

Review

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Effects of thyroid hormones in skeletal muscle protein turnover

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Abstract: Thyroid hormones (THs) are critical regulators of muscle metabolism in both healthy and unhealthy conditions. Acting concurrently as powerful anabolic and catabolic factors, THs are endowed with a vital role in muscle mass maintenance. As a result, thyroid dysfunctions are the leading cause of a wide range of muscle pathologies, globally identified as myopathies. Whether muscle wasting is a common feature in patients with hyperthyroidism and is mainly caused by THs-dependent stimulation of muscle proteolysis, also muscle growth is often associated with hyperthyroid conditions, linked to THs-dependent stimulation of muscle protein synthesis. Noteworthy, also hypothyroid status negatively impacts on muscle physiology, causing muscle weakness and fatigue. Most of these symptoms are due to altered balance between muscle protein synthesis and breakdown. Thus, a comprehensive understanding of THs-dependent skeletal muscle protein turnover might facilitate the management of physical discomfort or weakness in conditions of thyroid disease.

Herein, we describe the molecular mechanisms underlying the THs-dependent alteration of skeletal muscle structure and function associated with muscle atrophy and hypertrophy, thus providing new insights for targeted modulation of skeletal muscle dynamics.

Keywords: thyroid hormones; skeletal muscle; skeletal muscle atrophy; skeletal muscle hypertrophy

Introduction

Thyroid hormones homeostasis

Thyroid hormones (THs), produced and secreted from the thyroid gland in form of prohormone T4 (3,3',5,5'-Tetraiodo-L-thyronine) and bioactive hormone T3 (3,3',5-Triiodo-L-thyronine), emerge as notable tyrosine-based molecules with the potential to support a wide range of physiological processes, regulating metabolism, energy production, cellular growth, and tissue development [1–4]. Under physiological conditions, the thyroid gland produces T3 and T4 in a ratio of circa 1:14, and, through a central regulation mediated by the hypothalamic–pituitary–thyroid (HPT) axis, THs plasma homeostasis is maintained at remarkably stable levels [5]. Beyond THs circulating concentrations, their intracellular availability is tightly controlled via a fine-tuned interplay between transporters and modulating enzymes. Prior to THs metabolism within target cells, their cellular influx and efflux are mediated through transmembrane protein transporters, namely monocarboxylate transporters, MCT8 and MCT10, that show a cell-specific expression and different transport kinetics and specificity for THs (e.g., MCT8 preferentially transports T4, while MCT10 prefers T3) [3, 6–10]. Once inside the cells, THs can be directly metabolized by different pathways. Apart from the deamination, decarboxylation, sulfation, and glucuronidation, deiodination represents the most relevant process involved in THs metabolism [11]. Three different selenocysteine-dependent membrane enzymes, type 1 (D1), type 2 (D2), and type 3 (D3) deiodinases, contribute to the

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breakdown of THs, mediating the activation and inactivation of the initially released hormone precursor T4 into active or inactive metabolites in the target cells. Albeit with a different enzymatic efficiency (K_M of D1 is in micromolar range, 1–10 μ M, K_M of D2 is in the nanomolar range, 1–4 nM) [5], both D1 and D2, presenting outer ring deiodinase activity, can convert the prohormone T4 into the bioactive form T3. Conversely, D3, having inner ring deiodinase activity, acts as a physiological terminator of THs action, degrading both T4 and T3 into inactive molecules, respectively, rT3 and 3,3'-T2 [12]. Given that the human thyroid gland secretes only 20 % of the daily T3 requirement, the deiodinase-mediated T4-to-T3 peripheral conversion has a significant role in the maintenance of T3 plasma homeostasis in humans, providing the remaining requested 80 % [13, 14]. The bioactive hormone T3 exerts its biological activity either by binding to TH nuclear receptors (TR α encoded by *THRA* gene or TR β 1/TR β 2 encoded by *THRB* gene) [15–17] and regulating target gene expression (genomic action or type I action) or by binding to receptors on the cell membrane, as in the case of integrin α v β 3, and cytosolic partners, thus activating intracellular cascades transduced by extracellular signal regulated kinases 1/2 (ERK1/2) or phosphatidylinositol 3-kinase (PI3K) (nongenomic action or type II action) [1, 18, 19]. The nongenomic action of THs, which does not involve the binding of T3 to the nuclear receptors, differs from the classic genomic action of THs and regulates mechanisms that occur within seconds or minutes and are responsible for rapid effects. Understandably, THs signaling depends on the integrity and function of cellular THs transporters, deiodinases enzymes, and THs receptors that cooperate to elicit THs actions.

Thyroid hormones and metabolism

THs heavily impact the overall metabolic rate of each cell and tissue, affecting the entire sum of reactions occurring throughout the body [3]. It is well established that thyroid status correlates with body weight and energy expenditure [20–22]. Through both central and peripheral actions, THs play a key role in the maintenance of the basal metabolic rate (BMR). Indeed, patients with THs dysfunction often have symptoms of metabolic dysregulation: while an excess of THs levels (hyperthyroid state) raises the BMR and promotes a hypermetabolic state, characterized by increased resting energy expenditure, weight loss, reduced cholesterol levels, increased lipolysis, and gluconeogenesis [23, 24], a reduced THs availability (hypothyroid state) reduces the BMR and is associated with a hypometabolic state, characterized by reduced resting energy expenditure, weight

gain, increased cholesterol levels, reduced lipolysis, and gluconeogenesis [21].

THs stimulate metabolic cycles involving fat, glucose, and protein catabolism and anabolism. Thus, the physiological relevance of THs in coordinating short- and long-term cell energy needs sustains its critical role in tissue-specific metabolic control [25]. Many of THs actions in metabolic regulation involve modulation of other metabolic signaling pathways (Table 1).

Table 1: Metabolic processes modulated by thyroid hormone signaling.

Adaptive thermogenesis (in response to cold exposure and/or food intake)	THs effects Interacting pathways THs target	Stimulate Adrenergic/bile acids gluconeogenesis UCP1/PEPCK
Basal metabolic rate, BMR	THs effects Interacting pathways THs target	Stimulate Adrenergic Na ⁺ /K ⁺ ATPase SERCA-1/UCPs/LPL
Bile acid synthesis (in response to fat intake)	THs effects Interacting pathways THs target	Decrease TGR5/D2/FXR/PPAR α CYP7A1
Body weight regulation (in response to nutrient intake)	THs effects Interacting pathways THs target	Integrate balance with nutrient intake signals TRH/Leptin/Adrenergic CART/NPY/D2 TRH/TSH/Spot-14/D2
Cholesterol synthesis and efflux	THs effects Interacting pathways THs target	Promote cholesterol synthesis and efflux Sterol signaling (SREBP) PPAR α /LXR LDL-R/ABCA1
Fatty acid synthesis and oxidation (in response to fat intake/storage)	THs effects Interacting pathways THs target	Promote lipolysis and β -oxidation Adrenergic/PPAR α /LXR CPT1 α
Glucose metabolism (in response to carbohydrate intake and/or serum glucose/ insulin)	THs effects Interacting pathways THs target	Stimulate gluconeogen- esis Impair insulin secretion Glucose/Insulin/PPAR α LXR/SREBP/RXR ACC1/GLUT4/ChREBP

