study DMD and other human diseases, since the kinetics of juvenile growth are vastly different between these species and this correlates with the severity of the dystropathology (see Fig. 5 for further discussion).

Myofibre necrosis leads to inflammation and associated fibrosis, along with new muscle formation during regeneration. Satellite cells are required for new muscle formation. The proposal that this cell population is exhausted over time by the repeated demand for regeneration in dystrophic muscles, possibly due in part to shortened telomeres over time as indicated in muscles from DMD boys [67], is strengthened by long-term studies in mdx mice lacking telomerase where shortened telomeres exacerbated the severity of dystropathology [68]. A different explanation is that the myogenic regenerative potential is crippled in all species due to progressive accumulation of fibrosis that results in lineage conversion of myogenic precursors into non-myogenic fibroblasts, leading to replacement of muscle by fatty and fibrous connective tissue [21, 31, 69, 70]. Such resultant loss of muscle tissue is a progressive feature of the DMD condition and is pronounced even in young boys (due to ongoing necrosis over many years), well before the onset of the growth spurt associated with puberty. In extreme contrast, the growth phase is only a few weeks in mdx mouse and thus minimal fibrosis occurs before mdx mice stabilise as adults.

The proposed exacerbation of damage to dystrophic myofibres by growth raises the possibility that less intensive treatments might protect muscles during the adult phase, provided that a sufficient muscle mass can indeed be maintained until this time – clearly a much greater challenge in humans than mice. This proposal also emphasises that juvenile mdx mice may be a useful model to study the protective effects of drug and nutritional interventions for juvenile DMD boys, compared with the use of adult mdx mice.

Why are dysferlinopathies manifested after growth has ceased?

Dysferlinopathies involve progressive muscle wasting and have a variable phenotype. They typically begin in the late teenage years and include Limb-girdle muscular dystrophy 2B (LGMD 2B) and Miyoshi myopathy. They are caused by mutations in the gene encoding dysferlin, a transmembrane protein associated with T-tubules and the sarcolemma, involved in membrane vesicle trafficking and fusion events. Adult dysferlin-defective myofibres have mis-localised mutated dysferlin and impaired resealing of damaged sarcolemma [55, 71]. Dysferlinopathies affect both women and men. Prior to clinical onset, dysferlin patients are often very active in sports yet appear to be pre-symptomatic, indicating that skeletal muscles readily compensate for any problem during the growth phase. The gender differences in cessation of growth (Fig. 4C) correspond to the timing of most reports of clinical onset of dysferlinopthy soon after the growth spurt associated with puberty in females by 16 years and males by 17–20 years (with a mean onset age of 21 years); however, specific mutations of the dysferlin gene can lead to earlier or later clinical onset [72].

This post-growth onset suggests that the manifestation of defects in dysferlin may relate to different molecular mechanisms being used for sarcolemmal resealing of growing juvenile and adolescent myofibres (perhaps dysferlin independent) compared with mature adult muscles (dysferlin dependent). If this proves to be the case, then the need to use 'mature muscle' experimental models (isolated myofibres in culture or adult animals in vivo) is emphasised for this post-growth muscle disease, in order to identify the best targets for potential therapeutic interventions for dysferlinopathies in humans [for extensive information on dysferlinopathies see www.jain-foundation.org].

Conclusions

The scale and timing of post-natal growth is clearly very different between mice and humans. Reconciling data from disparate studies that use either immature myotubes in vitro or mature skeletal myofibres in situ can be challenging, and the relevance to clinical situations needs to be critically evaluated. What is surprising is that almost no consideration has been given to the properties of the growing sarcolemma during the extraordinary myofibre expansion in volume and length required to attain the full adult myofibre size. Skeletal myofibres are unique with respect to this impressive increase in cell size that occurs over many years in humans. The hypothesis that *the sarcolemma of an actively growing myofibre has different properties to the sarcolemma of a mature adult myofibre* appears completely novel. It seems likely that some properties of the sarcolemma (and maybe also T-tubules and other cell membranes) do indeed differ with growth, e.g. with reference to signalling response to growth factors such as IGF-1, calcium regulation, membrane fluidity, lipid raft distribution, lipid composition and the role of dysferlin. This has many implications for fundamental muscle biology, selection of models for research and, importantly, for insight into clinical muscle diseases and potential therapies. It will be fascinating to see what research into this topic reveals.

Acknowledgments

The research that forms the basis for this work was supported by funding over many years from the National Health & Medical Research Council of Australia. We thank various colleagues for their encouraging comments and Hannah Radley-Crabb for her assistance with preparation of the manuscript.

