Of bears, frogs, meat, mice and men: complexity of factors affecting skeletal muscle mass and fat

Thea Shavlakadze* and Miranda Grounds

Summary

Extreme loss of skeletal muscle mass (atrophy) occurs in human muscles that are not used. In striking contrast, skeletal muscles do not rapidly waste away in hibernating mammals such as bears, or aestivating frogs, subjected to many months of inactivity and starvation. What factors regulate skeletal muscle mass and what mechanisms protect against muscle atrophy in some species? Severe atrophy also occurs with ageing and there is much clinical interest in reducing such loss of muscle mass and strength (sarcopenia). In the meat industry, a key aim is optimizing the control of skeletal muscle growth and meat quality. The impaired response of muscle to insulin resulting in diabetes, that is a consequence of the metabolic impact of increasing obesity and fat deposition in humans, is also of increasing clinical concern. Intensive research in these fields, combined with mouse models, is reviewed with respect to the molecular control of muscle growth (myogenesis) and atrophy/hypertrophy and fat deposition (adipogenesis) in skeletal muscle, with a focus on IGF-1/insulin signaling. ***BioEssays*** **28:994–1009, 2006.**

School of Anatomy and Human Biology, The University of Western Australia, 6009, Western Australia

*Correspondence to: Thea Shavlakadze, Mail Delivery M309, School of Anatomy and Human Biology, The University of Western Australia, 35 Stirling Highway, Crawley, Western Australia, 6009.

E-mail: sthea@anhb.uwa.edu.au

DOI 10.1002/bies.20479

Published online in Wiley InterScience (www.interscience.wiley.com).

Abbreviations: CSA, cross sectional area; 4E-BP1, eukaryotic initiation factor 4E-binding protein 1; eIF, eukaryotic initiation factor; ADAM12, a disintegrin and metalloprotease 12; C/EBPα, CCAAT/enhancer binding protein α; HSP, heat shock protein; FABP, fatty acid binding protein; FOXO, forkhead box, sub-group O transcription factor; GSK-3β, glycogen synthase kinase 3β; ERK, extracellular signal regulated kinase; IGF-1, insulin like growth factor 1; IGF-1R, insulin like growth factor 1 receptor; IGFBP, insulin like growth factor binding protein; IRS-1, insulin receptor binding substrate 1; IMTG, intramyocellular triacylgycerols; JNK, c-jun N-terminal kinase; MAFbx, muscle atrophy F-box; MAPK, mitogen activated protein kinase; MAPKAPK, mitogen-activated protein kinase-activated protein kinase; mTOR, mammalian target of rapamycin; MuRF1, muscle RING finger 1; NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor kappa beta; PGC-1α, PPARγ co-activator 1α; PI3K, phosphoinositide 3-kinase; PDK-1, 3-phosphoinositide-dependent protein kinase; PKB or Akt, protein kinase B; PKC, protein kinase C; PPAR, peroxisome proliferation activated receptor; Runx1, myeloid leukemia 1 gene; S6K, S6 kinase; TSC, tuberous sclerosis complex; UCP, uncoupling protein.

Introduction

Skeletal muscle, which constitutes about 40% of the mass of the human body, is specialized contractile tissue that moves all parts of the skeleton and is essential for breathing; skeletal muscle is also important as a source of heat generation and as a regulator of metabolism. Maintenance of muscle mass is of critical importance for a healthy existence. Increase (hypertrophy) or loss (atrophy) of muscle mass has many consequences and comparative studies, over a range of vertebrates, provide insight into the complex molecular signaling that controls this important balance.

In humans, increasing the performance, strength and mass (hypertrophy) of skeletal muscle is of central interest to the science of sports medicine, and adequate skeletal muscle function is fundamental for normal healthy independent living. Loss of skeletal muscle mass and function occurs in disuse atrophy, situations of inflammation (cachexia), chronic heart failure and diseases like muscular dystrophy, with age-related loss of muscle mass and strength (sarcopenia) in humans becoming an increasing problem (Fig. 1). Avoidance of muscle atrophy after long periods of disuse in many species during dormancy raises the possibility of novel interventions to reduce or reverse muscle atrophy in humans with potential esoteric applications for extended space travel. Diet has an important influence on muscle biology and increasing obesity and fat deposition (adipogenesis) in humans, with dire consequences, is of much current clinical concern. Different issues occupy the meat and livestock industries where the key aims of commercial meat production (e.g. for poultry, pig, cattle, sheep) are optimizing the speed and efficiency of skeletal muscle (meat) deposition and improving meat quality (tender-

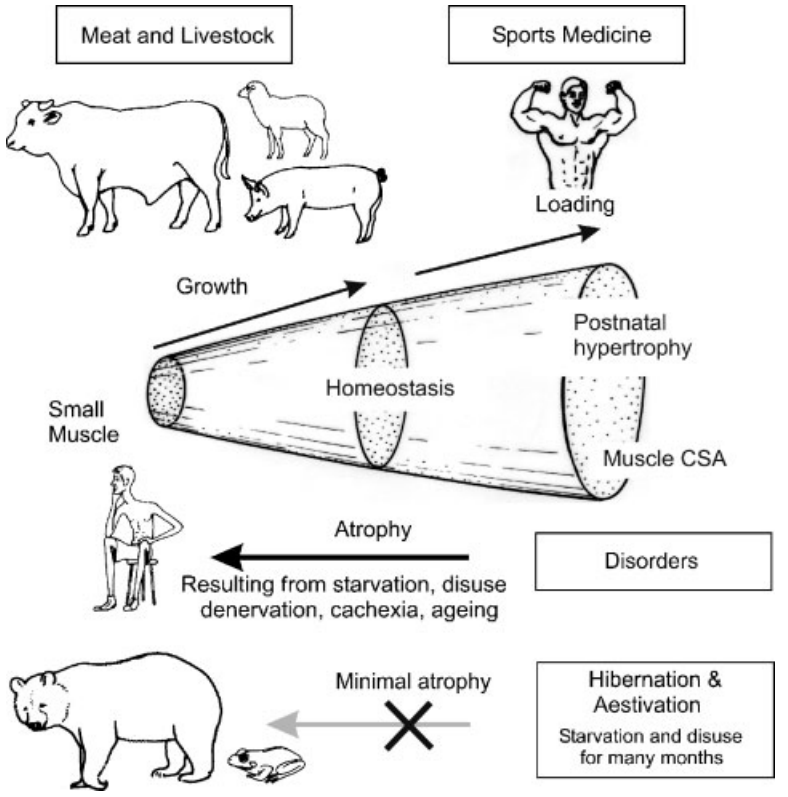

Figure 1. Animal farm: overview of research interests related to skeletal muscle atrophy/hypertrophy for various species. Factors controlling efficient early muscle growth and quality is a research focus of the Meat and Livestock Industries, whereas increased adult muscle size and strength (hypertrophy) is one goal of Sports Medicine. A loss of muscle mass and strength (atrophy) is a major concern in many human conditions and rapid atrophy results when muscles are not used. Yet this same atrophy does not occur in dormant animals (i.e., bears and frogs) even after many months of disuse and starvation. This review attempts to unravel and reconcile the complex signaling cascades that control these events in such diverse situations.

ness, fat content and flavor). Lean meat requires reduced fat, whereas high fat within skeletal muscle (marbling) produces highly priced premium beef. Thus, a greater understanding of the molecular interactions that control the balance between myogenesis and adipogenesis, which are often inversely related in skeletal muscle, has many applications. This review collates information from diverse fields to provide insight into the molecular mechanisms controlling these processes in skeletal muscle.

Skeletal muscle hypertrophy and atrophy

In postnatal life, an increase in skeletal muscle mass occurs during the growth phase and in response to physical activity (loading). Regulation of muscle mass depends on the balance between protein synthesis and degradation: for mass to increase, synthesis should exceed breakdown. Skeletal muscle growth and mass is controlled by nutritional, hormonal and mechanical factors. While nutrition and hormones are essential during the growth phase, increased mass (hypertrophy) of adult skeletal muscle is primarily driven by mechanical factors (exercise and physical loading). A wide range of conditions including disuse, starvation, disease and ageing, lead to a loss of muscle mass (atrophy) and strength. It is important to note that increased muscle size does not always correlate with increased strength.[1]

While protein synthesis increases myofibre protein content and size (cross-sectional area: CSA), fusion of muscle precursor cells (myoblasts derived from satellite cells) with the growing or hypertrophying myofibre maintains a relatively constant protein-to-DNA ratio.[2] Whether satellite cell proliferation is essential for increasing myofibre size is disputed since hypertrophy can be uncoupled from DNA synthesis in tissue culture[3] and in vivo.[4] It seems likely that increased net protein synthesis drives muscle hypertrophy and, in skeletal muscle, this stimulates activation of the satellite cells. In contrast, hypertrophy of heart muscle cells does not involve any cell division: however, discussion of cardiac muscle falls beyond the scope of this review.[5]

Despite common belief, more myoblasts do not always result in larger myotubes/myofibres (hypertrophy). For example, the sustained proliferation of myoblasts lacking MyoD does not generate larger myofibres either during development or regeneration in vivo.[6] In Piedmontese cattle[7] where the lack of myostatin can increase myoblast proliferation, myofibres are of normal or smaller size and more myofibres largely account for the huge increase in overall muscle mass (double muscling). Furthermore, increased fusion of myoblasts into myofibres that increases protein content by ~25% does not result in hypertrophy in MKR mice, which lack functional insulin-like growth factor 1 (IGF-1) and insulin receptors:[8]

these mice have reduced muscle mass at birth due to fewer myofibres per muscle (hypoplasia). Such increased myofibre protein content without hypertrophy (no increased myofibre CSA) also occurs in Rskα-actin/hIGF-1 mice, where skeletal-muscle-specific overexpression of human IGF-1 results in elevated IGF-1 in both muscle and blood.[9]

Myofibre hypertrophy can be disguised by myofibres splitting longitudinally when they reach a critically large CSA[10] and this complicates the analysis of hypertrophy. Such myofibre splitting occurs in quail muscles after stretch:[10] by 16 days after stretch the myofibre CSA increases by 141% (hypertrophy) but, at 28 days, the myofibres start to split and appear smaller ($\sim$39% larger compared to controls) and this results in more myofibre (CSA) profiles.[10] Since each syncitial myofibre develops from fusion of many myoblasts, it can be difficult to clearly distinguish between hypertrophy and hyperplasia.

Hyperplasia is defined as an increase in size of a tissue as a result of enhanced cell division: this easily applies to tissues composed of mononucleated cells. Whether skeletal muscle hypertrophy results from hyperplasia is complicated because skeletal muscle is composed of multinucleated cells (myofibres). It is widely agreed that the number of myofibres is fixed during embryogenesis and does not increase postnatally. However, an increase in absolute numbers of myonuclei within an individual myofibre does occur during growth due to fusion of myoblasts with the enlarging myofibre: this endpoint of an increase in myonuclear, but not cell (myofibre) number may be considered a form of hyperplasia. Due to such complexity, we here generally refer to muscle hypertrophy as increased CSA of individual myofibres leading to increased muscle mass.

