

Adaptive processes in skeletal muscle: Molecular regulators and genetic influences

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Abstract

Skeletal muscle is a highly adaptable tissue. It responds to environmental and physiological challenges by changes in size, fibre type and metabolism. All of these responses are underpinned by our genes and it is therefore generally assumed that genetic variation between individuals may account for the differences in musculature and athletic capabilities between people. Research into the genetic influences of our muscle is at an embryonic stage, but some early insight into potential regulators has recently emerged, which is reflected in this review. Broad heritability, which appears to affect muscle size and strength more than metabolism has been assessed in twin and sibling studies. It appears to account for more inter-individual variation in the young as opposed to older people. However, the studies reported to date do demonstrate a large degree of diversity, which is probably predominantly due to different methodological approaches being adopted as well as distinct populations being studied. At a molecular level, there has been enormous progress in identifying regulators of atrophy and hypertrophy through the study of knock-out and transgenic animals and also through the utilisation of cell culture models. Among others, the insulin-like growth factors, calcineurin, desmin, myf5, mrf4, MyoD and myogenin have been identified as positive regulators of muscle size, while TNF-alpha, myostatin and components of the ubiquitin pathway have been recognized as regulators of muscle wasting. However, given the ethical and mechanistic constraints of performing similar studies in humans, difficulties have arisen when attempting to translate the animal and cell culture findings to humans. However, the current search for target "exercise genes" in humans has yielded the first successful results. Variations in the genes encoding for: the angiotensin converting enzyme, alpha-actinin 3, bradykinin, ciliary neurotrophic factor, interleukin-15, insulin-like growth factor II, myostatin and the vitamin D-receptor have all been found to account for some of the inter-subject variability in muscle strength or size. However, the influences of these genetic variations are somewhat weak, and not always reproducible and furthermore they are predominantly based in young healthy people. Hence, a key topic, namely the molecular mechanisms of muscle frailty in the elderly still remains to be elucidated.

Keywords: Training, Exercise, Physiology, Exercise Genes, Hypertrophy, Atrophy

Background

Skeletal muscle is highly adaptable, responding to the stresses placed upon it. These demands vary from growth, the maintenance of posture, extreme athletic performance to the repair of injury. In addition to these positive physiologi-

cal responses, skeletal muscle declines in mass and function with age, disuse and disease. This extreme plasticity of skeletal muscle is in part regulated by genetic controls.

In humans, skeletal muscle is derived at approximately six weeks of gestation. Embryonic mesodermal stem cells originating from the myotome of the somites migrate into adjacent embryonic connective tissue, where they become determined as precursor skeletal muscle cells or myoblasts. After a period of proliferation, these cells fuse with one another to form multi-nucleated skeletal muscle cells, or myofibres. This change in phenotype is dependent on the tightly orchestrated activation of a number of muscle specific transcription factors¹⁻³ belonging to the myf family of genes and including: myf5, myoD, MRF4 and myogenin⁴. The transition of myoblasts to myotubes is counter-regulated by additional

The authors have no conflict of interest.

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Accepted 1 December 2005

Abbreviations (in alphabetical order)	
1-repetition maximum	1-RM
adenosine tri-phosphate	ATP
alpha-actinin 3	ACTN3
angiotensin converting enzyme	ACE
basic helix-loop-helix	bHLH
bradykinin β_2 receptor	B ₂ R
ciliary neurotrophic factor	CNTF
cross sectional area	CSA
dizygous	DZ
dystrophin glycoprotein complex	DGC
growth differentiation factor 8	gdf8
human immunodeficiency virus	HIV
insulin-like growth factor binding protein-3	IGFBP-3
insulin-like growth factor-I	IGF-I
insulin-like growth factor-II	IGF-II
interferon- γ	IFN- γ
interleukin-1	IL-1
interleukin-15	IL-15
interleukin-6	IL-6
monozygous	MZ
myocyte enhancer factor 2	MEF2
myosin heavy chain	MHC
nitric oxide synthase	NOS
nuclear factor of activated T cells	NFAT
protein phosphatase 2B	PP2B
single nucleotide polymorphism	SNP
transforming growth factor- β	TGF- β
tumour necrosis factor- α	TNF- α
vitamin D receptor	VDR

transcription factors and proteins including *id* and *myostatin*, which prevent the transcriptional activity of the *myf* group of proteins; indeed, mutations in the latter culminate in a double-muscle phenotype⁵⁻⁷.

In humans, the transition from myoblasts to multi-nucleated myotubes occurs between weeks seven and nine of gestation. At this time, the synthesis of the contractile proteins actin and myosin begin, and the first signs of cross striations are evident⁸. At approximately 10 weeks, innervation of the fibres occurs, and muscle gradually responds to the contractile activity by converting to adult-type myosins. From 11 weeks, the fibres grow in circumference and length, however, by 24 weeks of gestation it is believed that fibre numbers are set, with any further cross-sectional growth occurring as a consequence of hypertrophy and not hyperplasia; this is confirmed in rat models, where fibre numbers do not change but cross-sectional area increases 10-fold through life⁹. Hypertrophy occurs during the process of normal growth, but also following resistance exercise, and occurs as a consequence of increased protein synthesis¹⁰. Increases in muscle load stimulate the expression of a multipotent peptide growth factor, designated insulin-like growth factor-I (IGF-I), whose

activity is sufficient to stimulate hypertrophy of skeletal muscle. In addition to circulating factors inducing hypertrophy, a number has been shown to induce atrophy. These include interleukin-1 (IL-1), tumour necrosis factor- α (TNF- α)¹¹, and more recently the transforming growth factor- β (TGF- β) family member, myostatin, which causes atrophy in adult animals¹² and is associated with atrophy of microgravity¹³, AIDS¹⁴ and ageing¹⁵. In contrast to hypertrophy, conditions leading to atrophy are associated with increases in the rates of ubiquitin-mediated protein degradation. Indeed, recent studies have shown that the expression of two muscle-specific ubiquitin ligases *MURF-1* and *Atrogin-1* are upregulated in multiple situations of atrophy^{16,17}. Furthermore, preliminary studies in murine models of cachexia suggest that altered ACE activity culminates in elevated angiotensin-II levels, which predispose to enhanced protein degradation via the ubiquitin pathway, and ultimately culminate in muscle loss¹⁸.