ACC, Acetyl-CoA Carboxylase; CART, Cocaine- and Amphetamine-Regulated Transcripts; ChREBP, Carbohydrate Response Element Binding Protein; CPT1 α , Carnityl Palmotolyl Transferase 1 α ; CYP7A1, Cholesterol 7-hydroxylase; D2, 5'-deiodinase Type 2; FXR, Farnesoid X receptor; LPL, Lipoprotein Lipase; LXR, Liver X Receptor; NPY, Neuropeptide Y; PPAR α , Peroxisome Proliferator Activated Receptor α ; PEPCK, Phosphoenolpyruvate Carboxykinase; RXR, Retinoid X Receptor; SERCA, Sarcoplasmic Reticulum Calcium; TGR5, G protein-coupled receptor bile acid receptor; TRH, Thyrotropin Releasing Hormone; TSH, Thyroid Stimulating Hormone; UCP, Uncoupling Protein.

THs closely synergize with the adrenergic nervous system to produce heat in response to cold exposure and maintain body temperature, a reaction termed adaptive thermogenesis [26]. The thyroid–adrenergic synergy, most evident and better studied in the rodents' Brown Adipose Tissue (BAT) during cold adaptation, sustains the thermogenic responses involving genes such as Uncoupling Protein 1 (UCP1) and Peroxisome proliferator-activated receptor Gamma Cofactor 1 (PGC1) [27] and stimulating both mitochondrial biogenesis and upregulation of fatty acid oxidation.

THs regulate hepatic function by modulating the BMR of hepatocytes: through the nuclear hormone receptors interaction, THs control lipid metabolism and exert direct and indirect actions not only on the regulation of cholesterol production, disposal, and efflux, but also on bile acid synthesis and fatty acid metabolism [28, 29]. Furthermore, depending on the metabolic status, THs can induce both lipolysis and lipid synthesis (most known as lipogenesis). Although the THs direct action is lipolysis, lipogenesis is thought to be stimulated to restore fat stores [30].

THs can also stimulate the metabolism of carbohydrates. While not changing the blood glucose levels, THs can cause increased glucose reabsorption, gluconeogenesis, glycogen synthesis, and glucose oxidation. Indeed, THs directly impact glucose metabolism by (i) stimulating hepatic glucose production, (ii) reducing insulin levels, in part through accelerated insulin degradation, (iii) enhancing the expression of the Glucose Transporter GLUT4 in skeletal muscle, and (iv) stimulating the expression of additional factors, such as Carbohydrate Response Element Binding Protein (ChREBP), that then influence glucose response and insulin secretion [31–33].

The action of THs also has a significant effect on protein metabolism. Indeed, THs in high doses can additionally induce protein catabolism early and primary to overall metabolic reactions, increasing whole-body protein turnover and breakdown [34].

Remarkably, through an intricate signaling network, THs increase the sensitivity and the speed of responses to metabolic changes induced in response to intracellular and extracellular stimuli and contribute to regulating metabolic homeostasis in cells and tissues. Comprehensively, hyperthyroidism represents a catabolic state characterized by an increase of energy expenditure, glucose turnover [35], lipolysis [36], and protein turnover [37]. These metabolic effects are likely to be of clinical importance because hyperthyroidism reduces exercise performance [38] and increases risk of cardiovascular mortality [39, 40] and bone loss [41].

Thyroid hormones and regulation of protein catabolism and anabolism

Metabolism is the totality of the chemical processes that take place inside a living cell and are required for the maintenance of life. Whereas catabolism is the breakdown of complex molecules into smaller ones, anabolism is the process that creates complex molecules from simpler ones and entails the use of energy [42]. Proteins undergo catabolism within the cell, replenishing the intracellular pool of amino acids. Lysosomal proteases utilize protein to break it down into amino acids, which the cell can use to make new proteins when needed. During starvation, muscle protein can be broken down into amino acids, which can then be used as an energy source by gluconeogenesis. Conversely, when fed or in a state of metabolic acidosis, the kidneys can use glutamine as fuel. In case of an excess of amino acids, the human body lacks a method to store them. Therefore, they either disintegrate or change into glucose or ketones. Hydrocarbons and nitrogenous waste are produced by decomposition. Because nitrogen creates ammonium ions, the body may become toxically exposed to excessive nitrogen concentrations and the urea cycle aids in the digestion and elimination of nitrogen from our bodies.

The impact of exercise and dietary protein on the synthesis of new proteins is regulated by hormones. It is well known that the nervous and endocrine systems work together to ensure homeostasis by allowing other bodily systems to function in unison [42]. Among hormones with a catabolic or anabolic role, THs have a major influence on metabolism affecting both proteins, carbohydrates, and fats metabolism.

THs have a significant impact on protein metabolism explicating a variety and sometimes contradictory actions. In their physiological concentrations, THs stimulate protein synthesis as well as their breakdown, showing both anabolic and catabolic effects, whereas when oversecreted the protein catabolic action predominates [43]. This could be partially explained by that both hypo- and hyperthyroidism are characterized by changes in the levels of several hormones in the blood, such as insulin, glucagon, and glucocorticoids, in addition to an excess or deficiency of THs. Furthermore, it has been documented that other membrane receptors, such as those for glucagon and catecholamines, are modulated by the thyroid state [43]. As a result, hyperthyroidism increases the excretion of nitrogen and methyl-histidine in the urine indicating a decline in the protein stores found in the skeletal muscle probably as a consequence of a decreased muscular reuptake and increased protein catabolism [44]. A rise in the concentration of amino

acids in plasma from skeletal muscle in turn provides more substrate for improved hepatic gluconeogenesis. However, excess release of THs leads to cardiac hypertrophy, which is in contrast with atrophic skeletal muscle identified in patients suffering from hyperthyroidism [43]. Nevertheless, in euthyroid patients, it is observed that a “low T3 state” during starvation and replacing T3 within its physiological concentration either has no impact [45] or very slightly increases the excretion of nitrogen in the urine [46]. These findings clearly show that THs at physiological doses have little or only minor catabolic activity.

Skeletal muscle

Skeletal muscle (SKM) is the most prevalent tissue in healthy individuals, making up roughly approximately 40 % of human body mass [47]. Consequently, systemic physiology is significantly impacted by any modifications to the energy profile of SKM. Indeed, SKM is one of the tissues most crucial for energy expenditure, glucose, and lipid homeostasis.