Molecular insights into factors regulating skeletal muscle mass

Importance of PI3K/Akt signaling

There is intense interest in the molecular signals that control mammalian muscle growth and hypertrophy and much information comes from experiments using mouse models. Signaling through the PI3K/Akt pathway plays a fundamental role in controlling skeletal muscle mass and metabolism.[11] A particular emphasis has been placed on this pathway because it is regulated by exercise and, importantly, lies downstream of the IGF-1 and insulin receptors. Some of the main interactions of this complex signaling are summarized in Figure 2: here the mechanism of increased protein synthesis (Fig. 2A), combined with inhibition of protein degradation (Fig. 2B), which results in a net increase in protein content and skeletal muscle hypertrophy is presented. Akt seems to be a point of divergence for regulating growth and metabolism and Akt isoforms contribute to the somewhat different roles that IGF-1 and insulin play in skeletal muscles (Fig. 3). Three Akt isoforms Akt1, Akt2 and Akt3 are encoded by distinct genes[12,13] with Akt1 and Akt2 predominately expressed in skeletal muscle, thymus, brain, heart and lung, and Akt3 in brain and testes.[14] Studies using knockout mice show that different Akt isoforms function in a non-redundant way: Akt1 is an important regulator of growth, whereas Akt2 is integral to metabolic regulation. Loss of Akt1 expression in mice results in partial lethality and surviving Akt1 homozygous null mice are growth retarded, although they have normal insulin sensitivity and fat metabolism.[15] In contrast, mice lacking Akt2 develop without growth defects but display insulin resistance.[15] Such differences between Akt1 and Akt2 functions seem to parallel the divergence between IGF-1 and insulin, where the former primarily controls mammalian growth and the latter is one of the most important regulators of metabolism (Fig. 3). Phenotypes of IGF-1 and insulin receptor knockout mice clearly demonstrate such divergence in IGF-1 and insulin functions. IGF-1R homozygous null mice exhibit severe growth deficiency (45% of normal size) and die at birth of respiratory failure,[16] whereas mice lacking the insulin receptor have normal intrauterine growth and development but, at birth, they develop severe hyperglycaemia and hyperketonaemia and die within 48–72 hours.[17] Nonetheless, in skeletal muscle both insulin and IGF-1 stimulate protein synthesis[18,19] and IGF-1 is quite capable of compensating for insulin action in regulating glucose metabolism.[20] Mice with skeletal-muscle-specific inactivation of the insulin receptor develop only mild insulin resistance, whereas mice that lack functional receptors for both insulin and IGF-1 in skeletal muscle (MKR mice) manifest type 2 diabetes.[20] Functional receptors for both IGF-1 and insulin are required for skeletal muscle growth:[21] MKR mice have significantly reduced muscle mass and hypoplasia up to 3 weeks after birth,[20] but then the muscles undergo compensatory hyperplasia and the wet weight of adult hindlimb muscles is only $\sim$10% less than controls.[20] Comparative biology supports this overlapping function of insulin and IGF-1 since worms and flies have a single insulin/IGF-1 receptor. In vertebrates and mammals, divergence in insulin and IGF-1 is reflected by two types of receptors with overlapping functions.

The principal target downstream of Akt responsible for regulation of protein synthesis is mTOR (mammalian target of rapamycin)[22] (Fig. 2A). Homozygous deletion of mTOR in mice is lethal during embryogenesis.[23] In mammals, mTOR functions in association with other molecules and forms two complexes, mTORC1 and mTORC2. Rapamycin-sensitive mTORC1 controls several pathways involved in protein synthesis and determination of cell size. Rapamycin-insensitive mTORC2 controls the actin cytoskeleton and thereby determines the shape of the cell.[22] This review focuses on the function of mTORC1 in skeletal muscle which, for simplicity, we refer to as mTOR. It should be noted that mTOR is not simply a downstream target of Akt. Apart from insulin and IGF-1, which regulate mTOR via activation of Akt,

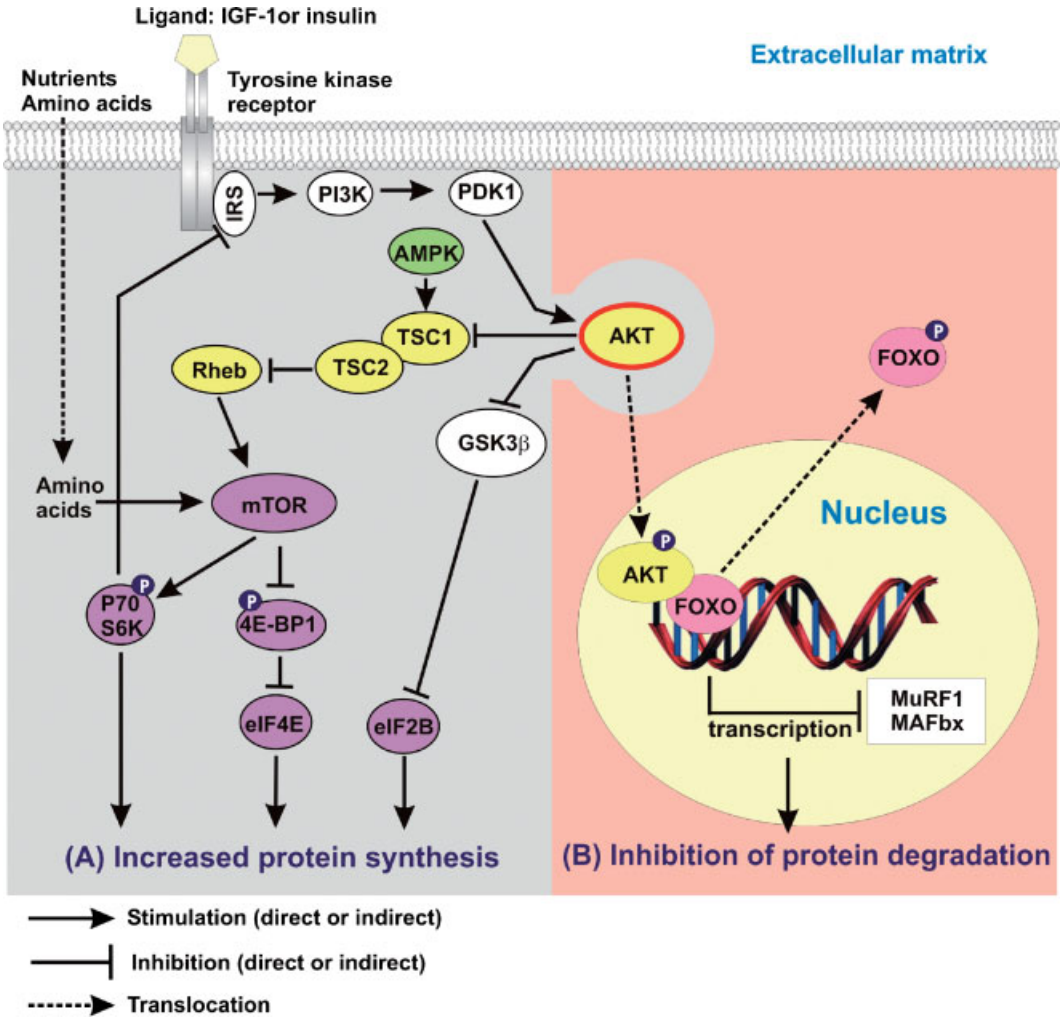

Figure 2. Regulation of protein metabolism by IGF-1 and insulin within skeletal muscle. This diagram summarizes signaling downstream of the IGF-1 or insulin receptor that results in **A:** increased protein synthesis and **B:** inhibition of protein degradation: this net signaling leads to muscle growth (hypertrophy). Akt plays a central role in determining the balance between protein synthesis and degradation. Atrophy is not a simple reversal of this signaling. Instead, atrophy results from activation of other pathways (not shown) that (i) directly activate the atrophy-related genes in the nucleus (by mechanisms independent of FOXO) and also (ii) inhibit Akt phosphorylation, hence FOXO is not phosphorylated and remains in the nucleus to activate the atrophy-related genes (MuRF1 and MAFbx). Comments on specific aspects of this signaling complexity follow. Binding of IGF-1 or insulin to their tyrosine kinase receptors phosphorylates IRS-1 and activates PI3K/Akt signaling that is a principle pathway involved in regulation of protein metabolism in skeletal muscle. mTOR is an important target that becomes activated by Akt. Activity of mTOR is negatively regulated by the tuberous sclerosis complex 1 and 2 (TSC1 and TSC2).[115] The TSC2 subunit triggers conversion of the active GTP-bound form of Rheb into the inactive GDP-bound state. The active Rheb is required for mTOR signaling and it is also possible that Rheb affects protein synthesis in parallel with mTOR.[115] Mammalian TSC2 is inactivated following phosphorylation by Akt, which results in activation of mTOR.[116] Two major targets that become phosphorylated by activated mTOR and promote protein synthesis are S6 kinase 1 (S6K1) and eukaryotic initiation factor 4E-binding protein 1 (4E-BP1).[22] It was commonly thought that activated S6K1 upregulates general translational capacity via phosphorylation of the 40S ribosomal protein S6 and increasing translation from the mRNAs that contain a 5′ tract of oligopyrimidine (TOP). However, translation of 5′TOP mRNAs does not depend on S6K (S6K1 and S6K2) activity nor on S6 phosphorylation.[22] How S6K controls translation remains to be determined. Phosphorylated 4E-BP1 releases eIF4E, which then associates with eIF4G and stimulates translation initiation. Activated S6K1 can reciprocally downregulate PI3K/Akt signaling by IRS-1 inhibition. Another downstream target that becomes phosphorylated by Akt is glycogen synthase kinase 3β (GSK-3β), which results in activation of initiation factor 2B (eIF2B) and stimulation of protein synthesis. Activated Akt phosphorylates FOXO transcription factors and causes their exclusion from the nucleus leading to transcriptional inactivation of the atrophy-related genes MuRF1 and MAFbx. This process leads to inhibition of protein degradation.

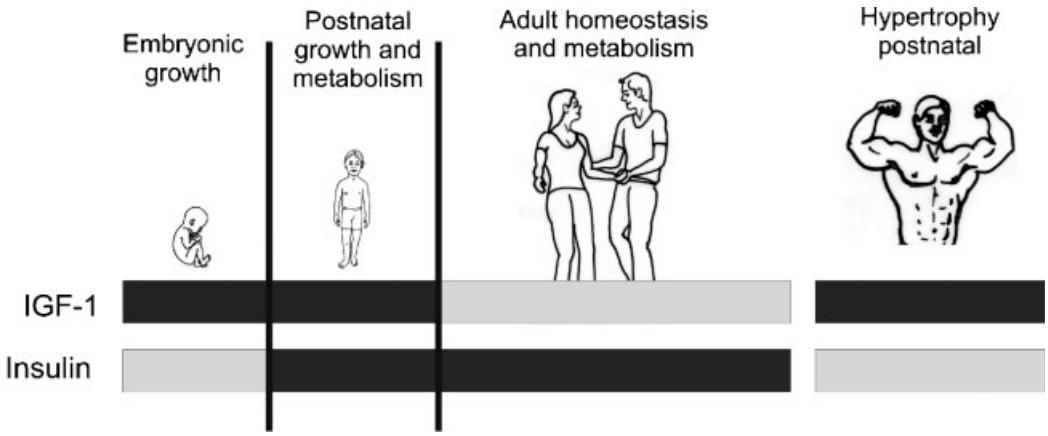

Figure 3. Simplified scheme showing relative importance of IGF-1 and insulin in skeletal muscle during development and adult life. IGF-1 is essential for normal skeletal muscle development during embryogenesis and postnatal growth. In adults when muscle mass reaches homeostasis the role of IGF-1 decreases, although it is required for muscle maintenance. IGF-1 is required for increased muscle mass and protein content during hypertrophy in response to loading. The importance of insulin is relatively low for muscle growth both during embryonic development and postnatally. However insulin is essential for skeletal muscle metabolism during postnatal life. Dark bars indicate the relatively high importance and the light bars indicate relatively low importance for both factors.

mTOR is also regulated by nutrients such as amino acids and intracellular energy metabolism (ATP levels).[24] Figure 2A shows downstream targets for mTOR that are involved in protein synthesis.