Two key contractile proteins essential for normal skeletal muscle function and alluded to above are actin and myosin. Actin filaments are variable in length and together with tropomyosin and the troponins form the thin filaments of myofibres. Actin filaments join at one end via α -actinin to form the z-line. Thick filaments consist of approximately 300 myosin molecules bound together, and arranged such that the actin filaments can slide between them. Each thick filament is surrounded by 6 actin thin filaments. A complete unit from z-line to z-line is known as a sarcomere and it is the addition of these units that enables muscle to grow in length⁸.

In addition to these contractile proteins, a number of additional structural proteins exist, whose function is to maintain the architecture of the sarcomere. Titin is an extremely large molecule and is thought to provide the template upon which myosin monomers condense as well as the longitudinal stability of the sarcomere¹⁹. Nebulin is another large protein, which is believed to act as a template for the formation of actin monomers, and also to provide stability for these filaments once formed. While α -actinin²⁰ binds the thin filaments together at the z-lines, desmin²¹ links the z-lines of adjacent myofibrils, maintaining sarcomere adhesion and structure.

It is not, however, sufficient to form these organised contractile structures, in order for them to function, they need to be attached to the lipid bilayer of the cell, which in turn needs to be anchored to the extracellular matrix of the basement membrane. Dystrophin is another extremely large protein, which anchors the sarcomeres to the cell surface and via interactions with cell membrane proteins including the dystroglycans and sarcoglycan, forms a link to laminin in the basement membrane²². In addition to its structural role of generating an anchor between actin of the sarcomeres and the extracellular environment, dystrophin is also linked to signalling molecules such as nitric oxide synthase (NOS) via the syntrophins²³ and caveolin-3 in the dystrophin glycoprotein complex (DGC), which plays an additional role in the organisation of T-tubules²⁴. The importance of all of these

proteins for muscle mass and function becomes rapidly evident in disease states, where absences or defects in any of these genes are associated with a range of the muscular dystrophies²⁴⁻²⁶.

Mechanical regulation of protein turnover

All biological materials are constantly in a state of flux. Every protein has its own steady state exchange rate that varies from seconds to weeks, with the contractile proteins among the most stable of all proteins, having half lives of 10 (MHC) to 20 (actin) days, when maintained within the sarcomere²⁷. However, if either of these molecules is removed from its protection within an intact filament, it is susceptible to rapid degradation, within a new half-life of minutes. This generates the question of what fosters the unravelling of filaments, in order that degradation may follow. Removal of load is believed to play a key role. Indeed, this is witnessed in situations of space flight, artificial dispersion of tissues into culture, or the inhibition of contraction, all of which are sufficient to reduce MHC and actin content of myocytes with an associated disappearance of sarcomeres²⁸. Thankfully, these are reversible effects, with an increase in load or activity, which can be influenced by altering calcium levels, enhancing reassembly of the sarcomere²⁹. Since protein turnover rates may vary under given mechanical conditions, it is possible to imagine a situation where such variation and hence atrophy or hypertrophy may be influenced by our genes.

Clinical studies of disease as well as exercise trials in humans together with modern molecular biological technologies involving gene transfer, protein tagging, transgenic and knock-out animals as well as primary stem cell cultures are beginning to provide clues as to the molecular and morphological responses of muscle cells to the altered demands placed upon them. While research into the genetic influences of growth and development has been topical for many years, more recent seminal investigations by Bouchard et al., have demonstrated that genes (and not simply exercise) determine our fitness³⁰. Fuelled by these data, studies investigating the impact of our genes on human muscle performance have evolved. "Fitness genetics" is a rapidly growing field with implications for both health and disease.

Broad heritability in skeletal muscle

What then is the relative importance of 'genes' for adaptive processes in skeletal muscles? To set up the system of co-ordinates, let us first consider a classical study. In a co-twin case-control study, Spungen et al. investigated the body composition of monozygotic twin pairs, one of whom was paralysed due to spinal cord injury³¹. Comparing the lean tissue mass, the authors found a reduction of ~50% in the legs of the paralysed sibling. It should be considered that, after spinal cord injury in voluntary muscle contractions are quite common. In conditions where the loss of neuromuscular traffic is complete, such as the Guillain-Barré syndrome,

muscle tissue is also lost more or less completely. These extreme examples suggest that maintenance of skeletal muscle crucially depends on neuromuscular activation and not upon our genes. However, in response to muscle activation (or training) only a small proportion of individuals boost up their muscle function to such an extent that they eventually win Olympic gold medals. There is accumulating evidence now that genetic variation may largely contribute to this.

In discussing skeletal muscle, the first, obvious questions usually are: how large are your muscles? And, how strong are they, i.e., how large is the maximal force at a given contraction velocity? We therefore initially want to discuss the current knowledge of genetic influences on muscle mass and muscle 'strength'^a. In this section, we review the most relevant twin (and some family) studies, in an attempt to give a general impression of the overall impact of our genes in pre-determining inter-individual variation in muscle mass.