The muscles are linked to bones by tendons through which the forces and movements developed during contractions are transmitted to the skeleton. Contraction is due to the activation of muscle fibers with a tendency of the fibers to shorten [48] and occurs when the cytosolic calcium concentration increases, triggering a series of molecular events that include the binding of calcium to the muscle-regulatory proteins, the interaction of myosin cross-bridges with actin filaments, and the production of the cross-bridge working stroke. For a long time, it was believed that the SKM served as the locomotor system's effector organs and confers stability and power for all body movements; thus, any impairment in SKM function results in at least some degree of instability or immobility. Due to their striped microscopic appearance, cardiac and skeletal muscles are both referred to as striated muscles as the result of the subcellular contractile components' uniform and organized arrangement [48]. Even though a large portion of their behavior is subconsciously regulated, they are under voluntary control. Unlike cardiac muscles, skeletal muscles do not exhibit intrinsic spontaneous activity because of lack of the ion channels that induce spontaneous membrane depolarization. As a result, a nerve impulse always serves as the trigger for physiological skeletal muscle activation, and muscle fibers receive from a single branch of a motor neuron their nerve inputs at single central swellings of the fibers known as motor endplates [49, 50].

Muscle fibers range in diameter from 10 to 100 μm . Most human muscles contain a mixture of fibers within this range, namely, I, IIa, IIx, and IIb. Type I fibers, which are thinner,

are adapted to generate submaximal strain during prolonged activity and are called slow-twitch fibers. The thickest fibers (type IIb) are best suited for short bursts of near-maximum activity and are called fast-twitch fibers [51]. Due to their high myoglobin content, muscles with a higher prevalence of type I fibers seem a deeper red color than muscles with fewer type I fibers. The heme moiety of myoglobin, which gives it the ability to bind oxygen, gives it its pigmentation. Myoglobin is a protein that allows muscle cells to store oxygen. It has a higher affinity for oxygen than hemoglobin but releases oxygen for aerobic metabolism when demand increases. While fast fibers are more glycolytic and have higher levels of glycogen and phosphocreatine, slow fibers are oxidative and have higher levels of mitochondria and myoglobin [52].

ATPase activity was found to be higher in fast type IIb fibers compared to IIa and IIx fibers, all of which have higher activity than slow type I fibers [53]. During contraction, the ATP hydrolysis rate is higher in fast fibers than in slow fibers proportionally to the ATP production speed of each fiber type. Moreover, regardless of their mitochondrial composition, type II fibers have well-developed sarcoplasmic reticulum, whereas type I fibers have poorly developed sarcoplasmic reticulum [54]. The expression of the Sarcoplasmic Reticulum Calcium (Ca^{2+})-ATPase (SERCA) varies across tissues, with SERCA1a being the primary isoform expressed in adult fast-twitch fibers (type II), associated with a faster Ca^{2+} storage compared to type 2a (SERCA2a) expressed in both in slow- and fast-twitch (type I and II) skeletal muscle fibers [55]. The most abundant protein in SKM is the myosin heavy chain (MYH), an important intrinsic factor for muscle twitch [56]. Myosin heavy chain 7 (MYH7) is expressed by type I fibers; myosin heavy chain 2 (MYH2) is found in type IIa fibers; myosin heavy chain 1 (MYH1) is expressed by type IIx fibers; and myosin heavy chain 4 (MYH4) is present in type IIb fibers [51].

Muscle atrophy and hypertrophy

Skeletal muscle mass and composition are continuously modulated during development or upon different stimuli, including pathological conditions that impact the musculo-skeletal or nervous system [57, 58]. The regulation of muscle mass and fiber size essentially reflects protein turnover, namely the balance between protein synthesis and degradation, which is a delicate equilibrium that, depending on the conditions, can promote muscle loss (atrophy) or muscle growth (hypertrophy). Changes in protein turnover leading to muscle atrophy or hypertrophy do not always proceed

according to the simplistic equations suggested by the balance analogy.

From a general point of view, skeletal muscle atrophy is caused by a decrease in the number of muscle cells and the size of preexisting muscle fibers and is characterized by an imbalance between protein synthesis and degradation in favor of the second one [59–61]. Indeed, skeletal muscle atrophy is associated with an increase in the rate of ATP-dependent ubiquitin-mediated proteolysis resulting in the enhancement of protein breakdown rates necessary as a source of amino acids for gluconeogenesis [62, 63]. Conversely, skeletal muscle hypertrophy is characterized by an increase in the size, as opposed to the number, of the preexisting muscle myofibers and is characterized by an enhanced rate of protein synthesis [64]. This increase in protein synthesis, a typical aspect of skeletal muscle hypertrophy occurring as an adaptive response to load-bearing exercise, enables new contractile filaments to be added to the preexisting muscle fibers, which in turn enables the muscle to generate greater force or resist fatigue.

Apart from functional changes, in atrophic or hypertrophic conditions, muscle cells and myofibers undergo also important structural changes [65]. The general muscle appearance in atrophic conditions is the wasting or thinning of muscle mass, due to a noticeable reduction in the muscle fiber cross-sectional area (CSA) that affects not only the maximal force and muscle power output but also the locomotor activity [66]. At the cellular level, muscle cells show characteristics such as sarcomere dissolution and endothelial degradation [67], a marked reduction in the mitochondria number [68], accumulation of connective tissue [67], elimination of apoptotic myonuclei [69], and a decrease in capillary density [70]. Conversely, the general muscle appearance in hypertrophic conditions is muscle building, due to an increase in the muscle fibers CSA associated with the radial enlargement of muscle fibers, which confers to the muscle a greater potential for maximal force production. At the cellular level, since a constant ratio of nuclei to cytoplasmic volume is maintained throughout all hypertrophic responses, the enlarged myofibers can only expand with the insertion of new myonuclei [71, 72]. Thus, hypertrophy is dependent on the proliferative activation of satellite cells and their myogenic differentiation before fusion with the existing myofibers [73, 74].

Thyroid hormones and the regulation of muscle mass

SKM is a major THs-target tissue, regulating oxygen consumption, fiber composition, calcium mobilization,

and glucose uptake [4, 75–77]. Adequate serum THs levels are crucial for SKM homeostasis since muscle performance is impaired in both hypo- and hyperthyroidism [78]. During the early phases of postnatal development, the maturation of SKM is induced by different stimuli. Muscle cell loses poly-neuronal innervations, mechanical strain to specific muscles increases, and THs levels rise simultaneously [79–81]. Both neuronal innervation and elevated serum THs cause muscle fiber profile transformations, such as the loss of embryonic and neonatal myosin and a rise in adult fast or slow myosin genes in certain muscles [82, 83]. Weight-bearing activity and electrical stimulation are essential for the postnatal development of slow fibers, while T3 signaling plays a critical role in the development of fast fibers, particularly in the transition of neonatal fibers to fibers IIb [56, 80, 84]. Thus, the normal pattern of fibers dispersion in each muscle is determined in part by physiological levels of THs [56, 85]. T3 suppresses the expression of MYH7, which is myosin from fibers type I, and stimulates the expression of MYH2, 1, and 4, which are myosin from fibers IIa, IIx, and IIb, respectively [86]. Furthermore, by causing the conversion of MYH7 to MYH2, MYH2 to MYH1, and MYH1 to MYH4, T3 promotes the slow-to-fast muscle fibers type conversion [76]. In the last years, different studies have demonstrated that the regulation of skeletal muscle myogenic development, regeneration, and metabolism is significantly influenced by the intracellular modulation of THs action, which is mediated by the deiodinases D2 and D3 and the TRs. THs promote the expression of myosin heavy chain (MHC) typical of the fast-twitch fibers, as well as the rate of relaxation and contraction and mitochondrial biogenesis. Thus, the concentration of THs inside cells is essential for the development of muscle progenitor cells, and it is precisely controlled by the joint action of D2 and D3 [87, 88]. Specifically, at the onset of the myogenic process, the intracellular concentration of THs should be kept low [89, 90]. Importantly, if D3 is highly expressed in activated and proliferating satellite cells (SCs), D2 is upregulated during differentiation [90], which causes an increase in intracellular THs concentration that propels the terminal differentiation of myocytes into myotubes/myofibers. Animal models with an alteration of THs signaling exhibited peculiar SKM characteristics, with profound changes in contractile and metabolic features both in hypo- and hyperthyroidism situations. Furthermore, most hypothyroidism patients showed myopathic alterations such as muscle weakness and pseudohypertrophy, myasthenic syndrome, and rhabdomyolysis, which are similar to the beginning of muscle weakness and atrophy