Activation of the PI3K/Akt pathway not only increases protein synthesis, but can also counteract the protein degradation in catabolic states and reduce loss of muscle protein (myofibre atrophy).[25–28] A common molecular mechanism that increases protein breakdown is revealed by microarray analysis of skeletal muscle undergoing atrophy induced by different factors (e.g. fasting, cancer, acute diabetes, renal failure):[26] such protein breakdown is inhibited by activation of PI3K/Akt signaling (Fig. 2B). The protein breakdown involves activation of the ATP dependent ubiquitin–proteasome proteolytic pathway where proteins are marked for degradation by covalent linkage with ubiquitin and subsequently degraded by the proteosome to yield small peptides.[29,30] Linkage of ubiquitin to the substrates involves ubiquitin–protein ligases and important ones for mammalian muscle atrophy are Muscle Atrophy F-box or Atrogen-1 (MAFbx) and Muscle RING Finger 1 (MuRF1).[30–32] Mice that lack MAFbx or MuRF1 are partially spared from the severe muscle atrophy following short-term (14 days) denervation that occurs in control mice.[31] Expression of MAFbx and MuRF1 is suppressed by activation of the PI3K/Akt signaling pathway (Fig. 2B) and it has been extensively shown that this pathway can counteract the protein degradation in catabolic states and reduce loss of muscle protein (myofibre atrophy).[25–28]

MAFbx transcription is inhibited by activated PI3K/Akt via phosphorylation (inactivation) of FOXO transcription factors[27,28] that excludes FOXO proteins from the nucleus and prevents their transcriptional functions.[33] The FOXO subfamily of forkhead transcription factors consists of four members, FOXO1, FOXO3, FOXO4, and FOXO6, that are negatively regulated by PI3K/Akt signaling.[33,34,117] Atrophying muscle cells are characterized by downregulation of the PI3K/Akt pathway, dephosphorylation (activation) of FOXOs and their translocation into the nucleus.[26,27] The role of FOXO1[28] and FOXO3[27] has been studied with respect to regulation of MAFbx and MRF1 gene expression. On the one hand, overexpression of FOXO1 alone does not induce MAFbx and MuRF1, but inactivation of FOXO1 via the PI3K/Akt pathway is a necessary step to block expression of MAFbx and

Figure 4. Myogenesis and adipogenesis. **A:** Relationship between myogenesis, adipogenesis and lipid content of muscle. A common precursor can give rise to cell lineages of muscle (myoblasts) or fat (adipocytes) under certain conditions. A shift in biochemical pathways to adipogenesis can also occur in muscle nuclei within myoblasts and possibly also in myofibres. Mature muscle cells (myofibres) contain lipid droplets in the sarcoplasm and the amount of this intramyofibrillar lipid increases in pathological conditions to interfere with healthy metabolism. This lipid content can be reduced by exercise. **B:** Signaling involved in regulation of adipogenesis shown for an adipocyte. Stabilization of β-catenin and its translocation into the nucleus results in inhibition of adipogenic factors. In contrast Akt, mTOR and S6K1 signaling is required for expression of adipogenic factors and accumulation of fat. The extent to which these pathways operate in muscle cells and interfere with protein synthesis mediated by PI3K/Akt signaling is unclear (see Fig. 2A and text for details).

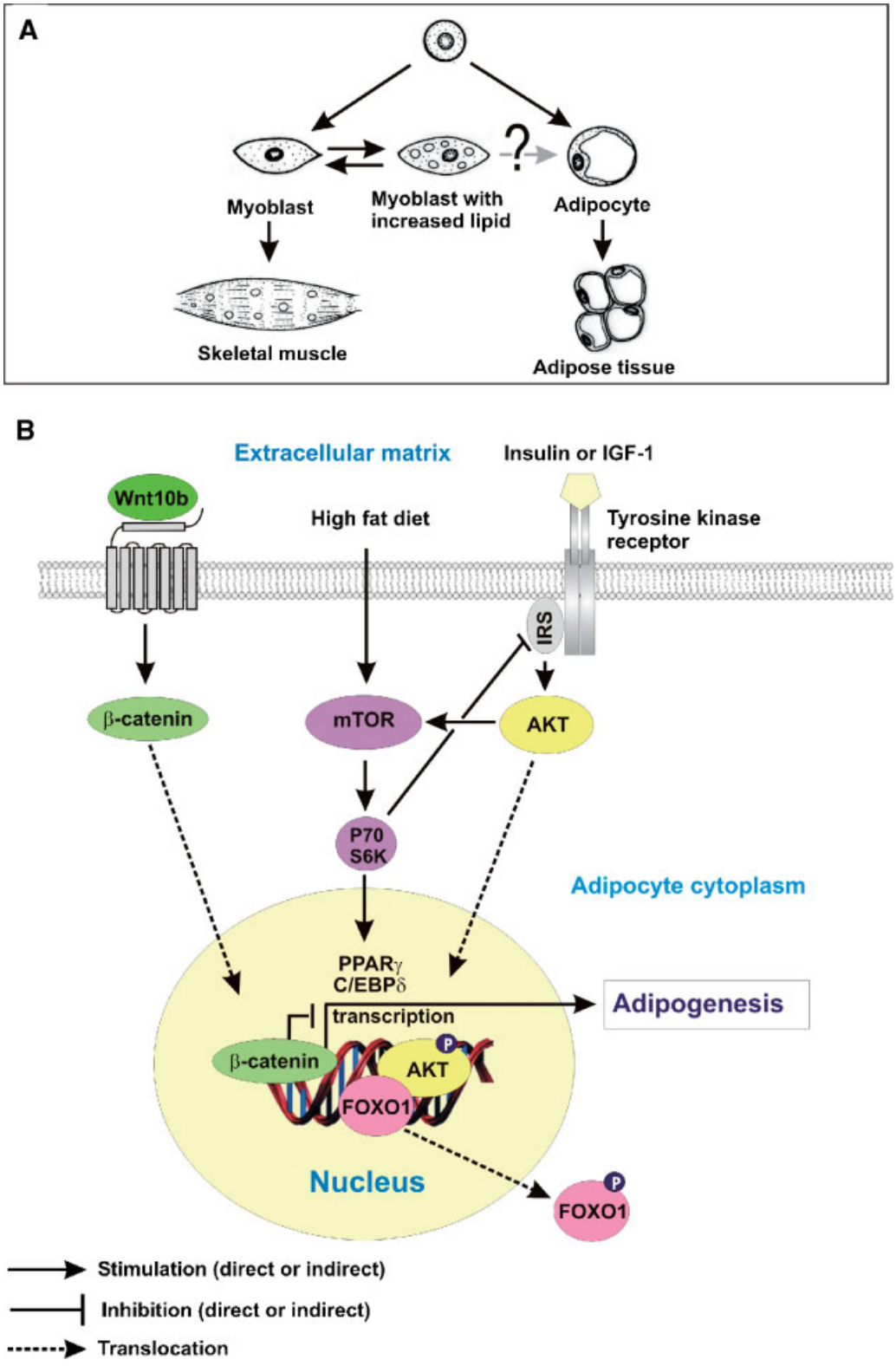
A
Myoblast
Myoblast with increased lipid
Adipocyte
Skeletal muscle
Adipose tissue
B
Extracellular matrix
Insulin or IGF-1
Wnt10b
High fat diet
Tyrosine kinase receptor
IRS
β-catenin
mTOR
AKT
P70 S6K
Adipocyte cytoplasm
PPARγ C/EBPδ
transcription
Adipogenesis
β-catenin
AKT
FOXO1
Nucleus
FOXO1
Stimulation (direct or indirect)
Inhibition (direct or indirect)
Translocation

Figure 4.

MuRF1 in skeletal muscle.[28] In C2C12 myotubes that overexpress FOXO1 mutated on Akt phophorylation sites, IGF-1 is unable to downregulate expression of MAFbx and MuRF1 genes.[28] On the other hand, activated FOXO3 translocates into the nucleus, where it directly induces transcription from the MAFbx gene promoter resulting in muscle atrophy in cell culture and in vivo. [27,35] Recent evidence suggests that activated FOXO1 can also reciprocally inhibit Akt, thus prioritizing the protein degradation pathway (Febbraio MA, personal communication).

Inhibition of PI3K alone is sufficient to induce expression of MAFbx, but not MuRF1 in C2C12 myotubes in culture.[36] Tissue culture studies also suggest that inhibition of mTOR by rapamycin increases expression of MAFbx and MuRF1.[36] How inhibition of mTOR, which lies downstream of Akt, stimulates expression of atrophy-related genes is unknown.

Thus activation of PI3K/Akt signaling downstream of the IGF-1 and insulin receptors increases protein content in skeletal muscle by increasing protein synthesis and also inhibiting protein degradation. The molecules Akt and mTOR are also involved in lipid metabolism in adipocytes (Fig. 4B). Whether interactions between Akt and mTOR with their downstream targets affect adipogenesis in skeletal muscle cells, and impact on protein synthesis, is not known (discussed later under *Control of myogenesis and adipogenesis*).

Reduced ATP and increased AMPK inhibit protein synthesis

Protein synthesis is an energy-consuming process and is downregulated when ATP is depleted, to limit any increase in muscle mass. ATP is the main source of cellular energy and any stress that depletes ATP results in activation of AMPK (adenosine monophosphate kinase) by AMP,[37] which then activates pathways involved in fatty acid oxidation and glucose uptake and glycolysis to restore ATP levels.[37]

The inhibitory effect of AMPK on protein signaling has been shown in rats where activation of AMPK by administration of the drug AICAR suppressed skeletal muscle protein synthesis by 45%, and this was associated with downregulation of Akt/mTOR-mediated signaling: specifically there was decreased phosphorylation of Akt on Ser473 and mTOR on Ser2448[38] (Fig. 2A). A later study in tissue culture mapped AMPK action downstream of Akt[39] and suggested that activated AMPK has no effect on Akt (Ser473) phosphorylation, but inhibits mTOR phosphorylation. The discrepancies between results from in vivo and in vitro studies might reflect more complex signaling interactions in vivo.