Muscle mass and 'strength'

Most human studies to date, investigating the role of our genes in determining muscle size and function have focused on our upper limbs. In young males (17-30 years), muscle mass, CSA and strength in flexion of the arm, are underpinned by strong heritable influences³². Studies of 1-RM and the muscle CSA demonstrated that additive and dominant genetic effects accounted for 80 to 90% of the observed variation in the muscles of study. Genetic contributions were also observed when studying peak torques in isometric and isokinetic contractions and the amplitude of the electromyogram. Interestingly, the genetic influences were specific to joint angle and contraction speed, reflecting supposedly anatomic features such as the joint angle-lever relationship and length-force relationship of skeletal muscle. Furthermore, the importance of genetic factors was greater for eccentric than for concentric contractions^b. In addition to this observation, MZ twins appear to be discordant for muscle damage induced by eccentric exercise³³, suggesting a non-genetic predisposition to muscle damage.

In a large sample of postmenopausal twin pairs, genetic components were found to contribute to 30% of the variability in grip strength seen within this group³⁴. A similar study performed in twins, in both males and females in their 7th decade of life, further demonstrated, after various

^a The term muscle strength is used ambiguously in the literature, to describe force, torque or power, either during voluntary contractions or upon electrical stimulation. In order to be consistent, we here use the term muscle "strength" (with speech marks), very much like a lay-word, in order to describe the general capability to generate joint torque against external resistance. Conversely, we use the term strength (without speech marks) for the maximum voluntary isometric force.

^b Importantly, it is the eccentric contraction during which the generated forces are largest.

"anthropometric" corrections, that grip strength depicted a broad heritability of 65%³⁵. A follow-up study 10 years later demonstrated that heritability of grip strength had decreased to only 22-35%³⁶. Still another study, again involving twins but over a broader age range (45 to 96 years) reported a heritability of strength of ~50%, without any impact of age³⁷.

Utilising the same sample population as in³² above the authors extended their studies by investigating the influence of genetic factors upon training³⁸. After a 10-week resistive exercise programme, the 1-RM in elbow flexion was enhanced by ~50%, the isometric torque was increased by ~10%, but CSA was changed by only 4%. According to the authors, 20% of the improvements in 'strength' performance were attributable to genetic factors. From this particular study, it would thus appear that the genetic contribution to baseline variation within the group was larger than the contribution to the variation observed in the training effects.

Not all studies have been restricted to the arms. Some have focused on the trunk musculature, where again, our genes appear to play a role in determining size. Here, while heritability was reported to account for ~70% of the variance in anatomical CSA of paraspinal muscles³⁹, it was found that levels of physical activity played a negligible role for variation in CSA. In another, large twin study (subject age between 35 to 70 years), however, genetic factors accounted for 60% of the variation in maximum isokinetic trunk extension⁴⁰.

Compared to the upper body, little is known about the heritability of muscle mass and strength in the lower limbs. In a family study by Zhai et al. muscle 'strength' in adult children of patients with osteoarthritis was found to depict a heritability of ~40%⁴¹, which seems to be comparable to the heritability reported in most of the studies on grip strength (see above). Although another study in 748 siblings yielded much higher estimates (82-96%), heritability was, again, found to be comparable for the lower and upper body and trunk isometric strength⁴². The same study reports somewhat lower heritability estimates of concentric strength (63-87%), which were likewise comparable for the upper and lower extremities. Contrasting these studies, in a population of twins age 63-76 years, a genetic component was found to account for only 14% of the variability in hand grip strength, and for 31% of knee extension strength⁴³.

Power and metabolism

Muscle power is the product of force and velocity of a muscle's contraction. Anaerobic power is the power that muscles can develop without relying on oxidative metabolism. It is crucial for brisk, rapid and forceful movement. In contrast to this, aerobic power relies upon the replenishment of energy stores through the oxidation of metabolites. Aerobic power is the basis for endurance performance.

While data pertaining to muscle mass and strength are already scant, even less is known about the heritability of muscle power. Genetic influences upon whole-body anaerobic power have been investigated by Calvo et al.⁴⁴. Using the

'Wingate'-test, the authors report heritabilities of 74% for the peak power and of 84% for the total power over 30 seconds. In a study in postmenopausal twins, genetic components were found to account for 46% of the variability in knee extension power³⁴. The same study found a heritability of lean body mass of 52%. Together, these data, while limited, do nevertheless suggest an element of genetic regulation of anaerobic power.

More data have been derived from investigations into the genetic influences of aerobic power. Engstrom and Fischbein assessed the heritability of isometric torque and aerobic capacity in 8 young male twins⁴⁵. They concluded from their studies that aerobic capacity is more influenced by environmental factors than is muscle strength. Similarly, when comparing MZ twins who were discordant for smoking, Larsson and Orlander found that fibre type distribution is more heavily affected by the habit of smoking than it is by the habits of exercise or genes⁴⁶. It should be noted that fibre transformation (from fast to slow types) is one of the most important changes for the adaptation of skeletal muscle during endurance training, suggesting in these studies at least, our genes are less crucial for the fiber type distribution than environment. By contrast, studies in prepubertal and pubertal twins (age 11 to 14 years) who underwent a 6-month running training trial revealed that the relative importance of hereditary factors for the improvement of aerobic power was approximately 45%⁴⁷. Finally, in the trunk, genetic influences have been found to account for only 5% of the variation in endurance of isometric extension⁴⁰. Interestingly, endurance of trunk extension is predictive of chronic lower back pain.