seen in hyperthyroid patients [91]. On the other hand, hyperthyroid individuals showed varying degrees of muscular weakness and atrophy [92]. Complying with the clinical manifestations of THRs gene mutation-based resistance to THs disorders in humans, global TR α - and TR β -knockout (KO) mice display developmental delay and mitochondrial dysfunction. In particular, TR α KO mice (both TR α 1 and TR α 2 isoforms are disrupted) exhibit progressive hypothyroidism, growth retardation, and several other disorders, including a lowered body temperature and significantly delayed intestinal and bone maturation, which ultimately results in death soon after the weaning period [93, 94]. This phenotype is consistent with the phenotype observed in subjects with *THRa* gene mutations, which are characterized by a wide range of hypothyroidism clinical characteristics, including constipation, low metabolic rate, poor growth, skeletal dysplasia, and neurodevelopmental delay [15, 95–97]. Furthermore, inactivating the *THR β* gene in mice impairs their ability to hear but does not affect their growth, metabolism, or neurological processes [98, 99]. The phenotype of the mouse model lacking both TRs isoforms (TR α / β KO) is drastically altered, displaying stunted growth and bone development, hyperactivity of the pituitary–thyroid axis, and low female fertility [100]. Another important concept is that the TRs deficiency causes mitochondrial dysfunctions and profound alterations of the lipid composition compared to wild-type mice. These observations support the concept that the TRs–THs complex in SKM is a key regulator of mitochondrial bioenergetics and lipid metabolism and that both TRs are needful for the THs-regulated metabolic rate Table 2 [15].

Table 2: THs effects on SKM properties.

Contractility	THs target	MYH7 (Myosin-7) ↓ MYH2 (Myosin-2) ↑ MYH1 (Myosin-1) ↑ MYH4 (Myosin-4) ↑
	THs effects	Increase the rate of contraction
	THs target	SERCA1a (Sarcoplasmic reticulum calcium 1a) ↑ SERCA2a (Sarcoplasmic reticulum calcium 2a) ↑
	THs effects	Increase the rate of contraction
Metabolism	THs target	Na ⁺ /K ⁺ -ATPase (sodium–potassium adenosine triphosphatase) ↑ SERCA1a (sarcoplasmic reticulum calcium 1a) ↑ SERCA2a (sarcoplasmic reticulum calcium 2a) ↑
	THs effects	Decreased energetic efficiency of contraction due to higher ATP consumption associated with fluxes of Na ⁺ /K ⁺ and Ca ²⁺ at rest and during activity

Thyroid hormones and the downstream pathways of muscle atrophy

Could one question whether skeletal muscle atrophy is simply the converse of skeletal muscle hypertrophy? From a macroscopic point of view, the atrophic and hypertrophic processes seem to be antithetical. However, the triggered molecular mechanisms are not necessarily opposite; on the contrary, unique transcriptional pathways are activated during muscle atrophy and hypertrophy [101, 102].

Genomic studies designed to underline the molecular mechanisms modulating muscle mass during atrophic processes identified two muscle-specific E3 ubiquitin ligases, whose expression is significantly upregulated in multiple settings of atrophy, *MuRF1* (muscle ring-finger protein-1) and *MAFbx* (muscle atrophy F-box, commonly known as Atrogin-1) [103, 104].

The involvement of the ubiquitin-proteasome pathway in skeletal muscle atrophy has been well characterized [105], as well as the effects of hypo- and hyperthyroidism in the regulation of muscle proteolysis [106–109]. Transcriptome analyses revealed that the expression of Atrogin-1 and MuRF1 is directly responsive to THs [110], which activate their nuclear transcription by upregulating cellular expression levels during muscle wasting. However, it is yet unknown whether the increase in protein degradation and the expression of Atrogin-1 and MuRF1 is exclusively due to direct effect of THs [41, 111] (Figure 1 and Table 3).

Accumulating evidence has demonstrated that THs also induce the autophagy-lysosome system, a second proteolytic mechanism that is activated in catabolic conditions and that is under transcriptional-dependent control. THs regulate protein degradation by increasing lysosomal enzyme activity [112]. Indeed, THs enhance autophagic fluxes in skeletal muscle through the induction of key autophagy genes, i.e., microtubule-associated proteins 1A/1B light chain 3B (MAP1LC3B, more simply LC3), Sequestosome 1 (or ubiquitin-binding protein p62), Unc-51-like kinase 1 (Ulk1), and Forkhead Box O1/3a (FoxO1/3a) [113] (Figure 1 and Table 3).

Both the ubiquitin-proteasome system and autophagy-lysosome system are simultaneously activated and coordinated by the FoxO transcription factors [114]. In physiological conditions, FoxO proteins are negatively regulated by the PI3K–AKT signaling pathway, which is normally involved in protein synthesis processes. In atrophic conditions, the decreased activity of the PI3K–AKT signaling pathway leads to the activation of FoxO3 that, inducing the *Atrogin-1* gene expression, is responsible for the fibers size decrease occurring during atrophy [115]. Importantly, FoxO3