References

1. **Harris AJ**, **Duxson MJ**, **Butler JE**, **Hodges PW**, et al. 2005. Muscle fiber and motor unit behavior in the longest human skeletal muscle. *J Neurosci* **25**: 8528–33.
2. **Paul AC.** 2001. Muscle length affects the architecture and pattern of innervation differently in leg muscles of mouse, guinea pig, and rabbit compared to those of human and monkey muscles. *Anat Rec* **262**: 301–9.

3. **von der Hagen M**, **Laval SH**, **Cree LM**, **Haldane F**, et al. 2005. The differential gene expression profiles of proximal and distal muscle groups are altered in pre-pathological dysferlin-deficient mice. *Neuromuscul Disord* **15**: 863–77.
4. **Shavlakadze T**, **Boswell JM**, **Burt DW**, **Asante EA**, et al. 2006. Rskalphaactin/hIGF-1 transgenic mice with increased IGF-I in skeletal muscle and blood: impact on regeneration, denervation and muscular dystrophy. *Growth Horm IGF Res* **16**: 157–73.
5. **Bogin B.** 2001. *The Growth of Humanity*. New York: Wiley–Liss. p. 319.
6. **Stickland NC.** 1981. Muscle development in the human fetus as exemplified by m. sartorius: a quantitative study. *J Anat* **132**: 557–79.
7. **Moore MJ**, **Rebeiz JJ**, **Holden M**, **Adams RD.** 1971. Biometric analyses of normal skeletal muscle. *Acta Neuropathol* **19**: 51–69.
8. **Beilharz MW**, **Lareu RR**, **Garrett KL**, **Grounds MD**, et al. 1992. Quantitation of muscle precursor cell activity in skeletal muscle by northern analysis of MyoD and myogenin expression – application to dystrophic (mdx) mouse muscle. *Mol Cell Neurosci* **3**: 326–31.
9. **White RB**, **Bierinx AS**, **Gnocchi VF**, **Zammit PS.** 2010. Dynamics of muscle fibre growth during postnatal mouse development. *MC Dev Biol* **10**: 21.
10. **Fiorotto ML**, **Schwartz RJ**, **Delaughter MC.** 2003. Persistent IGF-I overexpression in skeletal muscle transiently enhances DNA accretion and growth. *FASEB J* **17**: 59–60.
11. **Spalding KL**, **Bhardwaj RD**, **Buchholz BA**, **Druid H**, et al. 2005. Retrospective birth dating of cells in humans. *Cell* **122**: 133–43.
12. **Ponsot E**, **Lexell J**, **Kadi F.** 2008. Skeletal muscle telomere length is not impaired in healthy physically active old women and men. *Muscle Nerve* **37**: 467–72.
13. **Collins M**, **Renault V**, **Grobler LA**, **St Clair Gibson A**, et al. 2003. Athletes with exercise-associated fatigue have abnormally short muscle DNA telomeres. *Med Sci Sports Exerc* **35**: 1524–8.
14. **McLoon LK**, **Thorstenson KM**, **Solomon A**, **Lewis MP.** 2007. Myogenic precursor cells in craniofacial muscles. *Oral Dis* **13**: 134–40.
15. **Emery EH.** 2002. The muscular dystrophies. *Lancet* **359**: 687–95.
16. **White JD**, **Vuocolo T**, **McDonagh M**, **Grounds MD**, et al. 2008. Analysis of the callipyge phenotype through skeletal muscle development; association of Dlk1 with muscle precursor cells. *Differentiation* **76**: 283–98.
17. **Westerblad H**, **Bruton JD**, **Katz A.** 2010. Skeletal muscle: energy metabolism, fiber types, fatigue and adaptability. *Exp Cell Res* **316**: 3093–9.
18. **Christov C**, **Chretien F**, **Abou-Khalil R**, **Bassez G**, et al. 2007. Muscle satellite cells and endothelial cells: close neighbors and privileged partners. *Mol Biol Cell* **18**: 1397–409.
19. **Kjaer M.** 2004. Role of extracellular matrix in adaptation of tendon and skeletal muscle to mechanical loading. *Physiol Rev* **84**: 649–98.
20. **Yurchenco PD**, **Patton BL.** 2009. Developmental and pathogenic mechanisms of basement membrane assembly. *Curr Pharm Design* **15**: 1277–94.