The mechanical output of skeletal muscle does not only depend on its mass and fibre type, but also by its architecture, i.e., the angle of pennation and the fascicle length. For the gastrocnemius muscle, there appears to be a particularly large intra-pair correlation ($r=0.66$ to $r=0.98$) with regard to these measures in 9 pairs of adult twins of both genders⁴⁸. However, muscle architecture is also known to be affected by training, disuse, and ageing^{49,50}, factors that have not been considered in this study. Hence, it is not currently possible to give an estimate of the heritability of muscle architecture.

As with all studies of broad heritability, genetic effects may be under- or over-estimated by twin or family studies. Moreover, and as alluded to specifically above, direct effects of heritability cannot be discerned from indirect effects. That 'indirect' genetic effects may indeed play a role has been shown by Maia et al.⁵¹. In these studies, the authors showed that heritability of sports participation, as assessed by the Baecke questionnaire⁵², appears to be different in males, where genetic factors accounted for ~70% of the variation, and in females where it accounted for only ~40% of the variation. These findings therefore partly raise the question of interpretation of cross-sectional heritability findings as having direct effects in skeletal muscle responsiveness.

Interpretation

It is difficult to dissect simple, uncontroversial 'messages' from the above findings. This may reflect the different approaches and methods used in the studies, but also genetic and environmental differences in the study samples. Perhaps, the only uncontroversial summary would be to suggest that genetic influences upon skeletal muscle, be they large or small, were found in all studies. It is also possible that such genetic effects become less important with increasing age – at least as is suggested by some studies^{32,34,35,42,43}, while only one study appears to contradict this notion³⁷. Moreover, there is some evidence to suggest that endurance and metabolic adaptation of skeletal muscle is, by comparison, less affected by genetic influences than muscle mass and strength. This conclusion is supported by the heritability studies that suggest a genetic influence of approximately 70% for paraspinal muscle CSA³⁹ and of approximately 60% for maximum isokinetic trunk extension torque⁴⁰, but of only 5% for endurance of trunk extension⁴⁰.

Positive regulators of skeletal muscle in animal models

While the data described above, derived from human exercise studies are compelling and thought provoking, they currently provide more questions than answers. Although the field of "fitness genetics" is highly topical and becoming ever more popular, in order to carefully direct future studies in humans, it is imperative that we do not ignore the vast field of research that has been conducted in animal and cell models. Although these studies evolved from different questions, e.g., to gain an understanding of how specific molecules function, or to better comprehend the mechanisms underlying our successful growth and development, they provide us with core data at the level of muscle growth and function. Due to the fundamental importance of these studies to muscle biology, key findings are summarised in the sections below. By virtue of its nature, this is an enormous field of research, spanning decades, and to summarise all seminal findings would be beyond the scope of this review, therefore only a selection of core molecules, as alluded to in the background above, will be described here. We apologise in advance for numerous omissions that inevitably will have occurred.

Potential triggers: The insulin-like growth factors (IGFs)

The importance of peptide growth factors in growth and development is well established. Like other multifunctional peptide growth factors, the insulin-like growth factors (IGF-I & -II) elicit diverse effects on a variety of biological processes, and have a broad range of functions in the embryo, fetus and adult. For the full biological potential of the IGFs to be realised, we need a better understanding of

their modes of action. While multipotent, it is clear that they have key roles to play in skeletal muscle⁵³.

In direct contrast to the diminished muscle mass seen in mice lacking IGF-I or the IGF-I receptor^{54,55}, local overexpression of IGF-I in skeletal muscle stimulates myofibre hypertrophy and maintains muscle mass and strength during ageing^{56,57}. IGF-I mRNA and protein are expressed in newly replicating rat skeletal myoblasts following ischaemic or toxic injury⁵⁸⁻⁶⁰. Furthermore, gene expression of IGF-I and -II is induced as an early event of work-induced hypertrophy in pituitary intact and hypophysectomised rats⁶¹, implicating the IGFs as local regulators of growth and regeneration. Additional studies in rodents utilising the model of functional overload of specific muscle groups, following excision of, for example, the gastrocnemius muscle, confirm the early findings.

More recently, studies have investigated and identified the intracellular signalling pathways involved in IGF-induced hypertrophy. Bodine et al. demonstrated that two key enzymes Akt (a serine/threonine protein kinase, which is activated by a number of growth and survival factors) and p70S6 kinase (ribosomal protein S6 kinase (S6K) lies downstream of Akt signalling and is involved in the regulation of cell growth, proliferation and the translational up-regulation of mRNAs coding for components of the protein synthetic apparatus) were essential for hypertrophy to occur. As this signalling pathway regulates several aspects of protein synthesis, the data implicate translational control of protein production as a key component underlying the hypertrophic response⁶². Parallel studies examining the regulators of disuse atrophy induced by hind-limb suspension once again point to roles for Akt and p70S6 kinase as their levels decline during the development of atrophy but are restored during recovery; furthermore, forced expression of Akt culminated in increased muscle CSA even in the absence of weight-bearing activity⁶². Taken together, these studies support a role for IGF-stimulated signalling pathways that culminate in growth as a consequence of *de novo* protein synthesis.

Resistance training also culminates in increased muscle mass. Control mice subjected to regular sessions of treadmill running, display 25% increases in CSA of skeletal muscle; this response is doubled if studies are performed in transgenic mice overexpressing IGF-I in skeletal muscle⁶³. Responses are blocked in mice expressing an inactive IGF-I receptor⁶⁴.

In addition to playing a role in hypertrophy and recovery from disuse, the IGFs play important roles in muscle regeneration following loss or injury. The ability of mice to recover from injury induced by injection of cardiotoxin is significantly increased in IGF transgenic mice, compared with controls⁶⁵. Furthermore, in two animal models of disease, amyotrophic lateral sclerosis and Duchenne's dystrophy, local administration of the IGF-I transgene to muscle significantly delayed disease onset, progression and severity⁵³, and was thought to act in part at the level of the muscle satellite cell. The mechanisms underlying the ability of the IGFs to promote myoblast differentiation are not clear, but are thought to involve activation of myogenin⁶⁶. Although tempting to

speculate about the potential of the IGFs in muscle, introduction of IGF-I as a treatment for skeletal muscle atrophy is complicated, with issues of delivery and tissue selectivity needing to be solved before we can progress.