Autophagy

Autophagy is an alternative pathway in adult skeletal muscle for the degradation of cytoplasmic components including proteins.[40,41] Autophagy is also observed in neonates at birth when the *trans*-placental nutrient supply is interrupted and such autophagy is strongly induced in heart muscle, diaphragm, alveolar cells and skin in order to provide vital amino acids during this critical period of starvation.[41] In mammals, the importance of autophagy as an alternative mechanism of protein breakdown during starvation[40,41] and also in denervated muscle[42] is of increasing interest although the molecular control has barely been investigated (in yeast at least 16 genes are involved and these are conserved higher vertebrates).[41]

Nutritional control of muscle mass

The role of nutrition in muscle growth is a major focus of research in the meat and livestock industries and is clearly critical for normal human development and muscle homeostasis. Synthesis of new muscle proteins results from re-utilization of amino acids released during proteolysis within myofibres, but some amino acids are lost from skeletal muscle and need to be replaced from ingested protein. This protein intake is particularly important during growth, when significant accretion of muscle mass occurs: decreased blood amino acid concentrations override the positive effect of insulin on muscle protein synthesis even if excess energy (glucose) is provided.[43]

A high protein diet or amino acid (e.g. leucine) supplementation stimulates skeletal muscle protein synthesis in growing[44–46] and adult animals.[47,48] This responsiveness is especially high neonatally and decreases with age.[44,49] There is a threshold of protein required for maximal growth and, once this is reached, additional protein intake does not further stimulate protein synthesis in skeletal muscles.[45] Increased protein accretion in overnight-fasted animals following feeding with a protein-rich diet accords with activation of signaling involved in protein synthesis including increased phosphorylation of Akt, S6K1 and 4E-BP1.[47,49] Conversely, food deprivation decreases phosphorylation of Akt, S6K1 and 4E-BP1.[45] Amino acids do not simply supplement protein synthesis, but they also enhance protein translation by activating mTOR signaling independent of PI3K[50] (Fig. 2A), modulating signaling events that lead to binding of met-tRNAi to the 40S ribosomal subunit and binding of mRNA to the pre-initiation complex.[51]

Runx1 counteracts muscle atrophy associated with denervation

Skeletal muscle requires neural activity for contractile function and lack of nerve signaling (inactivity, denervation) generally results in rapid myofibre atrophy. The extent to which a loss of nerve function contributes to sarcopenia has barely been considered, but it may be a major factor.[52,53] One molecule in skeletal muscle that is regulated by innervation is Runx1, which normally has a protective effect and reduces the extent of denervation-induced muscle atrophy.[42] Runx1 mRNA in rat

skeletal muscle increases by 50–100 fold at 5 days following denervation, with a twofold increase detectable by 1 day.[54] At 2 weeks of denervation, skeletal myofibres of Runx1 null mice atrophy severely by about 10 fold, whereas control normal muscles atrophy far less, by only 1.5 fold.[42] Expression of Runx1 is required to prevent denervated myofibres from autophagy.[42]

Other important signaling pathways: NF-κB, MAPK and Calcineurin

The nuclear factor kappa beta (NF-κB) plays numerous roles in health and disease and is a key molecule in skeletal muscle atrophy resulting from disuse and inflammation (cachexia).[55–57] Under inflammatory conditions tumor necrosis factor-α (TNF-α) is a potent activator of NF-κB signaling and this is a major contributor to cachexia. However, disuse atrophy does not appear to be associated with TNF-α production. It seems that different NF-κB pathways are activated during cachexia and disuse atrophy and these mechanisms are the subject of an excellent recent review.[57]

NF-κB responds to low levels of oxygen (hypoxia) and upregulation of NF-κB in skeletal muscle and heart probably protects against oxidative damage in situations of ischaemia and also hibernation.[58,59]

Two other important signaling pathways involved in muscle hypertrophy are the mitogen-activated protein kinase (MAPK) pathway, mainly involved in muscle growth, and the calcineurin pathway mainly involved in muscle differentiation. These pathways have been extensively reviewed[11,60] and we discuss only key points here.

Three main subfamilies of MAPK involved in skeletal muscle growth are extracellular-signal-regulated kinase (ERK 1/2), c-jun N-terminal kinase (JNK) and p38 MAPK. Increased activity of these MAPK has been shown after physical loading[61,62] and also during hibernation.[63]

Calcineurin is a calcium-regulated, cyclosporine A-sensitive serine/threonine phosphatase involved in skeletal muscle differentiation[64] and upregulation of slow myofibre-type-specific gene expression.[65] Calcineurin activation and nuclear entry of its downstream target NFAT promotes cardiac hypertrophy;[66,67] however involvement of this pathway in skeletal muscle hypertrophy is controversial. Tissue-culture studies suggested that activation of calcineurin signaling may induce skeletal muscle hypertrophy[68,69] but this was not supported by in vivo studies where calcineurin activity decreased in functionally overloaded rat plantaris muscle (in vivo) and treatment with the calcineurin inhibitors cyclosporin A and FK506 did not prevent the compensatory increase in muscle mass and myofibre size.[25] Such disparities illustrate the complexity and multifunctional nature of the signaling pathways that control muscle mass in vivo.

Conclusions

Simplistically, the balance between protein synthesis and degradation, determines the size (CSA) of a myofibre. A shift in balance results in net protein loss (atrophy) or net protein gain (growth and hypertrophy). Whether these shifts reflect a simple reversal of the balance, or whether one aspect is more exaggerated in the different situations is unclear. The protein balance is controlled by complex signaling pathways that are affected by the energy status, nutrition (protein and fat intake) and nerve impulses, presenting many targets for possible therapeutic interventions.

Escape from atrophy in dormant bears, frogs and other animals

Muscle sparing in hibernating and aestivating animals

Inactivity and starvation results in loss of skeletal muscle mass and strength in most animals and humans, yet skeletal muscle of hibernating mammals such as bears are protected against atrophy during prolonged starvation and immobility for many months. Hibernation is a strategy for some mammals to survive when temperatures are very low and food supplies scarce, by allowing body temperatures to fall to near ambient (0°C), with a very slow heart beat with reduced blood flow and greatly reduced metabolism (less than 5% of normal). Many mammals from diverse orders show some capacity to hibernate with bears being an extreme example.[70,71] Hibernation requires an extensive reorganisation of metabolism with selective inhibition of many ATP-consuming activities and a change in fuel use to a primary dependence on the oxidation of lipid reserves. Preservation of muscle mass and function after very long periods of inactivity, associated with metabolic changes and suppressed metabolic rate, also occurs in aestivating[72] and freeze-tolerant frogs.[73] Such adaptations involve many changes related to the balance between myogenesis and adipogenesis and are briefly outlined below. Insight into such extreme adaptations may provide novel strategic interventions for human conditions ranging from muscle atrophy to obesity and ischaemic damage.

Black bears may hibernate for 5–7 months, during which time their body temperature drops about 4°C below normal, and yet their dormant muscles retain most of their mass and strength and appear fully functional upon awakening.[71] Overwintering black bears lose less then 23% of their strength: muscle biopsies show that some muscles retain the protein content completely, whereas others show only slightly reduced protein content.[71] In bears, after 130 days of inactivity, the force of lower-limb muscles was reduced by only 23% whereas the same period of inactivity causes about 90% loss of skeletal muscle strength in humans. The mechanisms that maintain muscle mass and strength in overwintering bears is not fully

understood, however it is hypothesized that skeletal muscle is conserved by recycling urea nitrogen back into protein synthesis or by shivering and rhythmically stimulating the muscles.[71]

There can be massive changes in oxygen consumption during the transition from hibernation: levels of oxygen consumption during hibernation are 1–5% of the normal rate but on arousal can rise by 100–300 fold within a few minutes to fuel the massive thermogenesis that rewarms the animal.[74] This rapid increase in oxygen consumption has the potential for tissue damage due to generation of reactive oxygen species (ROS), and thus hibernating tissues have very high levels of anti-oxidant enzymes in place to protect against the slow accumulation of ROS over the long periods of torpor. Studies in bats confirm that oxidative stress markers, specifically phosphorylated heat-shock protein HSP27 (Ser78/82) and IκB-α(Ser32), are upregulated in skeletal and heart muscle during hibernation, and that protection against oxidative stress occurs by selective upregulation of proteins such as a thioredoxin peroxidase like enzyme (PAG).[59] Elevated phospho-IκB-α(Ser32) results in its degradation and thus allows activation of NF-κB a transcription factor that responds to oxidative stress and is involved in resistance to ischaemic damage that is also a feature of hibernating muscle.[59]

Complex MAPK signaling cascades show different patterns of activation in various organs for ERKs, JNKs and p38MAPK in ground squirrels at 2 days after they enter hibernation.[63] In brief, ERK1/2 activity (and phosphorylation of downstream signaling molecules) increased significantly in muscle and brain (but decreased in kidney and liver), p38MAPK activity increased only in muscle and brain, whereas JNK activity increased in muscles, kidney and liver (but not brain). The activated p38MAPK correlated with increased activation of MAPKAKT2, which activates HSP27 that protects against stress and stabilizes the actin cytoskeleton; thus it may be important for maintaining sarcomere structure during hibernation. These different in vivo responses to hibernation suggest key roles for MAPK signaling pathways in regulating the different organ responses, although the precise roles of these MAPKs remains to be explored.

Protein synthesis decreases during hibernation as it uses much energy. Suppression of protein synthesis with concomitant inhibition the eukaryotic initiation factor 2α (eIF2α) and the eukaryotic elongation factor 2 (eEF2) occurs in skeletal muscles of hibernating bats.[59] A major sensor of cellular energy status is AMP (as discussed above) yet it does not seem to play a central role in metabolic depression in hibernating animals: while a transient change was observed in skeletal muscle of ground squirrels as they entered torpor, there was no sustained activation of AMPK with prolonged hibernation despite a huge net suppression of ATP turnover.[75] Downregulation of the IGF-1 system seems to take place during hibernation. In hibernating ground squirrels, blood levels of IGF-1 are reduced by 75% and the IGF-1 binding proteins IGFBP3 and IGFBP4 decrease to almost undetectable levels.[76] Whether IGF-1 expression in skeletal muscle also decrease with dormancy remains to be investigated.

Downregulation of Akt signaling, which is central to insulin and IGF-1 signaling, appears to play a regulatory role in tissue adaptation to hibernation in some animals. Dephosphorylation of Akt and downregulation of its kinase activity was shown in skeletal muscle, heart and brain of hibernating squirrels,[77] but not in skeletal muscles of hibernating bats.[78] Decreased Akt activity during hibernation might have a cytoprotective role in the conditions of starvation and hypoxia (Fig. 5) since, in tissue culture, decreased activity of Akt is protective against ischemia, possibly due to increased cell quiescence.[79] Decreased Akt activity in some hibernating animals has similarities to the altered homologous IGF-1/Akt signaling leading to activation of FOXO transcription factors in the arrested dauer larval stage of the nematode worm *C. elegans* under starvation.[80,81] FOXO1 FOXO2 and FOXO4 proteins also increase in fasting mammals, suggesting that, in the conditions of food scarcity, activation of FOXO transcription factors may be essential for survival.[82]

In ground squirrels, depression of protein translation is also regulated by mechanisms downstream of Akt. Seasonal regulation of 4IF-BP1 expression has been shown in the liver of these animals:[83] apparently, summer squirrels lack 4IF-BP1 and the control of eIF4E activity occurs through direct phosphorylation. Winter animals express 4IF-BP1, which binds and regulates eIF4E. During the euthermic periods that separate bouts of torpor (interbout arousal), 4E-BP1 is hyperphosphorylated to promote initiation. However, during torpor, 4E-BP1 is hypophosphorylated and cap-dependent initiation of translation is restricted.