21. **Grounds MD.** 2008. Complexity of extracellular matrix and skeletal muscle regeneration. In Schiaffino S, Partridge TA, ed; *Skeletal Muscle Repair and Regeneration*. The Netherlands: Springer. p. 269–302.
22. **Voermans NC**, **Bonnemann CG**, **Huijing PA**, **Hamel BC**, et al. 2008. Clinical and molecular overlap between myopathies and inherited connective tissue diseases. *Neuromuscul Disord* **18**: 843–56.
23. **Grounds MD**, **Sorokin L**, **White J.** 2005. Strength at the extracellular matrix-muscle interface. *Scand J Med Sci Sports* **15**: 381–91.
24. **Lapidos KA**, **Kakkar R**, **McNally EM.** 2004. The dystrophin glycoprotein complex: signalling strength and integrity for the sarcolemma. *Circ Res* **94**: 1023–31.
25. **Jani K**, **Schock F.** 2009. Molecular mechanisms of mechanosensing in muscle development. *Dev Dyn* **238**: 1526–34.
26. **Kanagawa M**, **Toda T.** 2006. The genetic and molecular basis of muscular dystrophy: roles of cell-matrix linkage in the pathogenesis. *J Hum Genet* **51**: 915–26.
27. **Bozzi M**, **Morlacchi S**, **Bigotti MG**, **Sciandra F**, et al. 2009. Functional diversity of dystroglycan. *Matrix Biol* **28**: 179–87.
28. **Lai FP**, **Mutalif RA**, **Phua SC**, **Stewart CL.** 2010. Informatics-based analysis of mechanosignaling in the laminopathies. *Methods Cell Biol* **98**: 323–35.
29. **Hallmann R**, **Horn N**, **Selg M**, **Wendler O**, et al. 2005. Expression and function of laminins in the embryonic and mature vasculature. *Physiol Rev* **85**: 979–1000.
30. **Hernandez L**, **Roux KJ**, **Wong ES**, **Mounkes LC**, et al. 2010. Functional coupling between the extracellular matrix and nuclear lamina by Wnt signaling in progeria. *Dev Cell* **19**: 413–25.
31. **Serrano AL**, **Munoz-Canoves P.** 2010. Regulation and dysregulation of fibrosis in skeletal muscle. *Exp Cell Res* **316**: 3050–8.
32. **Butler GS**, **Overall CM.** 2009. Updated biological roles for matrix metalloproteinases and new ''intracellular'' substrates revealed by degradomics. *Biochemistry* **48**: 10830–45.
33. **Davis TA**, **Fiorotto ML.** 2009. Regulation of muscle growth in neonates. *Curr Opin Clin Nutr Metab Care* **12**: 78–85.
34. **Suryawan A**, **Orellana RA**, **Fiorotto ML**, **Davis TA.** 2010. Leucine acts as a nutrient signal to stimulate protein synthesis in neonatal pigs. *J Anim Sci* in press, DOI: 10.2527/jas.2010-3400.
35. **Glass DJ.** 2005. Skeletal muscle hypertrophy and atrophy signaling pathways. *Int J Biochem Cell Biol* **37**: 1974–84.
36. **Sandri M.** 2008. Signaling in muscle atrophy and hypertrophy. *Physiology (Bethesda)* **23**: 160–70.
37. **Shavlakadze T**, **Chai J**, **Maley K**, **Cozens G**, et al. 2010. A growth stimulus is needed for IGF-1 to induce skeletal muscle hypertrophy in vivo. *J Cell Sci* **123**: 960–71.
38. **Phillips SM.** 2011. The science of muscle hypertrophy: making dietary protein count. *Proc Nutr Soc* **70**: 100–3.
39. **Raffaello A**, **Milan G**, **Masiero E**, **Carnio S**, et al. 2010. JunB transcription factor maintains skeletal muscle mass and promotes hypertrophy. *J Cell Biol* **191**: 101–13.
40. **Chan S**, **Seto JT**, **Houweling PJ**, **Yang N**, et al. 2010. Properties of extensor digitorum longus muscle and skinned fibers from adult and aged male and female Actn3 knockout mice. *Muscle Nerve* **43**: 37–48.
41. **Zammit PS**, **Partridge TA**, **Yablonka-Reuveni Z.** 2006. The skeletal muscle satellite cell: the stem cell that came in from the cold. *J Histochem Cytochem* **54**: 1177–91.