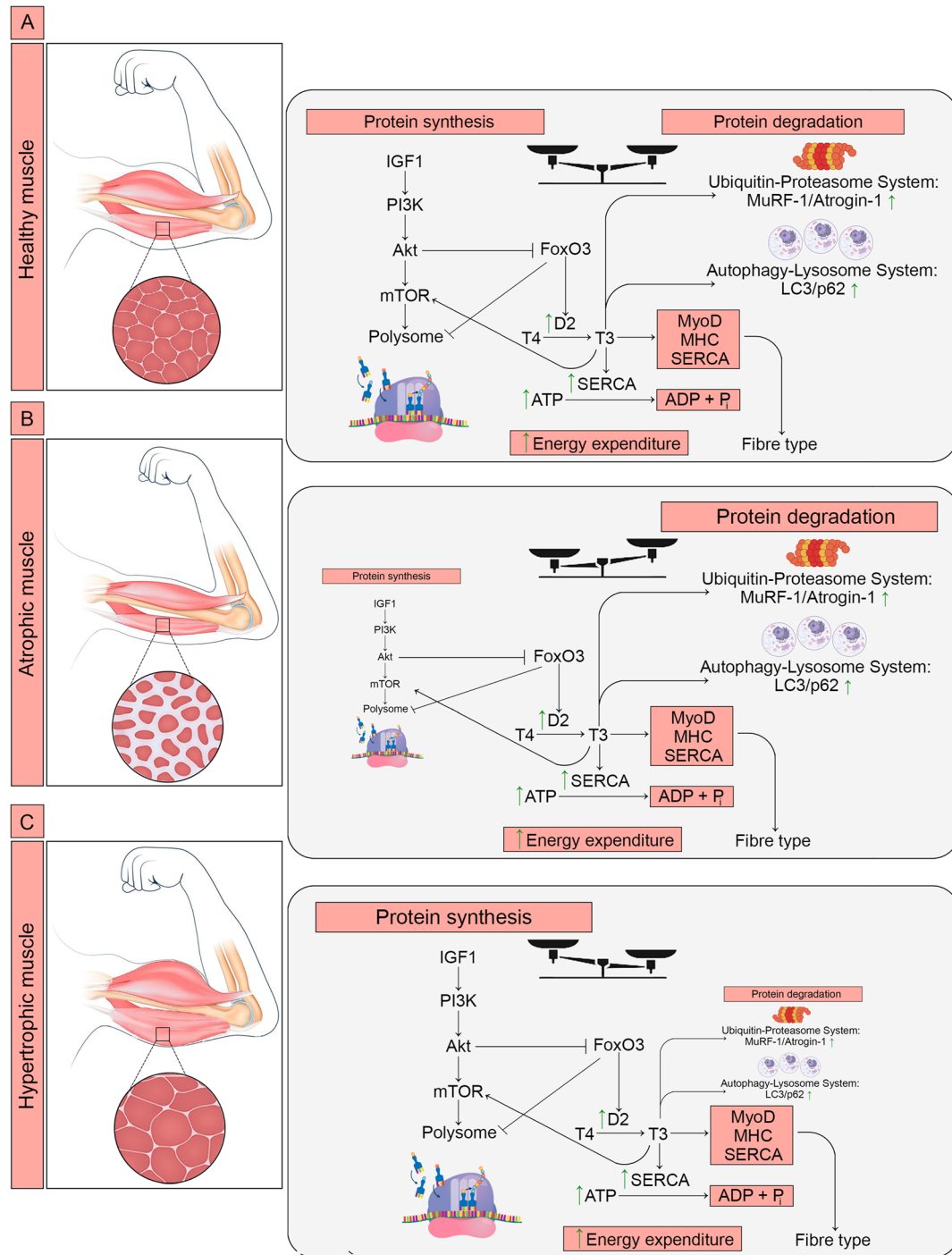


Figure 1: Schematic overview of the signaling pathways under thyroid hormones control involved in the regulation of muscle mass in healthy and unhealthy condition. FoxO3 (Forkhead Box O3) induces D2 expression, thus promoting the T4-to-T3 conversion and increasing local T3 in skeletal muscle. T3 acts promoting THs-target gene expression, in detail MyoD, MHC (myosin heavy chain) and SERCA (sarcoplasmic reticulum calcium (Ca²⁺)-ATPase), responsible of muscle fiber types and contraction. T3 stimulates SERCA, which hydrolyzes ATP and increases energy expenditure. (A) In healthy condition, THs regulate the balance between protein synthesis and degradation. (B) In atrophic condition, this balance is lost in favor of protein degradation process; thus, THs enhance the expression of genes and proteins involved both in ubiquitin-proteasome pathway and autophagy-lysosome system. (C) By contrast, in hypertrophic condition, the balance between protein synthesis and degradation is lost in favor of the first one; thus, THs enhance the expression of genes and proteins involved in the activation of IGF1–PI3K–Akt/PKB–mTOR pathway, resulting in the positive regulation of polysome and enhanced protein synthesis.

Table 3: THs and the downstream pathways of muscle atrophy and hypertrophy.

Pathways controlling muscle atrophy	Gene/proteins involved	THs effects
Ubiquitin-proteasome system	MuRF1 (muscle ring-finger protein-1)	MuRF1 ↑ [110]
	MAFbx (muscle atrophy F-box or Atrogin-1)	Atrogin-1 ↑ [110]
Autophagy-lysosome system	Microtubule-associated proteins 1A/1B light chain 3B (MAP1LC3B or LC3)	LC3 ↑ [113]
	Sequestosome 1 (or ubiquitin-binding protein p62)	p62 ↑ [113]
	Unc-51-like kinase 1 (Ulk1)	Ulk1 ↑ [113]
	Forkhead box O1/3a (FoxO1/3a)	FoxO1/3a ↑ [90]
Pathways controlling muscle hypertrophy	Gene/proteins involved	THs effects
IGF1–PI3K–Akt/PKB–mTOR	Insulin-like growth factor 1 (IGF1)	IGF1 ↑ [117]
	Phospho-inositide-3-kinase (PI3K)	PI3K ↑ [116–119]
	Akt (akt)	Akt ↑ [123–124]
	Protein kinase B (PKB)	PKB ↑
	Mammalian target of rapamycin (mTOR)	mTOR ↑ [123–124]
Myostatin-Smad3	Myostatin	Myostatin ↓ [125, 126]
	Small mother against decap-entaplegic 3 (Smad3)	Smad3 ↓ [125, 126]

is positively regulated by THs level: if on one hand T3 directly induces the transcriptional expression of *FoxO3* gene, on the other FoxO3 indirectly sustains T3 concentration by inducing D2 [90] (Figure 1 and Table 3).

Thyroid hormones and the downstream pathways of muscle hypertrophy

Muscle growth, whether it is for development, regeneration, or overload-induced hypertrophy, is generally controlled by two divergent signaling pathways, the Insulin-like Growth Factor 1–Phospho-Inositide-3-Kinase–Akt/Protein Kinase B–mammalian Target Of Rapamycin (IGF1–PI3K–Akt/PKB–mTOR) pathway [116–119] and the myostatin–Smad3 pathway, which act respectively as a positive and negative regulator of muscle growth (Figure 1 and Table 3).