Protein-degradation machinery seems to also adapt to hibernation: e.g. proteosomes of hibernating squirrels have altered kinetic properties at 8–10°C (but not at 23°C) that are more suited for low-temperature degradation of oxidatively damaged proteins.[84] It is suggested that low body temperature during hibernation depresses proteolysis via inhibition of the proteolytic activity of the proteasome.[85]

Lipid metabolism is of central importance during hibernation. Large stores of adipose tissue are produced before hibernation commences and, during dormancy, most organs depend primarily on lipid catabolism for ATP production. Key molecules in this lipogenesis are the PPAR (peroxisome proliferation activated receptor) family of transcription factors. Both PPAR-γ and its co-activator PGC-1α are increased in muscles (where red/slow myofibres are especially protected from atrophy), fat and other tissues of ground squirrels hibernating for 2–5 days.[86]

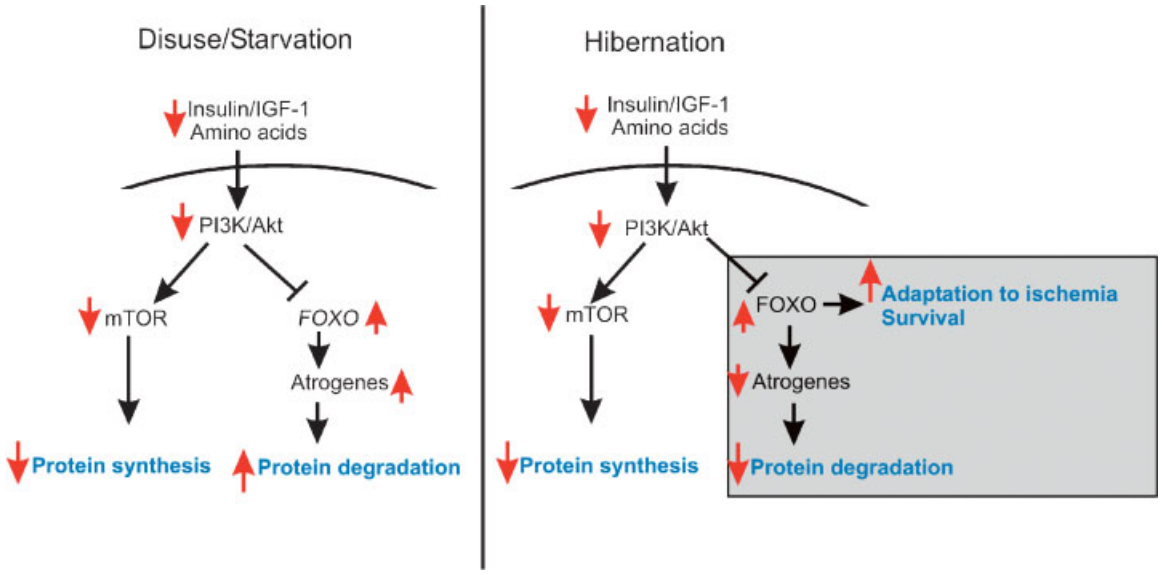

Figure 5. Summary of key differences between skeletal muscle response to disuse and starvation in humans and non-hibernating mammals (where severe atrophy takes place) and dormant animals, specifically in hibernation (with minimal atrophy). Note: the potential role of autophagy has not been included in this summary. Red arrows show upregulation or downregulation of molecules and processes.

A striking avoidance of atrophy in immobile starved muscles is also seen in aestivating green-striped burrowing frogs (*C. alboguttata*). During the intense heat of summer, these frogs bury themselves in the ground in a cocoon where they remain inert for up to a year, yet even after 6–9 months of dormancy the frogs appear to retain almost full muscle mass and strength.[87] It is suggested that this sparing of muscles from atrophy is largely due to the modulation of oxidative stress, with mitochondrial quiescence and reduced oxidant production while antioxidant enzymes are maintained. In striking contrast with cold-induced mammalian hibernation, lipid metabolism does not increase to help spare the hypometabolic muscles during this deep metabolic depression.[87] In addition, spontaneous release of the neurotransmitter acetylcholine is maintained at the neuromuscular junction of the aestivating frog, permitting activity between nerve and muscle even in the absence of electrical activity.[87] The issue of synaptic activity in resting or ageing muscle has received remarkably little attention to date. Similarly, the regulation of autophagy in dormancy has not been addressed.

Conclusion

In dormant animals, there is downregulation of PI3K/Akt signaling, due to starvation or possibly due to lack of nerve-mediated muscle contraction. Such decreased PI3K/Akt signaling in skeletal muscle normally results rapidly in protein breakdown. The fact that such protein degradation does not occur in hibernating bears and aestivating frogs after many months seems to be a central issue (Fig. 5). What stops protein breakdown in dormant animals?

Modulation of signaling has been studied experimentally using induced dormancy in animals such as ground squirrels and bats over very short periods, days to weeks: here protein breakdown occurs but is reduced. Suppression of protein breakdown must be much more extreme in bears and frogs, yet signaling analyses have not been carried out in these long-term dormant animals, due in part to lack of suitable molecular tools. While, in hibernating animals, depression of protein breakdown might depend on the inhibition of proteolysis due to the low body temperature, this cannot be the case during aestivation that occurs at high temperature. In addition, mechanisms that prevent skeletal muscle atrophy, possibly by preventing activation of MAFbx and MuRF1 by FOXO in dormant animals have yet to be identified. The roles of nerve and synaptic activity, and also of autophagy, during dormancy also remain to be elucidated.

Relationship between molecular control of myogenesis and adipogenesis

A close, and at many times inverse, relationship between the control of myogenesis/hypertrophy and adipogenesis is supported by phenotypes of many new genetically engineered mice. Some of the molecules that affect both myogenesis and adipogenesis are Akt,[88,89] S6K1,[90] myostatin,[91] ADAM12[92] and DLK1.[93] Critical regulators of skeletal

muscle lipid and energy metabolism are orphan and adopted nuclear receptors, such as peroxisome proliferator-activated receptors (PPARs), liver X receptors (LXRs), estrogen-related receptors (EERs), RORs, Rev-erbs and NR4As.[118] These receptors represent emerging targets for treatment of metabolic diseases.

The pathways that link regulation of muscle and fat formation are not well understood (Fig. 4A, B). In committed muscle cells, a shift towards adipogenesis represents a biochemical process i.e. there is increased accumulation of lipid within the muscle cell rather than transformation into an adipocyte lineage. Two signaling pathways that relate to both myogenesis and adipogenesis involve Wnt10 and mTOR (Fig. 4B).

Wnt signaling

Wnts are a family of secreted glycoproteins that affect both adipogenic and myogenic programs. There are 19 Wnts and 10 frizzled (Fz) receptors in humans. In adults, Wnts are implicated in maintenance of stem-cell-like fates in various tissues[94] and act through different pathways, with the main signaling involving β-catenin. In brief, in the β-catenin-dependent pathway, binding of Wnt to a Fz receptor stabilizes and increases the free cytoplasmic pool of β-catenin; β-catenin translocates into the nucleus and converts T-cell factor (TCF) into a transcription activator.[94,95] Alternatively, in the absence of Wnt, β-catenin is assembled into an 'Axin complex', where it is phosphorylated and becomes degraded through the ubiquitin pathway.[95–97] However, recent evidence suggests that GSK-3 also plays a role in upstream signaling events leading to β-catenin stabilization.[98] An alternative Wnt pathway, independent of β-catenin, appears to be mediated by calcineurin since blocking calcineurin downregulates Wnt signaling.[99]

It is suggested that β-catenin-mediated Wnt signaling (Fig. 4B) inhibits adipogenic differentiation, since Wnt (likely Wnt-10b) signaling, prevents conversion of 3T3-L1 pre-adipocytes into adipocytes by inhibiting factors required for adipogenic differentiation, including CCAAT enhancer-binding protein α (C/EBPα), peroxisome proliferator-activated receptor gamma (PPARγ) and fatty-acid-binding protein 4 (FABP4).[100] Furthermore, myoblasts respond to inhibition of this canonical Wnt signaling pathway by activation of adipogenesis[100] and it is suggested that decreased Wnt signaling is one of the reasons for increased adipogenic potential of ageing muscle.[101] Primary myoblast cultures from young adult (8 month) mice accumulated threefold more cytosolic β-catenin compared to myoblasts isolated from old (24 month) mice, with an inverse relationship between expression of Wnt-10b and transcription factors involved in adipogenesis (C/EBPα and FABP4). In addition, PPARγ2 was hyperphosphorylated (inactive) in young compared with old myoblasts.[101] Enhanced myogenesis at the expense of adipogeneis by Wnt signaling, is further supported by retroviral overexpression of Wnt-10b in old myoblasts that increases cytosolic β-catenin to levels characteristic of adult myoblasts, inhibits expression of adipogenic genes (C/EBPα, PPARγ and FAB4) and decreases the lipid content.[102]

Further insight in Wnt-10b function was obtained from in vivo studies on Wnt-10b null mice.[102] Skeletal muscle morphology of adult Wnt-10b null mice was the same as littermate controls but, when Wnt-10b null mice were fed a high fat diet (45% fat) and their muscles injured, the activated myoblasts showed lipid accumulation, and adiposity in regenerated Wnt-10b null muscle was apparent even at 1 month after injury but not in control mice.[102] Accelerated myoblast fusion is seen in Wnt-10b null cultures and this may reflect compensatory upregulation of the more potent myogenic Wnt-7b.[102] It is interesting that such upregulation of Wnt-7b expression does not occur in normal ageing myoblasts that exhibit defects in Wnt10b signaling.[102] It has recently been suggested that β-catenin may play a role in skeletal muscle hypertophy since nuclear levels of β-catenin increase by ~4.4 fold in skeletal muscle of mice overloaded for 7 days by synergist ablation, and it forms a complex with Lef-1 to activate transcription of target genes involved in growth control: c-Myc, cyclin D1 and paired-like homeodomain transcription factor 2.[103]

These data show a strong and consistent association between (1) Wnt-10b signaling and myogenesis and (2) decreased Wnt-10b signaling and increased expression of adipogenic genes, with this pattern becoming more pronounced with ageing.

Akt, mTOR and S6K1

PI3K/Akt signaling and its downstream targets such as mTOR and S6K1 are essential for regulation of protein metabolism in skeletal muscle (Fig. 2A) and also play a role in adipogenic differentiation. Akt is absolutely required for adipocyte diffentiation.[89] Mice that lack Akt1 and Akt2 fail to upregulate the key regulators of adipogenesis PPARγ and C/EBPα and have severely impaired adipogenesis.[89] One of the mechanisms by which Akt upregulates expression of adipogenic genes is by phosphorylation of FOXO1, thus removing this inhibitor of PPARγ expression[89] (Fig. 4B).