Potential effectors: calcineurin

Muscles use calcium as a second messenger to respond and adapt to environmental stimuli. Elevation of intracellular calcium levels culminates in the activation of a serine/threonine phosphatase, calcineurin (also called protein phosphatase 2B (PP2B)), which regulates the expression of genes essential for the remodelling of skeletal muscle⁶⁷. Calcineurin, like the IGFs, impacts on a wide variety of tissue types. However, while many of its effectors are ubiquitous, some are restricted to skeletal and cardiac muscle. As discussed above, the myogenic regulatory transcription factors MyoD, myf5, myogenin and MRF4 are upregulated during myoblast differentiation. It is well established that calcium regulates many pathways involved in myogenesis⁶⁷. Similarly, calcineurin plays a key regulatory role early in myogenesis, leading to the transcriptional activation of the myf family of muscle-specific regulatory genes^{68,69}.

Although the role of calcineurin in cardiac hypertrophy is well documented, its role in skeletal muscle hypertrophy is less evident. Indeed, transgenic mice expressing activated calcineurin in their skeletal muscle do not show any features of hypertrophy⁷⁰. However, the inhibition of calcineurin activity in skeletal muscle fibres has not been directly assessed using knock-out technology. What is, however, clear from transgenic studies is that activated calcineurin is associated with increased abundance of oxidative, slow twitch type I fibres⁷⁰. In addition, mice lacking calcineurin have a decrease in oxidative slow fibres⁶⁷. These data suggest that calcineurin is essential for the induction and maintenance of the slow muscle gene programme. These studies were enhanced by data demonstrating that calcineurin lies upstream of NFAT and MEF2, transcription factors critical to the regulation of myf5, myoglobin, MHC and troponin I. Muscle of calcineurin transgenic mice displayed enhanced insulin-sensitivity. This was associated with a 60 % increase in insulin-stimulated glucose uptake and a doubling of GLUT4 transporters, when compared with wild-type littermates⁷¹.

If calcineurin is important for the initiation of differentiation, it may also be important for muscle repair. Indeed, inhibition of calcineurin activity following injury, prevents new fibre formation⁶⁷. Few data exist regarding a role for calcineurin in atrophy. However, following release of hind limb suspension in rats, calcineurin levels increased significantly after 3 days of recovery. Unfortunately, however, calcineurin activity was not measured in these studies⁷².

Together, these findings suggest that following changes in calcium levels, as a consequence of environmental stimuli, calcineurin becomes activated and facilitates remodelling of striated muscle. While it may not play a key role in hypertrophy of skeletal muscle, it appears to be essential for the initiation of

differentiation, repair of damage and the transition of myofibres to an oxidative phenotype. Calcineurin activity also appears to play a pivotal role in the responsiveness of skeletal muscle to insulin, and if targeted correctly, could possibly be utilised as a drug therapy regime to minimise the pathogenesis of insulin resistance, which is becoming a global problem with the increasing worldwide epidemic of obesity and diabetes.

Potential targets: desmin

Desmin is an intermediate filament protein located predominantly within the z-line of striated muscle, and plays a fundamental role in maintaining the cytoarchitecture of muscle. Desmin knock-out mice develop normally and are fertile. Neither early stages of muscle development, nor subsequent maturation of fibres are affected in its absence, or are anatomical or behavioural defects evident at birth²¹. However, after birth, mice lacking desmin display features of cardiomyopathy, skeletal myopathy and smooth muscle dysfunction. Together, these disorders culminate in a reduced lifespan of the animals and render them less tolerant to exercise²¹. Despite the early stages of muscle development being normal in desmin knock-out mice, alterations in function become apparent shortly after birth. The muscles that are most clearly affected are the weight-bearing muscles such as the soleus and the constant use muscles of the diaphragm. Mice lacking desmin are weaker and fatigue more easily than wild-type littermates^{73,74}. Structural organisation of the myofibres is irregular and anchorage of the myofibrils to the plasma membrane at the costameres is lost²¹. Myofibrillogenesis during regeneration is often abortive and disorganised, indicating that desmin is important in repair processes of skeletal muscle; furthermore, cycles of degeneration and regeneration result in increased relative amounts of slow and decreased amounts of fast type myosin heavy chains.

The physiological consequences of decreased desmin expression have been assessed by measuring isometric tension generated in skinned fibres and intact muscle. Tension declined faster than normal in high frequency fatigue situations and was associated with altered calcium release in the knock-out fibres⁷⁵. Furthermore, stretched skeletal muscles of desmin knock-out mice develop greater passive stiffness than controls while skinned fibres develop less active force^{76,77}.

Together, these findings suggest that desmin filaments are involved in the generation of active and passive forces, possibly by supporting sarcomere alignment or force transmission. They suggest that desmin is essential for the structural integrity of skeletal muscle, for optimal excitation-contraction coupling and for long-term maintenance and/or repair of muscle. It does not, however, appear to be involved in commitment, differentiation or fusion of skeletal muscle.