The members of IGF1–PI3K–Akt/PKB–mTOR pathway act as a cascade, positively regulating muscle growth: in this flow, IGF1 activates PI3K–Akt pathway, which in turn

activates mTOR kinase, that stimulates protein synthesis by simultaneously inhibiting the protein breakdown and blocking autophagy via Ulk1. Genetic and pharmacological evidence supported the crucial role of mTOR in mediating muscle growth. Indeed, muscle-specific inactivation of mTOR causes reduced postnatal growth, due to metabolic and dystrophin defects, which are reflected in the reduced size of fast but not slow muscle fibers leading to severe myopathy [120]. Furthermore, the Akt–mTOR pathway is also a point of convergence for additional signaling pathways that are recognized to promote muscle growth, as in the case of androgens and β -adrenergic agents, both known to have anabolic effects on skeletal muscle [121, 122]. The mTOR kinase integrates multiple stimuli coming not only from cytokines, nutrients, ATP/AMP ratio but also from hormones, among which THs. It has been well documented that THs, beside their role in mediating muscle catabolism through the induction of FoxO3a [90], also exert proanabolic action and stimulate protein synthesis by activating the Akt–mTOR pathway [123, 124].

Conversely, myostatin-Smad3 pathway negatively regulates muscle growth, by inhibiting protein synthesis. In detail, myostatin signaling in myofibers is mediated by phosphorylation and nuclear translocation of Smad2 or Smad3 transcription factors, and formation of heterodimers with Smad4. Importantly, just inhibition of Smad2/3 is sufficient to promote muscle growth, suggesting that genes involved in protein turnover are the target of these transcription factors [125, 126]. Although the transcriptional targets of the Smad2/Smad4 and Smad3/Smad4 complexes that mediate the inhibitory effect on growth are not known, it is possible that myostatin signaling interferes with the Akt–mTOR pathway [125, 127].

Conclusions

Thyroid hormones are fine regulators of the balance between muscle mass loss and synthesis. The cellular mechanisms underlying the THs-dependent skeletal muscle physiology have been widely investigated. However, still many aspects of the complex relationship between THs and the maintenance of skeletal muscle composition remain to be fully elucidated, as well as the impact of hypo- and hyperthyroidism in muscle dysfunctions.

Multiple aspects contribute to such a complexity, in particular the dual ability of THs to act as both proatrophic and prohypertrophic agents. Indeed, although an excess of THs is responsible for the accelerated skeletal muscle catabolism exceeding anabolic processes during muscle atrophy, in the meantime, an excess of THs is also associated

with functional skeletal muscle anabolism during muscle hypertrophy.

The whole picture emerging indicates that skeletal muscle mass and composition are target of a divergent spectrum of metabolic functions regulated by the THs. Thus, THs can profoundly impact muscle forces and functionality in pathophysiological conditions such as those following muscle wasting syndromes or load-bearing exercise. In conclusion, future studies are required to clarify the multiple functions of THs in controlling skeletal muscle physiology, to provide critical benefits in counteracting skeletal muscle pathology.

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References

- Cheng SY, Leonard JL, Davis PJ. Molecular aspects of thyroid hormone actions. *Endocr Rev* 2010;31:139–70.
- Brent GA. Mechanisms of thyroid hormone action. *J Clin Invest* 2012;122:3035–43.
- Mullur R, Liu YY, Brent GA. Thyroid hormone regulation of metabolism. *Physiol Rev* 2014;94:355–82.
- Cicatiello AG, Di Girolamo D, Dentice M. Metabolic effects of the intracellular regulation of thyroid hormone: old players, new concepts. *Front Endocrinol (Lausanne)* 2018;9:474.
- Bianco AC. Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocr Rev* 2002;23:38–89.
- Friesema EC, Jansen J, Visser T. Thyroid hormone transporters. *Biochem Soc Trans* 2005;33:228–32.
- Gereben B, Zavacki AM, Ribich S, Kim BW, Huang SA, Simonides WS, et al. Cellular and molecular basis of deiodinase-regulated thyroid hormone signaling. *Endocr Rev* 2008;29:898–938.
- Van der Spek AH, Fliers E, Boelen A. The classic pathways of thyroid hormone metabolism. *Mol Cell Endocrinol* 2017;458:29–38.
- Bianco AC, Dumitrescu A, Gereben B, Ribeiro MO, Fonseca TL, Fernandes GW, et al. Paradigms of dynamic control of thyroid hormone signaling. *Endocr Rev* 2019;40:1000–47.
- Groeneweg S, Van Geest FS, Peeters RP, Heuer H, Visser WE. Thyroid hormone transporters. *Endocr Rev* 2020;41. <https://doi.org/10.1210/edrev/bnz008>.
- Peeters RP, Visser TJ. Metabolism of thyroid hormone. In: Feingold KR, editor. *Endotext*. South Dartmouth, MA: MDText.com, Inc.; 2000.
- Dentice M, Salvatore D. Deiodinases: the balance of thyroid hormone: local impact of thyroid hormone inactivation. *J Endocrinol* 2011;209:273–82.
- Pilo A, Iervasi G, Vitek F, Ferdeghini M, Cazzuola F, Bianchi R. Thyroidal and peripheral production of 3,5,3'-triiodothyronine in humans by multicompartmental analysis. *Am J Physiol* 1990;258:E715–26.
- Maia AL, Kim BW, Huang SA, Harney JW, Larsen PR. Type 2 iodothyronine deiodinase is the major source of plasma T3 in euthyroid humans. *J Clin Invest* 2005;115:2524–33.
- Nappi A, Murolo M, Cicatiello AG, Sagliocchi S, Di Cicco E, Raia M, et al. Thyroid hormone receptor isoforms alpha and beta play convergent roles in muscle physiology and metabolic regulation. *Metabolites* 2022;12. <https://doi.org/10.3390/metabo12050405>.
- Miro C, Di Giovanni A, Murolo M, Cicatiello AG, Nappi A, Sagliocchi S, et al. Thyroid hormone and androgen signals mutually interplay and enhance inflammation and tumorigenic activation of tumor microenvironment in prostate cancer. *Cancer Lett* 2022;532. <https://doi.org/10.1016/j.canlet.2022.215581>.
- Torabinejad S, Miro C, Barone B, Imbimbo C, Crocetto F, Dentice M. The androgen-thyroid hormone crosstalk in prostate cancer and the clinical implications. *Eur Thyroid J* 2023;12. <https://doi.org/10.1530/etj-22-0228>.
- Davis PJ, Leonard JL, Davis FB. Mechanisms of nongenomic actions of thyroid hormone. *Front Neuroendocrinol* 2008;29:211–8.
- Nappi A, Murolo M, Sagliocchi S, Miro C, Cicatiello AG, Di Cicco E, et al. Selective inhibition of genomic and non-genomic effects of thyroid hormone regulates muscle cell differentiation and metabolic behavior. *Int J Mol Sci* 2021;22. <https://doi.org/10.3390/ijms22137175>.
- Fox CS. Relations of thyroid function to body weight: cross-sectional and longitudinal observations in a community-based sample. *Arch Intern Med* 2008;168:587–92.
- Iwen KA, Schröder E, Brabant G. Thyroid hormones and the metabolic syndrome. *Eur Thyroid J* 2013;2:83–92.
- Knudsen N, Laurberg P, Rasmussen LB, Bülow I, Perrild H, Ovesen L, et al. Small differences in thyroid function may be important for body mass index and the occurrence of obesity in the population. *J Clin Endocrinol Metab* 2005;90:4019–24.
- Brent GA. Clinical practice. Graves' disease. *N Engl J Med* 2008;358:2594–605.
- Motomura K, Brent GA. Mechanisms of thyroid hormone action. Implications for the clinical manifestation of thyrotoxicosis. *Endocrinol Metab Clin N Am* 1998;27:1–23.
- Oetting A, Yen PM. New insights into thyroid hormone action. *Best Pract Res Clin Endocrinol Metab* 2007;21:193–208.
- Silva JE, Bianco SD. Thyroid-adrenergic interactions: physiological and clinical implications. *Thyroid* 2008;18:157–65.
- Silva JE. Thermogenic mechanisms and their hormonal regulation. *Physiol Rev* 2006;86:435–64.
- Liu YY, Brent GA. Thyroid hormone crosstalk with nuclear receptor signaling in metabolic regulation. *Trends Endocrinol Metab* 2010;21:166–73.
- Webb P. Thyroid hormone receptor and lipid regulation. *Curr Opin Invest Drugs* 2010;11:1135–42.
- Oppenheimer JH, Schwartz HL, Lane JT, Thompson MP. Functional relationship of thyroid hormone-induced lipogenesis, lipolysis, and thermogenesis in the rat. *J Clin Invest* 1991;87:125–32.
- Crunkhorn S, Patti ME. Links between thyroid hormone action, oxidative metabolism, and diabetes risk? *Thyroid* 2008;18:227–37.