Accumulation of body fat is also influenced by mTOR signaling (Fig. 4B).[90,104] Inhibition of mTOR with rapamycin blocks differentiation and lipid accumulation in 3T3-L1 preadipocytes, possibly by inhibition of transcriptional activity of PPAR-γ and disruption of the positive transcriptional feedback loop between PPAR-γ and C/EBPα[104] Furthermore, *melted* mutant flies, which are defective in TOR signaling, are lean due to loss of fat.[105] Regulation of body fat deposition involves S6K1, a downstream target of mTOR (Fig. 4B).[90] S6K1 null mice accumulate less fat and are protected against diet-induced obesity due to increased

oxidative phosphorylation and increased insulin sensitivity. Expression of genes involved in energy utilization and oxidative phosphorylation uncoupling protein 1 (UCP1), UCP3, carnitine palmitoyltransferase 1 (CPT1) and PPARγ co-activator 1α (PGC1α) are strongly upregulated in adipocytes of S6K1 null mice. It is suggested that activation of S6K1 by a high-fat diet inhibits signaling downstream of IRS1 (by phosphorylating IRS1 at Ser307 and Ser636/639) and thus suppresses insulin signaling leading to insulin resistance;[90] this is supported by a high-fat diet suppressing Akt phosphorylation in fat, liver and skeletal muscle of wild-type but not S6K1 null mice, resulting in insulin resistance.[90]

Whether Akt, mTOR and S6K1 are involved in regulating the adipogenic program in skeletal muscle cells under various conditions, e.g. high fat diet, is not known. The fact that a high fat diet suppresses Akt phosphorylation in skeletal muscle of wild-type but not S6K1 null mice supports the existence of a negative loop of regulation between S6K1 and IRS1 in skeletal muscle cells similar to adipocytes.[90]

Conclusion

While conversion of a common precursor cell into myogenic and adipogenic lineages is readily demonstrated under tissue-culture conditions, the in vivo significance is unclear. Also, it is not known whether increased activation of adipogenic pathways within muscle cells can ultimately switch off the myogenic program and convert muscle into adipocytes. There is increasing interest in factors controlling the balance between myogenic and adipogenic signaling programs, especially within myofibres in relation to obesity, disease and ageing, although many fundamental questions remain unanswered.

Fat in muscle tissue and influence on metabolism

The balance between fat and muscle content is of great interest to the meat industry and to clinical conditions in humans. In the meat industry, the extreme case of fat deposition between muscle fibres produces marbled beef in

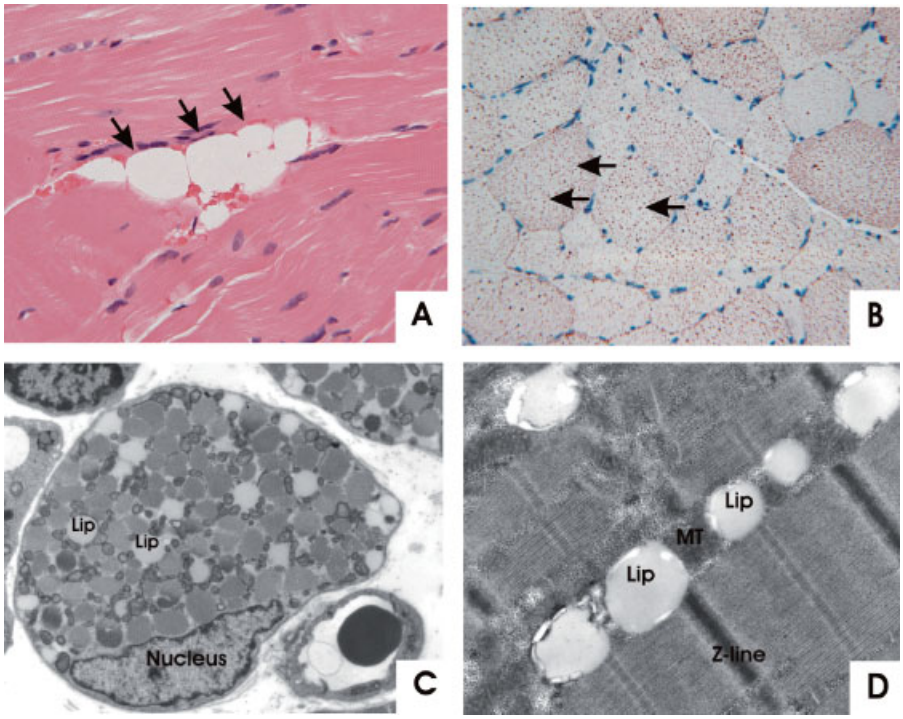

Figure 6. Lipid deposition in human skeletal muscle. **A:** Lipid accumulation in adipocytes (arrows) located in the connective tissue between myofibres; H&E-stained section from a 68-year-old patient with active necrotising myopathy (×16). **B:** Very small lipid droplets (arrows) within all myofibres; lipid stained red with Oil Red O in cross section of muscle (×16). **C:** Electron microscopy shows lipid droplets (Lip) located between myofibrills in the proximity of mitochondria (MT) of a transverse section of a myofibre; a myonucleus (Nucleus) is located at the periphery: this is more clearly demonstrated in the high power image **D:** of a myofibre in longitudinal section, where sarcomere structure and Z-lines are also clearly shown. Magnifications (A) ×9,000 (B) ×42,000. The low-power images illustrating inter- and intramyofibrillar lipid were kindly provide by Dr. Phil Morling, and electron micrographs of human skeletal muscle with pathologically increased intramyofibrillar lipid by Dr. Vicki Fabian and Ms. Lisa Griffiths, from the Section of Neuropathology, Department of Anatomical Pathology, Royal Perth Hospital, Western Australia.

Japanese Black cattle,[106] where marbling results in beef tenderization.[107] Premium-quality marbled beef can cost over US$300 per pound in Japan: thus there is intensive research into factors controlling adipocytes in such commercial muscle. Adiposity and fat content is also of central interest in humans as it relates to obesity, type 2 diabetes and many other conditions including the replacement of muscle tissue by fatty connective tissue in dystrophic and severely damaged muscles.

Fat or lipid (triacylglycerols; TG) is mostly stored in adipocytes located in subcutaneous and deep visceral tissues. Lipid is also present in the circulation and within the cytoplasm of myofibres.[108] Lipid content in skeletal muscle has major implication for the metabolic status of this tissue.

In skeletal muscle, lipid is present in adipocytes located in the interstitial connective tissue between myofibres (Fig. 6A) and within the sarcoplasm of myofibres (as lipid droplets) where they are referred to as intramyocellular triacylgycerols (IMTG) (Fig. 6B, C, D). Adipocytes within the interstitial connective tissue are often closely associated with blood vessels and this is certainly pronounced in marbled beef.[107] Microarray analysis of gene expression in skeletal muscles of Japanese Black cattle shows elevated expression of genes involved in lipid metabolism and adipocyte differentiation.[106]

With respect to lipid droplets within myofibres, the IMTGs are located adjacent to the mitochondria (Fig. 6D) and their content is about three times higher in oxidative slow type 1 myofibres compared to glycolytic fast type 2 myofibres.[109] Many observations support the role of IMTG as an energy source during physical exercise:[108] for example, a >60% reduction in IMTG content occurs in type I myofibres in humans after a 2 hour exercise bout.[110] Exercise seems to play a crucial role in depleting and reversing the adverse effects of IMTGs in muscles e.g in patients tending towards type 2 diabetes.[111] Developing a drug that substitutes for the benefits of exercise presents a challenge.

In humans, increased lipid content within myofibres correlates with skeletal muscle insulin resistance, and is independent of total body adiposity.[111] This correlation is pronounced in patients with type 2 diabetes (insulin is initially present but the cell fails to respond and glucose uptake is impaired), where myofibres display insulin resistance and significantly increased lipid content.[112] Paradoxically, IMGT content is also significantly increased in endurance-trained athletes, who are highly sensitive to insulin;[112,113] however, despite this elevated IMGT storage, skeletal muscles of endurance-trained athletes have higher oxidative capacity compared to the sedentary lean healthy individuals and diabetic patients.[112] Based on these findings, the following were suggested: (1) Increased lipid deposition in myofibres per se does not affect insulin sensitivity, but rather represents a marker for the increase of other lipid molecules (such as ceramide, diglyceride, or long-chain acyl-CoA) that may induce defects in the insulin-signaling pathway and muscle insulin resistance.[111,112] (2) Insulin sensitivity may be influenced by the oxidative capacity of skeletal muscle with a tendency towards increased oxidation of fatty acids, rather than synthesis of the metabolites.[111,112] Ageing is also associated with increased fat within myofibres. Magnetic resonance spectroscopy on healthy non-diabetic subjects showed that IMGT increases in ageing and this correlates with insulin resistance.[114]

Closing comments

Beyond the powerful example of hibernation and aestivation, there are other fascinating animal situations that provide the opportunity for comparative analysis of the molecular control of muscle atrophy/hypertrophy. It is hoped that information gleaned from such diverse animal systems, which relate largely to postnatal muscle, can provide novel insights with applications to human conditions. This approach, combined with exploitation of high-throughput molecular and protein analysis technology, the wealth of new molecules and mutant organisms generated by models such as zebrafish and *Drosophila*, and the emergent field of microRNAs, will provide much new information into factors controlling muscle hypertophy/atrophy and myogenesis/adipogenesis.

Acknowledgments

We thank Nadia Rosenthal (European Molecular Biology Laboratory, Rome, Italy) and Marie Bogoyevitch (The University of Western Australia, WA) for their constructive comments on the review.

References

1. Hoffman EP, Escolar D. 2006. Translating mighty mice into neuromuscular therapeutics: is bigger muscle better? Am J Pathol 168:1775–1778.
2. Roy RR, Monke SR, Allen DL, Edgerton VR. 1999. Modulation of myonuclear number in functionally overloaded and exercised rat plantaris fibers. J Appl Physiol 87:634–642.
3. Jacquemin V, Furling D, Bigot A, Butler-Browne GS, Mouly V. 2004. IGF-1 induces human myotube hypertrophy by increasing cell recruitment. Exp Cell Res 299:148–158.
4. Lowe DA, Alway SE. 1999. Stretch-induced myogenin, MyoD, and MRF4 expression and acute hypertrophy in quail slow-tonic muscle are not dependent upon satellite cell proliferation. Cell Tissue Res 296:531–539.
5. Frey N, Katus HA, Olson EN, Hill JA. 2004. Hypertrophy of the heart: a new therapeutic target? Circulation 109:1580–1589.
6. White JD, Scaffidi A, Davies M, McGeachie J, Rudnicki MA, et al. 2000. Myotube formation is delayed but not prevented in MyoD-deficient skeletal muscle: studies in regenerating whole muscle grafts of adult mice. J Histochem Cytochem 48:1531–1544.
7. Kambadur R, Sharma M, Smith TP, Bass JJ. 1997. Mutations in myostatin (GDF8) in double-muscled Belgian Blue and Piedmontese cattle. Genome Res 7:910–916.
8. Fernandez AM, Dupont J, Farrar RP, Lee S, Stannard B, et al. 2002. Muscle-specific inactivation of the IGF-1 receptor induces compensatory hyperplasia in skeletal muscle. The Journal of Clinical Investigation 109(3):347–355.
9. Shavlakadze T. 2006. Rskalpha-actin/hIGF-1 transgenic mice with increased IGF-I in skeletal muscle and blood: impact on regeneration,