Potential targets: myogenic regulatory factors

Muscle development is the result of the combined action of several factors ultimately culminating in the generation of

a heterogeneous population of slow and fast twitch fibres. Potential mediators of fibre specific transcription include the muscle-specific myogenic regulatory factors myf5, MRF4, MyoD and myogenin; all members of a more ubiquitous family of basic helix-loop-helix (bHLH) proteins. Gene targeting studies have provided important insights into the role of these proteins during specification and differentiation. The four factors are expressed in temporally and spatially distinct patterns, controlling different stages of the myogenic programme⁷⁸. MyoD and myf5 are required for the commitment of stem cells to the myogenic lineage, whereas myogenin and MRF4 regulate the transition of these committed cells to multinucleated myofibres.

It has been demonstrated that MyoD transcripts are preferentially expressed in fast-type fibre types, however, little fibre specificity has been ascribed to the other factors⁷⁹. Moreover, removal of MyoD via knock-out technology resulted in little perturbation in fibre type, while mice carrying null mutations of the other 3 regulatory factors, either failed to form differentiated fibres or showed no obvious phenotype⁷⁸.

Although the role of these regulatory factors has been extensively studied during embryogenesis, as well as in cell culture models of differentiation, little is actually known about their roles during post-natal life. MRF4 levels remain elevated post-partum and throughout adult life, whereas the abundance of MyoD, myf5 and myogenin all decline within one week of birth. However, during ageing, disease and injury, these regulatory factors are re-activated in an attempt to reinitiate the myogenic programme and repair the damage or loss⁷⁸. The relative success of these reinitiated processes depend largely on the age of the subject and the extent of the damage.

Regulators of skeletal muscle atrophy in animal models

Having examined a select few of the key players involved in skeletal muscle hypertrophy, commitment, repair and function, we would now like to turn our attention to factors involved in the progression of atrophy. Based on the findings of knock-out studies above, one could imagine a situation where atrophy was simply the converse of hypertrophy. However, this would be remiss, because during atrophy, a distinct and progressive process of protein degradation and turnover is triggered. It is therefore not too surprising, given the high rate of basal protein turnover in muscle (250-300g/day) that any small but persistent changes in protein degradation will culminate in loss. Skeletal muscle atrophy occurs as a result of disuse, ageing and catabolic diseases. Regardless of the inciting event, physiologically, atrophy is characterised by decreased protein content of the affected muscles, decreased fibre diameter and force production and decreased fatigue resistance. Currently no therapeutic interventions prevail to slow skeletal muscle loss. The precise cascade of events leading to muscle loss is not known. However, many potential triggers and signalling molecules have been identified and are being studied.

Potential triggers: tumour necrosis factor- α (TNF- α)

Extensive studies have been performed investigating the potential roles of TNF- α in skeletal muscle loss. TNF- α is believed to be important in the pathogenesis of muscle wasting, with acute administration causing severe, transient weight loss in rodents⁸⁰, and prolonged exposure resulting in profound wasting^{80,81}. Site-specific TNF- α production also alters the pattern of tissue wasting⁸¹, suggesting that local production may be sufficient to induce degeneration. Neutralising TNF- α antibodies administered to tumour-bearing mice reduced protein and fat losses, slowed tumour growth, and prevented TNF- α -induced cytokine/hormonal cascades (glucagon, cortisol, interleukins-1 and -6 (IL-1 and -6), and interferon- γ (IFN- γ))^{81,82}. Furthermore, implantation of Lewis lung carcinoma cells into wild-type and TNF- α type 1 receptor knock-out mice, increased circulating levels of TNF- α in both groups, but had little effect in the knock-out animals⁸³. Finally, intravenous infusion of TNF- α into rats caused a significant reduction in circulating levels of IGF-I and IGFBP-3, confirming that TNF- α directly impinges on the IGF system⁸³, which is critical for positively regulating muscle mass. While the molecular basis of muscle wasting in cancer is still being investigated, these animal models are supported by the observation that TNF- α levels may be elevated in the circulation of cancer patients.

In addition to the disease state, altered IGF and TNF- α levels are witnessed in ageing, where muscle mass also declines, albeit over several decades. In this scenario, IGF-I declines with age, while TNF- α and IL-6 levels increase⁸⁴. High circulating levels of TNF- α and IL-6 with low circulating levels of IGF are synergistic risk factors for poor muscle strength and independent risk factors for mortality. Indeed, the circulating and intramuscular concentrations of IGF-I, and the responsiveness of muscle to IGF-I are reduced in most medical conditions where circulating cytokines are elevated (to levels often seen in the elderly) and muscle wasting is evident⁸⁴.

Despite this large and persuasive body of evidence, there is currently no evidence to suggest that TNF- α plays a key role in either disuse atrophy or in unloaded muscle⁸⁵. A potential explanation for this discrepancy is the fact that ageing and cachexia (as well as damage, when TNF- α levels are also elevated) are associated with systemic, immunological changes in the body, whereas the disuse or suspension of given limbs are not as yet associated with inflammatory responses.

Potential triggers: myostatin

Myostatin, a family member of the TGF- β superfamily, functions as a negative regulator of muscle growth⁸⁶. It was first identified in a strain of cattle which displayed a double muscled phenotype, which was associated with a mutation in the myostatin gene, rendering it non-functional⁸⁷. Confirming

an important role for myostatin in muscle, mdx mice injected with neutralising antibodies to myostatin displayed increased muscle mass following 3 months of treatment⁸⁸.