32. Potenza M, Via MA, Yanagisawa RT. Excess thyroid hormone and carbohydrate metabolism. *Endocr Pract* 2009;15:254–62.
33. Hashimoto K, Ishida E, Matsumoto S, Okada S, Yamada M, Satoh T, et al. Carbohydrate response element binding protein gene expression is positively regulated by thyroid hormone. *Endocrinology* 2009;150:3417–24.
34. Riis AL, Jørgensen JOL, Ivarsen P, Frystyk J, Weeke J, Møller N. Increased protein turnover and proteolysis is an early and primary feature of short-term experimental hyperthyroidism in healthy women. *J Clin Endocrinol Metab* 2008;93:3999–4005.
35. Møller N, Nielsen S, Nyholm B, Pøksen N, George K, Alberti MM, et al. Glucose turnover, fuel oxidation and forearm substrate exchange in patients with thyrotoxicosis before and after medical treatment. *Clin Endocrinol (Oxf)* 1996;44:453–9.
36. Riis AL, Gravholt CH, Djurhuus CB, Nørrelund H, Jørgensen JOL, Weeke J, et al. Elevated regional lipolysis in hyperthyroidism. *J Clin Endocrinol Metab* 2002;87:4747–53.
37. Tauveron I, Charrier S, Champredon C, Bonnet Y, Berry C, Bayle G, et al. Response of leucine metabolism to hyperinsulinemia under amino acid replacement in experimental hyperthyroidism. *Am J Physiol* 1995;269:E499–507.
38. Miro C, Nappi A, Sagliocchi S, Di Cicco E, Murolo M, Torabinejad S, et al. Thyroid hormone regulates the lipid content of muscle fibers, thus affecting physical exercise performance. *Int J Mol Sci* 2023;24. <https://doi.org/10.3390/ijms241512074>.
39. Biondi B. Effects of subclinical thyroid dysfunction on the heart. *Ann Intern Med* 2002;137:904–14.
40. Murolo M, Di Vincenzo O, Cicatiello AG, Scalfi L, Dentice M. Cardiovascular and neuronal consequences of thyroid hormones alterations in the ischemic stroke. *Metabolites* 2022;13. <https://doi.org/10.3390/metabo13010022>.
41. Faber J. Normalization of serum thyrotrophin by means of radioiodine treatment in subclinical hyperthyroidism: effect on bone loss in postmenopausal women. *Clin Endocrinol (Oxf)* 1998;48:285–90.
42. Judge A, Dodd MS. *Metabolism. Essays Biochem* 2020;64:607–47.
43. Muller MJ, Seitz HJ. Thyroid hormone action on intermediary metabolism. Part II: lipid metabolism in hypo- and hyperthyroidism. *Klin Wochenschr* 1984;62:49–55.
44. Burini R, Santidrian S, Moreyra M, Brown P, Munro HN, Young VR. Interaction of thyroid status and diet on muscle protein breakdown in the rat, as measured by N tau-methylhistidine excretion. *Metabolism* 1981;30:679–87.
45. Schwartz HL, Lancer SR, Oppenheimer JH. Thyroid hormones influence starvation-induced hepatic protein loss in the rat: possible role of thyroid hormones in the generation of labile protein. *Endocrinology* 1980;107:1684–92.
46. Gardner DF, Kaplan MM, Stanley CA, Utiger RD. Effect of tri-iodothyronine replacement on the metabolic and pituitary responses to starvation. *N Engl J Med* 1979;300:579–84.
47. Kim KM, Jang HC, Lim S. Differences among skeletal muscle mass indices derived from height-weight-and body mass index-adjusted models in assessing sarcopenia. *Korean J Intern Med* 2016;31:643–50.
48. Faulkner JA. Terminology for contractions of muscles during shortening, while isometric, and during lengthening. *J Appl Physiol* 2003;95:455–9.
49. Sanes JR, Lichtman JW. Development of the vertebrate neuromuscular junction. *Annu Rev Neurosci* 1999;22:389–442.
50. Pratt SJP, Shah SB, Ward CW, Inacio MP, Stains JP, Lovering RM. Effects of in vivo injury on the neuromuscular junction in healthy and dystrophic muscles. *J Physiol* 2013;591:559–70.
51. Schiaffino S, Reggiani C. Fiber types in mammalian skeletal muscles. *Physiol Rev* 2011;91:1447–531.
52. Sagliocchi S, Cicatiello AG, Di Cicco E, Ambrosio R, Miro C, Di Girolamo D, et al. The thyroid hormone activating enzyme, type 2 deiodinase, induces myogenic differentiation by regulating mitochondrial metabolism and reducing oxidative stress. *Redox Biol* 2019;24. <https://doi.org/10.1016/j.redox.2019.101228>.
53. Stienen GJ, Kiers JL, Bottinelli R, Reggiani C. Myofibrillar ATPase activity in skinned human skeletal muscle fibres: fibre type and temperature dependence. *J Physiol* 1996;493:299–307.
54. Schiaffino S, Hanzlíková V, Pierobon S. Relations between structure and function in rat skeletal muscle fibers. *J Cell Biol* 1970;47:107–19.
55. Wu KD, Lytton J. Molecular cloning and quantification of sarcoplasmic reticulum Ca(2+)-ATPase isoforms in rat muscles. *Am J Physiol* 1993;264:C333–41.
56. Baldwin KM, Haddad F. Effects of different activity and inactivity paradigms on myosin heavy chain gene expression in striated muscle. *J Appl Physiol* 2001 90, 345–57.
57. Thompson LV. Skeletal muscle adaptations with age, inactivity, and therapeutic exercise. *J Orthop Sports Phys Ther* 2002;32:44–57.
58. Baldwin KM, Haddad F, Pandorf CE, Roy RR, Edgerton VR. Alterations in muscle mass and contractile phenotype in response to unloading models: role of transcriptional/pretranslational mechanisms. *Front Physiol* 2013;4:284.
59. Glass DJ. Molecular mechanisms modulating muscle mass. *Trends Mol Med* 2003;9:344–50.
60. Glass DJ. Skeletal muscle hypertrophy and atrophy signaling pathways. *Int J Biochem Cell Biol* 2005;37:1974–84.
61. Bonaldo P, Sandri M. Cellular and molecular mechanisms of muscle atrophy. *Dis Model Mech* 2013;6:25–39.
62. Mitch WE, Goldberg AL. Mechanisms of muscle wasting. The role of the ubiquitin-proteasome pathway. *N Engl J Med* 1996;335:1897–905.
63. Jagoe RT, Lecker SH, Gomes M, Goldberg AL. Patterns of gene expression in atrophying skeletal muscles: response to food deprivation. *FASEB J* 2002;16:1697–712.
64. Goldspink DF, Garlick PJ, McNurlan MA. Protein turnover measured in vivo in muscles undergoing compensatory growth and subsequent denervation atrophy. *Biochem J* 1983;210:89–98.
65. Boonyarom O, Inui K. Atrophy and hypertrophy of skeletal muscles: structural and functional aspects. *Acta Physiol (Oxf)* 2006;188:77–89.
66. Hudson NJ, Franklin CE. Maintaining muscle mass during extended disuse: aestivating frogs as a model species. *J Exp Biol* 2002;205:2297–303.
67. Oki S, Desaki J, Matsuda Y, Okumura H, Shibata T. Capillaries with fenestrae in the rat soleus muscle after experimental limb immobilization. *J Electron Microscop* (Tokyo) 1995;44:307–10.
68. Mujika I, Padilla S. Muscular characteristics of detraining in humans. *Med Sci Sports Exerc* 2001;33:1297–303.
69. Smith HK, Maxwell L, Martyn JA, Bass JJ. Nuclear DNA fragmentation and morphological alterations in adult rabbit skeletal muscle after short-term immobilization. *Cell Tissue Res* 2000;302:235–41.
70. Hudson NJ, Franklin CE. Preservation of three-dimensional capillary structure in frog muscle during aestivation. *J Anat* 2003;202:471–4.
71. McCall GE, Allen DL, Linderman JK, Grindeland RE, Roy RR, Mukku VR, et al. Maintenance of myonuclear domain size in rat soleus after overload and growth hormone/IGF-I treatment. *J Appl Physiol* 1998;84:1407–12.