denervation and muscular dystrophy. Growth Hormone & IGF Research (Epub ahead of print).
10. Antonio J, Gonyea WJ. 1993. Progressive stretch overload of skeletal muscle results in hypertrophy before hyperplasia. J Appl Physiol 75:1263–1271.
11. Glass DJ. 2005. Skeletal muscle hypertrophy and atrophy signaling pathways. Int J Biochem Cell Biol 37:1974–1984.
12. Coffer PJ, Jin J, Woodgett JR. 1998. Protein kinase B (c-Akt): a multifunctional mediator of phosphatidylinositol 3-kinase activation. Biochem J 335:1–13.
13. Murthy SS, Tosolini A, Taguchi T, Testa JR. 2000. Mapping of AKT3, encoding a member of the Akt/protein kinase B family, to human and rodent chromosomes by fluorescence in situ hybridization. Cytogenet Cell Genet 88:38–40.
14. Nader GA. 2005. Molecular determinants of skeletal muscle mass: getting the ''AKT'' together. Int J Biochem Cell Biol 37:1985–1996.
15. Cho H, Thorvaldsen JL, Chu Q, Feng F, Birnbaum MJ. 2001. Akt1/PKBalpha is required for normal growth but dispensable for maintenance of glucose homeostasis in mice. J Biol Chem 276:38349–38352.
16. Liu JP, Baker J, Perkins AS, Robertson EJ, Efstratiadis A. 1993. Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). Cell 75:59–72.
17. Accili D, Drago J, Lee EJ, Johnson MD, Cool MH, et al. 1996. Early neonatal death in mice homozygous for a null allele of the insulin receptor gene. Nat Genet 12:106–109.
18. Bolster DR, Kimball SR, Jefferson LS. 2003. Translational control mechanisms modulate skeletal muscle gene expression during hypertrophy. Exerc Sport Sci Rev 31:111–116.
19. Bolster DR, Jefferson LS, Kimball SR. 2004. Regulation of protein synthesis associated with skeletal muscle hypertrophy by insulin-, amino acid- and exercise-induced signalling. Proc Nutr Soc 63:351–356.
20. Fernandez AM, Kim JK, Yakar S, Dupont J, Hernandez-Sanchez C, et al. 2001. Functional inactivation of the IGF-1 and insulin receptors in skeletal muscle causes type 2 diabetes. Genes and Development 15:1926–1934.
21. Kim H, Barton E, Muja N, Yakar S, Pennisi P, et al. 2005. Intact insulin and insulin-like growth factor-I receptor signaling is required for growth hormone effects on skeletal muscle growth and function in vivo. Endocrinology 146:1772–1779.
22. Wullschleger S, Loewith R, Hall MN. 2006. TOR signaling in growth and metabolism. Cell 124:471–484.
23. Gangloff YG, Mueller M, Dann SG, Svoboda P, Sticker M, et al. 2004. Disruption of the mouse mTOR gene leads to early postimplantation lethality and prohibits embryonic stem cell development. Mol Cell Biol 24:9508–9516.
24. Hay N, Sonenberg N. 2004. Upstream and downstream of mTOR. Genes Dev 18:1926–1945.
25. Bodine SC, Stitt TN, Gonzalez M, Kline WO, Stover GL, et al. 2001. Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. Nature Cell Biology 3:1014–1019.
26. Latres E, Amini AR, Amini AA, Griffiths J, Martin FJ, et al. 2004. IGF-1 inversely regulates atrophy-induced genes via the PI3K/Akt/mTOR pathway. J Biol Chem 280:2737–2744.
27. Sandri M, Sandri C, Gilbert A, Skurk C, Calabria E, et al. 2004. Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. Cell 117:399–412.
28. Stitt TN, Drujan D, Clarke BA, Panaro F, Timofeyva Y, et al. 2004. The IGF-1/PI3K/Akt pathway prevents expression of muscle atrophy-induced ubiquitin ligases by inhibiting FOXO transcription factors. Mol Cell 14:395–403.
29. Jagoe RT, Goldberg AL. 2001. What do we really know about the ubiquitin-proteasome pathway in muscle atrophy? Curr Opin Clin Nutr Metab Care 4:183–190.
30. Lecker SH, Jagoe RT, Gilbert A, Gomes M, Baracos V, et al. 2004. Multiple types of skeletal muscle atrophy involve a common program of changes in gene expression. Faseb J 18:39–51.
31. Bodine SC, Latres E, Baumhueter S, Lai VK, Nunez L, et al. 2001. Identification of ubiquitin ligases required for skeletal muscle atrophy. Science 294:1704–1708.
32. Jagoe RT, Lecker SH, Gomes M, Goldberg AL. 2002. Patterns of gene expression in atrophying skeletal muscles: response to food deprivation. Faseb J 16:1697–1712.
33. Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, et al. 1999. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell 96:857–868.
34. Anderson MJ, Viars CS, Czekay S, Cavenee WK, Arden KC. 1998. Cloning and characterization of three human forkhead genes that comprise an FKHR-like gene subfamily. Genomics 47:187–199.
35. Skurk C, Izumiya Y, Maatz H, Razeghi P, Shiojima I, et al. 2005. The FOXO3a transcription factor regulates cardiac myocyte size downstream of AKT signaling. J Biol Chem 280:20814–20823.
36. Sacheck JM, Ohtsuka A, McLary SC, Goldberg AL. 2004. IGF-I stimulates muscle growth by suppressing protein breakdown and expression of atrophy-related ubiquitin ligases, atrogin-1 and MuRF1. Am J Physiol Endocrinol Metab 287:E591–E601.
37. Hardie DG. 2004. The AMP-activated protein kinase pathway–new players upstream and downstream. J Cell Sci 117:5479–5487.
38. Bolster DR, Crozier SJ, Kimball SR, Jefferson LS. 2002. AMP-activated protein kinase suppresses protein synthesis in rat skeletal muscle through down-regulated mammalian target of rapamycin (mTOR) signaling. J Biol Chem 277:23977–23980.
39. Inoki K, Zhu T, Guan KL. 2003. TSC2 mediates cellular energy response to control cell growth and survival. Cell 115:577–590.
40. Mizushima N, Yamamoto A, Matsui M, Yoshimori T, Ohsumi Y. 2004. In vivo analysis of autophagy in response to nutrient starvation using transgenic mice expressing a fluorescent autophagosome marker. Mol Biol Cell 15:1101–1111.
41. Kuma A, Hatano M, Matsui M, Yamamoto A, Nakaya H, et al. 2004. The role of autophagy during the early neonatal starvation period. Nature 432:1032–1036.
42. Wang X, Blagden C, Fan J, Nowak SJ, Taniuchi I, et al. 2005. Runx1 prevents wasting, myofibrillar disorganization, and autophagy of skeletal muscle. Genes Dev 19:1715–1722.
43. Bell JA, Fujita S, Volpi E, Cadenas JG, Rasmussen BB. 2005. Short-term insulin and nutritional energy provision do not stimulate muscle protein synthesis if blood amino acid availability decreases. Am J Physiol Endocrinol Metab 289:E999–E1006.
44. Davis TA, Burrin DG, Fiorotto ML, Nguyen HV. 1996. Protein synthesis in skeletal muscle and jejunum is more responsive to feeding in 7-than in 26-day-old pigs. Am J Physiol 270:E802–E809.
45. Frank JW, Escobar J, Suryawan A, Kimball SR, Nguyen HV, et al. 2005. Protein synthesis and translation initiation factor activation in neonatal pigs fed increasing levels of dietary protein. J Nutr 135:1374–1381.
46. Escobar J, Frank JW, Suryawan A, Nguyen HV, Kimball SR, et al. 2005. Physiological rise in plasma leucine stimulates muscle protein synthesis in neonatal pigs by enhancing translation initiation factor activation. Am J Physiol Endocrinol Metab 288:E914–E9121.
47. Svanberg E, Jefferson LS, Lundholm K, Kimball SR. 1997. Postprandial stimulation of muscle protein synthesis is independent of changes in insulin. Am J Physiol 272:E841–E847.
48. Anthony JC, Reiter AK, Anthony TG, Crozier SJ, Lang CH, et al. 2002. Orally administered leucine enhances protein synthesis in skeletal muscle of diabetic rats in the absence of increases in 4E-BP1 or S6K1 phosphorylation. Diabetes 51:928–936.
49. Davis TA, Fiorotto ML, Burrin DG, Reeds PJ, Nguyen HV, et al. 2002. Stimulation of protein synthesis by both insulin and amino acids is unique to skeletal muscle in neonatal pigs. Am J Physiol Endocrinol Metab 282:E880–E890.
50. Hara K, Yonezawa K, Weng QP, Kozlowski MT, Belham C, Avruch J. 1998. Amino acid sufficiency and mTOR regulate p70 S6 kinase and eIF-4E BP1 through a common effector mechanism. J Biol Chem 273:14484–14494.
51. Kimball SR, Jefferson LS. 2005. Role of amino acids in the translational control of protein synthesis in mammals. Semin Cell Dev Biol 16: 21–27.
52. Shavlakadze T, Grounds MD. 2003. Therapeutic interventions for age-related muscle wasting: importance of innervation and exercise for preventing sarcopenia. In: Rattan S, editor. Modulating aging and