Interestingly, mice generated to be null for myostatin, like the cattle, had larger muscles than their wild-type littermates, but upon closer examination, it was found that the increased size was attributable to an increase in fibre numbers and not to increased CSA⁸⁹. These data suggest that myostatin acts early in muscle development, perhaps by preventing the proliferation of muscle precursor cells, hence ultimately reducing fibre number. However, contrasting this theory, administration of neutralising myostatin antibodies to adult animals, caused features of hypertrophy and not hyperplasia, while mice administered with myostatin displayed features of atrophy⁹⁰, implicating a direct role for myostatin on the differentiated fibres. Substantiating this latter finding, murine skeletal muscle cells exposed to myostatin displayed decreased protein content and had reduced rates of protein synthesis compared with controls⁹¹. Furthermore, increased myostatin activity is associated with: muscle wasting in HIV-infected men, prolonged bed-rest in young men, disuse atrophy in older patients and muscle loss associated with age⁸⁶. Strength training, which leads to increased muscle mass is associated with decreased myostatin levels in both old and young people⁹².

Regardless of how myostatin is functioning in these models, they all suggest that its activity is associated with decreased muscle mass. Data contravening these studies do however exist. In rat models of denervation atrophy, myostatin levels were not increased until several days after atrophy was first witnessed. Furthermore, in models of hindlimb suspension, myostatin knock-out mice lost more muscle mass than did controls. Clarifying these differences in responses to myostatin will be essential if it is to be utilised as a therapeutic target, as has been suggested. Given that rates of atrophy vary, it is perhaps not too surprising that myostatin plays different roles in different models.

Effectors: ubiquitin pathway

The ubiquitin proteasome pathway plays a key role in the turnover of muscle protein and is activated in several catabolic conditions which culminate in muscle loss. The addition of ubiquitin chains by ATP requiring enzymes marks the conjugated proteins for degradation by the proteasome into small peptide fragments⁹³. The involvement of this pathway in skeletal muscle atrophy is well established and associated with many pathologies, as well as with models of disuse (unloading, denervation or immobilisation), where inhibitors of the pathway are capable of altering the rate of proteolysis and atrophy. More recently, two genes have been identified whose expression increases significantly in multiple models of skeletal muscle atrophy. These genes are designated *MuRF1* (muscle ring finger 1) and *MAFbx* (muscle atrophy f box or Atrogin1)^{16,17}, and encode ubiquitin ligases, which catalyse the covalent interaction between ubiquitin and the protein substrate.

Skeletal muscle denervation, immobilisation or treatment with glucocorticoids, as well as sepsis all lead to atrophy and the expression of both *MuRF1* and *MAFbx*¹⁶. *MuRF1*, in addition to being a ubiquitin ligase has been shown to bind to Titin and is thought to be involved in its turnover⁹⁴. *MuRF1* and *MAFbx* null mice are phenotypically normal, however, under atrophic conditions these animals lose less muscle mass than their wild-type littermates¹⁶. These seminal studies demonstrated for the first time, that the inhibition of specific ligases was able to directly moderate levels of muscle loss during atrophy, suggesting that these ligases, more so than other ubiquitin pathway members, may provide good early markers of atrophy, as well as potential targets for therapeutic interventions.

Interpretation

The progress made over the last decade, identifying regulators of atrophy and hypertrophy was made possible because of advances in our understanding and use of cell culture, molecular tools and genomic and proteomic studies. Information generated utilising these technologies provide a wealth of knowledge pertaining to skeletal muscle. Difficulties do, however, occur when attempting to compare or translate the findings to humans. When studying transgenic animals, genetic backgrounds often alter responses. In addition, the gene of interest is frequently over-expressed, raising further complications. Reconciliation of data from cumulative models, even of only one gene, must therefore be performed with extreme care. Consideration must be given to genetic isoforms of proteins, expression patterns, promoter strength/duration in driving the transgenes, local vs. systemic expression of the gene etc. In order that this plethora of knowledge can be effectively extended to clinical environments, it is critical that we do not over-interpret single findings, but that we re-interpret results as new findings become available. With this cautionary note in mind, future animal studies should progress in parallel with data arising from human studies of health and disease.

Evidence for genetic regulators: first results in humans

The plethora of data in existence and emerging from the above studies are indeed supported by evidence arising in humans. These data do provide substance to the theory that our responses to training and to disease are indeed regulated in part at the level of our genes.

Angiotensin converting enzyme (ACE)

Angiotensin was initially identified for its role in the regulation of blood pressure. More recently the ACE insertion/deletion (I/D) polymorphism has become the most intensely studied gene polymorphism known to affect skeletal muscle adap-

tation to exercise⁹⁵. The longer allele of the polymorphism co-ordinates with a reduced enzyme activity and seems to be associated with an enhanced efficiency of muscle contractions⁹⁶. Moreover, it appears more frequently in successful endurance athletes⁹⁷. The D-allele has been found to be associated with enhanced quadriceps responses to resistance training in young adults⁹⁸. While promising, controversy does exist in these human studies. A 10-week resistive training program in young adults demonstrated that there was no association between the ACE I/D genotype and either baseline values (pre-exercise) or gains in elbow flexor strength or muscle cross-sectional area (post-exercise)⁹⁹. Likewise, in training studies with 204 elderly subjects, the I/D polymorphism depicted no difference with regards to pre- and post-training levels of aerobic capacity, muscle strength, walking speed or body composition¹⁰⁰. The jury is therefore still hung with regard to the role that the ACE polymorphisms may play in exercise.

Alpha-actinin 3 (ACTN3)

The α -actinins belong to an ancient family of actin binding proteins that play structural and regulatory roles in cytoskeletal organisation. The ACTN3 gene encodes the most highly specialised protein of the four mammalian α -actinins. It anchors the actin filament (the 'passive' strand) in the Z-disk (end of sarcomere). ACTN3 is only expressed in fast twitch fibres (leading it to be dubbed as a gene for speed), and ~20% of the population are homozygous for an allele that leads to a loss of function¹⁰¹. A cross-sectional study demonstrated that the ACTN3 null-genotype is frequently observed in endurance, but less frequently in power athletes¹⁰². A recent interventional study found no association between the ACTN3 genotype and maximal elbow muscle flexor torque or size in men, but a lower muscle strength was reported in women with the null-genotype¹⁰³. After a 12-week program of elbow flexor resistance training, women with the ACTN3 null genotype depicted greater gains in torque compared with controls. This finding corroborates the original hypothesis of the null genotype favouring an 'endurance'-like phenotype. Moreover, the overall genotype contribution to baseline torque was only 2% of the observed variance.