42. **Han R**, **Grounds MD**, **Bakker AJ.** 2006. Measurement of sub-membrane Ca^{2+} in adult myofibers and cytosolic Ca^{2+} in myotubes from normal and mdx mice using the Ca^{2+} indicator FFP-18. *Cell Calcium* **40**: 299–307.
43. **Horwitz A**, **Schotland D.** 1986. The plasma membrane of the muscle fiber. In Engel AG, Banker BQ, ed; *Myology*. USA: McGraw-Hill. p. 177–207.
44. **Mayor S**, **Rao M.** 2004. Rafts: scale-dependent, active lipid organization at the cell surface. *Traffic* **5**: 231–40.
45. **Draeger A**, **Monastyrskaya K**, **Burkhard FC**, **Wobus AM**, et al. 2003. Membrane segregation and downregulation of raft markers during sarcolemmal differentiation in skeletal muscle cells. *Dev Biol* **262**: 324–34.
46. **Moral-Naranjo MT**, **Montenegro MF**, **Munoz-Delgado E**, **Campoy FJ**, et al. 2010. The levels of both lipid rafts and raft-located acetylcholinesterase dimers increase in muscle of mice with muscular dystrophy by merosin deficiency. *Biochim Biophys Acta* **1802**: 754–64.
47. **Franzini-Armstrong C.** 1991. Simultaneous maturation of transverse tubules and sarcoplasmic reticulum during muscle differentiation in the mouse. *Dev Biol* **146**: 353–63.
48. **Vigh L**, **Escriba PV**, **Sonnleitner A**, **Sonnleitner M**, et al. 2005. The significance of lipid composition for membrane activity: new concepts and ways of assessing function. *Prog Lipid Res* **44**: 303–44.
49. **Schermelleh L**, **Heintzmann R**, **Leonhardt H.** 2010. A guide to super resolution fluorescence microscopy. *J Cell Biol* **190**: 165–75.
50. **Brameshuber M**, **Weghuber J**, **Ruprecht V**, **Gombos I**, et al. 2010. Imaging of mobile long-lived nanoplatforms in the live cell plasma membrane. *J Biol Chem* **285**: 41765–71.
51. **Owen DM**, **Magenau A**, **Majumdar A**, **Gaus K.** 2010. Imaging membrane lipid order in whole, living vertebrate organisms. *Biophys J* **99**: L7–9.
52. **Han R**, **Campbell KP.** 2007. Dysferlin and muscle membrane repair. *Curr Opin Cell Biol* **19**: 409–16.
53. **Lek A**, **Lek M**, **North KN**, **Cooper ST.** 2010. Phylogenetic analysis of ferlin genes reveals ancient eukaryotic origins. *BMC Evol Biol* **10**: 231.
54. **Evesson FJ**, **Peat RA**, **Lek A**, **Brilot F**, et al. 2010. Reduced plasma membrane expression of dysferlin mutants is attributed to accelerated endocytosis via a syntaxin-4-associated pathway. *J Biol Chem* **285**: 28529–39.
55. **Lo HP**, **Cooper ST**, **Evesson FJ**, **Seto JT**, et al. 2008. Limb-girdle muscular dystrophy: diagnostic evaluation, frequency and clues to pathogenesis. *Neuromuscul Disord* **18**: 34–44.
56. **Cai C**, **Weisleder N**, **Ko JK**, **Komazaki S**, et al. 2009. Membrane repair defects in muscular dystrophy are linked to altered interaction between MG53, caveolin-3, and dysferlin. *J Biol Chem* **284**: 15894–902.
57. **Noonan KJ**, **Farnum CE**, **Leiferman EM**, **Lampl M**, et al. 2004. Growing pains: are they due to increased growth during recumbency as documented in a lamb model? *J Pediatr Orthop* **24**: 726–31.
58. **Grounds MD.** 2008. Two-tiered hypotheses for Duchenne muscular dystrophy. *Cell Mol Life Sci* **65**: 1621–5.
59. **Biggar WD.** 2006. Duchenne muscular dystrophy. *Pediatr Rev* **27**: 83–8.
60. **Quinlivan R**, **Shaw N**, **Bushby K.** 2010. 170th ENMC International Workshop: bone protection for corticosteroid treated Duchenne muscular dystrophy. 27-29 November 2009, Naarden, The Netherlands. *Neuromuscul Disord* **20**: 761–9.