longevity. Kluwer Academic Publishers. Printed in Great Britain. p 139–166.
53. Kajaer M, Krogsgaard M, Magnusson P, Engebretsen L, Ross H, et al. (Editors). 2003. Textbook of sports medicine: Basic science and clinical aspects of sports injury and physical activity. Blackwell Publishing.
54. Zhu X, Yeadon JE, Burden SJ. 1994. AML1 is expressed in skeletal muscle and is regulated by innervation. Mol Cell Biol 14:8051–8057.
55. Kumar A, Takada Y, Boriek AM, Aggarwal BB. 2004. Nuclear factor-kappaB: its role in health and disease. J Mol Med 82:434–448.
56. Jackman R, Kandarian SC. 2004. The molecular basis of skeletal muscle atrophy. Am J Physiol Cell Physiol 287:C834–C843.
57. Kandarian SC, Jackman RW. 2006. Intracellular signaling during skeletal muscle atrophy. Muscle Nerve 33:155–165.
58. Cummins EP, Taylor CT. 2005. Hypoxia-responsive transcription factors. Pflugers Arch 450:363–371.
59. Eddy SF, McNally JD, Storey KB. 2005. Up-regulation of a thioredoxin peroxidase-like protein, proliferation-associated gene, in hibernating bats. Arch Biochem Biophys 435:103–111.
60. Bassel-Duby R, Olson EN. 2006. Signaling Pathways in Skeletal Muscle Remodeling. Annu Rev Biochem 75:19–37.
61. Koulmann N, Bigard AX. 2006. Interaction between signalling pathways involved in skeletal muscle responses to endurance exercise. Pflugers Arch 452(2):125–139.
62. Lluis F, Perdiguero E, Nebreda AR, Munoz-Canoves P. 2006. Regulation of skeletal muscle gene expression by p38 MAP kinases. Trends Cell Biol 16:36–44.
63. MacDonald JA, Storey KB. 2005. Mitogen-activated protein kinases and selected downstream targets display organ-specific responses in the hibernating ground squirrel. Int J Biochem Cell Biol 37:679–691.
64. Friday BB, Mitchell PO, Kegley KM, Pavlath GK. 2003. Calcineurin initiates skeletal muscle differentiation by activating MEF2 and MyoD. Differentiation 71:217–227.
65. Liu Y, Shen T, Randall WR, Schneider MF. 2005. Signaling pathways in activity-dependent fiber type plasticity in adult skeletal muscle. J Muscle Res Cell Motil 26:13–21.
66. Molkentin JD, Lu JR, Antos CL, Markham B, Richardson J, et al. 1998. A calcineurin-dependent transcriptional pathway for cardiac hypertrophy. Cell 93:215–228.
67. McKinsey TA, Olson EN. 2005. Toward transcriptional therapies for the failing heart: chemical screens to modulate genes. J Clin Invest 115:538–546.
68. Musaro A, Rosenthal N. 1999. Maturation of the myogenic program is induced by postmitotic expression of insulin-like growth factor I. Mol Cell Biol 19:3115–3124.
69. Semsarian C, Wu MJ, Ju YK, Marciniec T, Yeoh T, et al. 1999. Skeletal muscle hypertrophy is mediated by a Ca2+-dependent calcineurin signalling pathway. Nature 400:576–581.
70. Rourke BC, Yokoyama Y, Milsom WK, Caiozzo VJ. 2004. Myosin isoform expression and MAFbx mRNA levels in hibernating golden-mantled ground squirrels (Spermophilus lateralis). Physiol Biochem Zool 77:582–593.
71. Harlow HJ, Lohuis T, Beck TD, Iaizzo PA. 2001. Muscle strength in overwintering bears. Nature 409:997.
72. Hudson NJ, Lavidis NA, Choy PT, Franklin CE. 2005. Effect of prolonged inactivity on skeletal motor nerve terminals during aestivation in the burrowing frog, Cyclorana alboguttata. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 191:373–379.
73. Storey KB. 2004. Strategies for exploration of freeze responsive gene expression: advances in vertebrate freeze tolerance. Cryobiology 48:134–145.
74. Eddy SF, Storey KB. 2004. Up-regulation of fatty acid-binding proteins during hibernation in the little brown bat, Myotis lucifugus. Biochim Biophys Acta 1676:63–70.
75. Horman S, Hussain N, Dilworth SM, Storey KB, Rider MH. 2005. Evaluation of the role of AMP-activated protein kinase and its downstream targets in mammalian hibernation. Comp Biochem Physiol B Biochem Mol Biol 142:374–382.
76. Schmidt KE, Kelley KM. 2001. Down-regulation in the insulin-like growth factor (IGF) axis during hibernation in the golden-mantled ground squirrel, Spermophilus lateralis: IGF-I and the IGF-binding proteins (IGFBPs). J Exp Zool 289:66–73.
77. Cai D, McCarron RM, Yu EZ, Li Y, Hallenbeck J. 2004. Akt phosphorylation and kinase activity are down-regulated during hibernation in the 13-lined ground squirrel. Brain Res 1014:14–21.
78. Eddy SF, Storey KB. 2003. Differential expression of Akt, PPARgamma, and PGC-1 during hibernation in bats. Biochem Cell Biol 81:269–274.
79. Hillion JA, Li Y, Maric D, Takanohashi A, Klimanis D, et al. 2006. Involvement of Akt in preconditioning-induced tolerance to ischemia in PC12 cells. J Cereb Blood Flow Metab Mar 1; [Epub ahead of print].
80. Ogg S, Paradis S, Gottlieb S, Patterson GI, Lee L, et al. 1997. The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in C. elegans. Nature 389:994–999.
81. Long X, Spycher C, Han ZS, Rose AM, Muller F, et al. 2002. TOR deficiency in C. elegans causes developmental arrest and intestinal atrophy by inhibition of mRNA translation. Curr Biol 12:1448–1461.
82. Imae M, Fu Z, Yoshida A, Noguchi T, Kato H. 2003. Nutritional and hormonal factors control the gene expression of FoxOs, the mammalian homologues of DAF-16. J Mol Endocrinol 30:253–262.
83. van Breukelen F, Sonenberg N, Martin SL. 2004. Seasonal and state-dependent changes of eIF4E and 4E-BP1 during mammalian hibernation: implications for the control of translation during torpor. Am J Physiol Regul Integr Comp Physiol 287:R349–R353.
84. Woods AK, Storey KB. 2005. Effects of hibernation on multicatalytic proteinase complex in thirteen-lined ground squirrels, Spermophilus tridecemlineatus. Mol Cell Biochem 271:205–213.
85. Velickovska V, Lloyd BP, Qureshi S, van Breukelen F. 2005. Proteolysis is depressed during torpor in hibernators at the level of the 20S core protease. J Comp Physiol [B] 175:329–335.
86. Eddy SF, Morin P Jr, Storey KB. 2005. Cloning and expression of PPAR-gamma and PGC-1alpha from the hibernating ground squirrel, Spermophilus tridecemlineatus. Mol Cell Biochem 269:175–182.
87. Hudson NJ, Lehnert SA, Ingham AB, Symonds B, Franklin CE, et al. 2005. Lessons from an Estivating Frog: Sparing Muscle Protein Despite Starvation and Disuse. Am J Physiol Regul Integr Comp Physiol 240(3):R836–R843.
88. Lai KM, Gonzalez M, Poueymirou WT, Kline WO, Na E, et al. 2004. Conditional activation of akt in adult skeletal muscle induces rapid hypertrophy. Mol Cell Biol 24:9295–9304.
89. Peng XD, Xu PZ, Chen ML, Hahn-Windgassen A, Skeen J, et al. 2003. Dwarfism, impaired skin development, skeletal muscle atrophy, delayed bone development, and impeded adipogenesis in mice lacking Akt1 and Akt2. Genes Dev 17:1352–1365.
90. Um SH, Frigerio F, Watanabe M, Picard F, Joaquin M, et al. 2004. Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. Nature 431:200–205.
91. McPherron AC, Lawler AM, Lee SJ. 1997. Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. Nature 387:83–90.
92. Kawaguchi N, Xu X, Tajima R, Kronqvist P, Sundberg C, et al. 2002. ADAM 12 protease induces adipogenesis in transgenic mice. Am J Pathol 160:1895–1903.
93. Davis E, Jensen CH, Schroder HD, Farnir F, Shay-Hadfield T, et al. 2004. Ectopic expression of DLK1 protein in skeletal muscle of padumnal heterozygotes causes the callipyge phenotype. Curr Biol 14:1858–1862.
94. Cadigan KM, Liu YI. 2006. Wnt signaling: complexity at the surface. J Cell Sci 119:395–402.
95. Miller JR, Hocking AM, Brown JD, Moon RT. 1999. Mechanism and function of signal transduction by the Wnt/beta-catenin and Wnt/Ca2+ pathways. Oncogene 18:7860–7872.
96. Cadigan KM, Nusse R. 1997. Wnt signaling: a common theme in animal development. Genes Dev 11:3286–3305.
97. Kikuchi A. 1999. Modulation of Wnt signaling by Axin and Axil. Cytokine Growth Factor Rev 10:255–265.
98. Heeg-Truesdell E, LaBonne C. 2006. Wnt signaling: a shaggy dogma tale. Curr Biol 16:R62–R64.
99. Kennell JA, MacDougald OA. 2005. Wnt signaling inhibits adipogenesis through beta-catenin-dependent and -independent mechanisms. J Biol Chem 280:24004–24010.

100. Ross SE, Hemati N, Longo KA, Bennett CN, Lucas PC, et al. 2000. Inhibition of adipogenesis by Wnt signaling. Science 289:950–953.
101. Taylor-Jones JM, McGehee RE, Rando TA, Lecka-Czernik B, Lipschitz DA, et al. 2002. Activation of an adipogenic program in adult myoblasts with age. Mech Ageing Dev 123:649–661.
102. Vertino AM, Taylor-Jones JM, Longo KA, Bearden ED, Lane TF, et al. 2005. Wnt10b deficiency promotes coexpression of myogenic and adipogenic programs in myoblasts. Mol Biol Cell 16:2039–2048.
103. Armstrong DD, Esser KA. 2005. Wnt/beta-catenin signaling activates growth-control genes during overload-induced skeletal muscle hypertrophy. Am J Physiol Cell Physiol 289:C853–C859.
104. Kim JE, Chen J. 2004. Regulation of peroxisome proliferator-activated receptor-gamma activity by mammalian target of rapamycin and amino acids in adipogenesis. Diabetes 53:2748–2756.
105. Teleman AA, Chen YW, Cohen SM. 2005. Drosophila Melted modulates FOXO and TOR activity. Dev Cell 9:271–281.
106. Wang YH, Byrne KA, Reverter A, Harper GS, Taniguchi M, et al. 2005. Transcriptional profiling of skeletal muscle tissue from two breeds of cattle. Mamm Genome 16:201–210.
107. Nishimura T, Hattori A, Takahashi K. 1999. Structural changes in intramuscular connective tissue during the fattening of Japanese black cattle: effect of marbling on beef tenderization. J Anim Sci 77:93–104.
108. van Loon LJ. 2004. Use of intramuscular triacylglycerol as a substrate source during exercise in humans. J Appl Physiol 97:1170–1187.
109. van Loon LJ, Schrauwen-Hinderling VB, Koopman R, Wagenmakers AJ, Hesselink MK, et al. 2003. Influence of prolonged endurance cycling and recovery diet on intramuscular triglyceride content in trained males. Am J Physiol Endocrinol Metab 285:E804–E811.
110. van Loon LJ, Koopman R, Stegen JH, Wagenmakers AJ, Keizer HA, et al. 2003. Intramyocellular lipids form an important substrate source during moderate intensity exercise in endurance-trained males in a fasted state. J Physiol 553:611–625.
111. Goodpaster FH, Brown FF. 2005. Skeletal muscle lipid and its association with insulin resistance: what is the role for exercise? Exerc Sport Sci Rev 33:150–154.
112. Goodpaster BH, He J, Watkins S, Kelley DE. 2001. Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance-trained athletes. J Clin Endocrinol Metab 86:5755–5761.
113. Pruchnic R, Katsiaras A, He J, Kelley DE, Winters C, et al. 2004. Exercise training increases intramyocellular lipid and oxidative capacity in older adults. Am J Physiol Endocrinol Metab 287:E857–E862.
114. Cree MG, Newcomer BR, Katsanos CS, Sheffield-Moore M, Chinkes D, et al. 2004. Intramuscular and liver triglycerides are increased in the elderly. J Clin Endocrinol Metab 89:3864–3871.
115. Abraham RT. 2005. TOR signaling: an odyssey from cellular stress to the cell growth machinery. Curr Biol 15:R139–R141.
116. Astrinidis A, Henske EP. 2005. Tuberous sclerosis complex: linking growth and energy signaling pathways with human disease. Oncogene 24:7475–7481.
117. Jacobs FM, van der Heide LP, Wijchers PJ, Burbach JP, Hoekman MF, Smidt MP. 2003. FoxO6, a novel member of the FoxO class of transcription factors with distinct shuttling dynamics. J Biol Chem 278(38):35959–35967.
118. Smith AG, Muscat GE. 2006. Orphan nuclear receptors: therapeutic opportunities in skeletal muscle. Am J Physiol Cell Physiol 291(2): C203–C217.