Bradykinin

A role for bradykinin in vasodilation is well established. In the exercise world, interest in this protein has arisen following the discovery that it is degraded by ACE and hence inversely linked with the angiotensin II system. The bradykinin β_2 receptor (B_2R) gene is associated with a polymorphism. This observation raises the pertinent hypothesis that this genotypic variant is associated with phenotypic muscle function, which has been tested in two cross-sectional studies¹⁰⁴. The muscular efficiency of young adults was associated with both the ACE and B_2R genotypes, and among 81 Olympic track athletes, the 'high kinin receptor activity' combination of genotypes was more frequent in endurance

than in power runners, suggesting that the actions of the two systems are intrinsically linked.

Ciliary neurotrophic factor (CNTF)

The gene for the CNTF may be subjected to a null mutation in humans that has been related to the onset of multiple sclerosis¹⁰⁵. CNTF appears to have direct influences on motoneuron survival and differentiation of myotubes and muscle function¹⁰⁶, suggesting a critical regulator of motoneuron function and maintenance. This being said, heterozygous carriers of the null allele reportedly have an enhanced muscle strength, when compared with controls¹⁰⁷. In a cross-sectional study in ~500 subjects aged 20 to 91 years, a CNTF receptor polymorphism has been shown to be associated with an increased lean body mass and knee extensor strength, a result predominantly seen in men¹⁰⁶.

Interleukin-15 (IL-15)

IL-15 is one of the most abundant cytokines found in skeletal muscle, and is believed to be a positive regulator of skeletal muscle maintenance¹⁰⁸. The suggestion of this positive role was derived from studies demonstrating that IL-15 could prevent the muscle wasting of cachexia¹⁰⁹. In an interventional study in young adult men and women, 7% and 3% of the variance in the response to a 10-week program of resistive exercise were independently accounted for by two different polymorphisms of the gene for the α sub-type of the IL-15 receptor¹⁰⁹. Interestingly, these polymorphisms affected muscle mass and muscle functions conversely, the main difference in genotypes thus being a gain in training-induced muscle 'quality'.

Insulin-like growth factor II (IGF-II)

The IGFs are both well recognised for their key functions in muscle (see the section on "Potential triggers: the Insulin-like growth factors (IGFs)" above). They have gained specific interest due to the fact that they are the only peptide growth factors identified to date that can influence both growth and differentiation of skeletal muscle cells. A polymorphism in the gene for IGF-II causing increased IGF-II levels in the blood was found to be associated with increased birth weight and grip strength in a cross-sectional study of men and women aged 64 to 74¹¹⁰. However, only ~1% of the overall variance in grip strength could be accounted for by the IGF-II polymorphism, suggesting that it does not play a directive role in this process.

Myostatin

Myostatin, also known as growth differentiation factor 8 (gdf8), is a potent inhibitor of muscle hypertrophy and, as such, has gained enormous interest in the academic milieu of knock-out and knock-in engineering, generating a plethora

of information as to the roles it elicits in atrophy vs. hypertrophy. In humans, six different polymorphisms have been described¹¹¹, one of which (R153) is reported to affect muscle phenotypes¹¹². This observation has been further substantiated in one small, cross sectional study¹¹¹, however, larger numbers are required and exercise trials remain to be performed to assess the impact of these polymorphisms on basal and stimulated muscle mass.

Vitamin D

A large area of research has focused on the genomic and non-genomic effects of Vitamin D on skeletal muscle¹¹³. It has been reported to impact not only on the transmembranous flows of Ca^{++} and phosphate in skeletal muscle, but also on the synthesis rate of contractile proteins. The vitamin D receptor (VDR) was one of the first for which polymorphisms were reported to be functionally relevant in bone¹¹⁴. In a cross-sectional study of non-obese women, the VDR BsmI polymorphism was associated with differences in quadriceps muscle strength, but not in grip strength¹¹⁵. Another recent, population-based cross-sectional study, has found an association of the poly A repeat and the BsmI polymorphisms with hamstring CSA, but not with quadriceps or grip strength¹¹⁶. Again, these are preliminary data, but point to an important role for our genes in dictating our muscular responses.

Conclusion

While heritability studies suggest a relatively important role of genetics in the biological variation of muscle mass and muscle function, the search for 'candidate' genes is still in its infancy. As of yet, studies into the effects on gene polymorphisms and skeletal muscle have only been able to attribute minor or moderate genetic influences, and different studies have often obtained contradicting results. This is probably due to:

1. The pool of genes involved being larger than those investigated so far;
2. The most important genes not having been studied so far;
3. Models of study (e.g., mostly cross-sectional studies in different populations).

The data to date provide us with insight and challenges, as there is obviously extensive and rewarding work still to be done. From a medical and public health perspective, one of the key questions with regard to skeletal muscle is its interaction with genetics and ageing. As outlined above, heritability studies may indicate a 'wash out' of genetic influences with ageing. On the other hand, ageing itself is at least partly under genetic influence, with different individuals ageing at different rates. However, to the best of our knowledge, there is no single study that related the effects of training to genetics in old age. Future studies, currently missing in the literature, therefore ought to focus on the genetic influences of physical activity, and the subjects' responses, particularly in the elderly population.

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