61. **Biggar WD**, **Harris VA**, **Eliasoph L**, **Alman B.** 2006. Long-term benefits of deflazacort treatment for boys with Duchenne muscular dystrophy in their second decade. *Neuromuscul Disord* **16**: 249–55.
62. **Zatz M**, **Betti RT**, **Levy JA.** 1981. Benign Duchenne muscular dystrophy in a patient with growth hormone deficiency. *Am J Med Genet* **10**: 301–4.
63. **Zatz M**, **Betti RT.** 1986. Benign Duchenne muscular dystrophy in a patient with growth hormone deficiency: a five years follow-up. *Am J Med Genet* **24**: 567–72.
64. **Zatz M**, **Betti RT**, **Frota-Pessoa O.** 1986. Treatment of Duchenne muscular dystrophy with growth hormone inhibitors. *Am J Med Genet* **24**: 549–66.
65. **Porter JD**, **Merriam AP**, **Khanna S**, **Andrade FH**, et al. 2003. Constitutive properties, not molecular adaptations, mediate extraocular muscle sparing in dystrophic mdx mice. *FASEB J* **10**: 893–901.
66. **Zeiger U**, **Mitchell CH**, **Khurana TS.** 2010. Superior calcium homeostasis of extraocular muscles. *Exp Eye Res* **91**: 613–22.
67. **Decary S**, **Hamida CB**, **Mouly V**, **Barbet JP**, et al. 2000. Shorter telomeres in dystrophic muscle consistent with extensive regeneration in young children. *Neuromuscul Disord* **10**: 113–20.
68. **Sacco A**, **Mourkioti F**, **Tran R**, **Choi J**, et al. 2010. Short telomeres and stem cell exhaustion model Duchenne muscular dystrophy in mdx/mTR mice. *Cell* **143**: 1059–71.
69. **Alexakis C**, **Partridge T**, **Bou-Gharios G.** 2007. Implication of the satellite cell in dystrophic muscle fibrosis: a self-perpetuating mechanism of collagen overproduction. *Am J Physiol Cell Physiol* **293**: C661–9.
70. **Brack AS**, **Conboy MJ**, **Roy S**, **Lee M**, et al. 2007. Increased Wnt signalling during aging alters muscle stem cell fate and increases fibrosis. *Science* **317**: 807–10.
71. **Albrecht DE**, **Garg N**, **Rufibach LE**, **Williams BA**, et al. 2009. 3rd Annual Dysferlin Conference 2–5 June 2009, Boston, Massachusetts, USA. *Neuromuscul Disord* **19**: 867–73.
72. **Albrecht DE**, **Garg N**, **Rufibach LE**, **Williams BA**, et al. 2011. 4th Annual Dysferlin Conference 11–14 September 2010, Washington, USA. *Neuromuscul Disord* **21**: 304–10.
73. **Shavlakadze T**, **Grounds MD.** 2003. Therapeutic interventions for age related muscle wasting: importance of innervation and exercise for preventing sarcopenia. In Rattan S, ed; *Modulating Aging and Longevity*. Singapore: Kluwer Academic Publisher. p. 139–66.
74. **Cruz-Jentoft AJ**, **Baeyens JP**, **Bauer JM**, **Boirie Y**, et al. 2010. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age Ageing* **39**: 412–23.
75. **Chan S**, **Head SI.** 2010. Age- and gender-related changes in contractile properties of non-atrophied EDL muscle. *PLoS One* **5**: e12345.
76. **Sayer AA**, **Syddall H**, **Martin H**, **Patel H**, et al. 2008. The developmental origins of sarcopenia. *J Nutr Health Aging* **12**: 427–32.
77. **Patel H**, **Jameson K**, **Syddall H**, **Martin H**, et al. 2011. Developmental influences, muscle morphology and sarcopenia in community dwelling older men. *J Gerontol A Biol Sci Med Sci*, in press, DOI: 10.1093/gerona/glr020.
78. **Bogin B.** 1999. *Patterns of Human Growth*. Cambridge: Cambridge University Press. p. 455.
79. **Grounds MD**, **Radley HG**, **Lynch GS**, **Nagaraju K**, et al. 2008. Towards developing standard operating procedures for pre-clinical testing in the mdx mouse model of Duchenne muscular dystrophy. *Neurobiol Dis* **31**: 1–19.
80. **Willmann R**, **Possekel S**, **Dubach-Powell J**, **Meier T**, et al. 2009. Mammalian animal models for Duchenne muscular dystrophy. *Neuromuscul Disord* **19**: 241–9.
81. **Smith BF**, **Yue Y**, **Woods PR**, **Kornegay JN**, et al. 2011. An intronic LINE-1 element insertion in the dystrophin gene aborts dystrophin expression and results in Duchenne-like muscular dystrophy in the corgi breed. *Lab Invest* **91**: 216–31.