72. Barton-Davis ER. Contribution of satellite cells to IGF-I induced hypertrophy of skeletal muscle. *Acta Physiol Scand* 1999;167:301–5.
73. Seale P, Rudnicki MA. A new look at the origin, function, and “stem-cell” status of muscle satellite cells. *Dev Biol* 2000;218:115–24.
74. Garry DJ, Meeson A, Elterman J, Zhao Y, Yang P, Bassel-Duby R, et al. Myogenic stem cell function is impaired in mice lacking the forkhead/winged helix protein MNF. *Proc Natl Acad Sci U S A* 2000;97:5416–21.
75. Salvatore D, Simonides WS, Dentice M, Zavacki AM, Larsen PR. Thyroid hormones and skeletal muscle – new insights and potential implications. *Nat Rev Endocrinol* 2014;10:206–14.
76. Simonides WS, van Hardeveld C. Thyroid hormone as a determinant of metabolic and contractile phenotype of skeletal muscle. *Thyroid* 2008;18:205–16.
77. Bloise FF, Cordeiro A, Ortiga-Carvalho TM. Role of thyroid hormone in skeletal muscle physiology. *J Endocrinol* 2018;236:R57–68.
78. Lee JW, Kim NH, Milanesi A. Thyroid hormone signaling in muscle development, repair and metabolism. *J Endocrinol Diabetes Obes* 2014;2:1046.
79. Slater CR. Postnatal maturation of nerve-muscle junctions in hindlimb muscles of the mouse. *Dev Biol* 1982;94:11–22.
80. Gambke B, Lyons GE, Haselgrove J, Kelly AM, Rubinstein NA. Thyroidal and neural control of myosin transitions during development of rat fast and slow muscles. *FEBS Lett* 1983;156:335–9.
81. Cormery B, Beaumont E, Csukly K, Gardiner P. Hindlimb unweighting for 2 weeks alters physiological properties of rat hindlimb motoneurons. *J Physiol* 2005;568:841–50.
82. Schiaffino S, Gorza L, Pitton G, Saggin L, Ausoni S, Sartore S, et al. Embryonic and neonatal myosin heavy chain in denervated and paralyzed rat skeletal muscle. *Dev Biol* 1988;127:1–11.
83. Schiaffino S, Reggiani C, Kostrominova TY, Mann M, Murgia M. Mitochondrial specialization revealed by single muscle fiber proteomics: focus on the Krebs cycle. *Scand J Med Sci Sports* 2015;25:41–8.
84. Adams GR, Haddad F, Baldwin KM. The interaction of space flight and thyroid state on somatic and skeletal muscle growth and myosin heavy chain expression on neonatal rodents. *J Gravit Physiol* 2000;7: P15–8.
85. Mahdavi V, Izumo S, Nadal-Ginard B. Developmental and hormonal regulation of sarcomeric myosin heavy chain gene family. *Circ Res* 1987;60:804–14.
86. Larsson L, Li X, Teresi A, Salviati G. Effects of thyroid hormone on fast- and slow-twitch skeletal muscles in young and old rats. *J Physiol* 1994;481:149–61.
87. Boelen A, Van der Spek AH, Bloise F, de Vries EM, Surovtseva OV, Van Beeren M, et al. Tissue thyroid hormone metabolism is differentially regulated during illness in mice. *J Endocrinol* 2017;233: 25–36.
88. Ambrosio R, De Stefano MA, Di Girolamo D, Salvatore D. Thyroid hormone signaling and deiodinase actions in muscle stem/progenitor cells. *Mol Cell Endocrinol* 2017;459:79–83.
89. Dentice M, Marsili A, Ambrosio R, Guardiola O, Sibilio A, Paik JH, et al. The FoxO3/type 2 deiodinase pathway is required for normal mouse myogenesis and muscle regeneration. *J Clin Invest* 2010;120:4021–30.
90. Dentice M, Ambrosio R, Damiano V, Sibilio A, Luongo C, Guardiola O, et al. Intracellular inactivation of thyroid hormone is a survival mechanism for muscle stem cell proliferation and lineage progression. *Cell Metab* 2014;20:1038–48.
91. Udayakumar N, Rameshkumar AC, Srinivasan AV. Hoffmann syndrome: presentation in hypothyroidism. *J Postgrad Med* 2005;51: 332–3.
92. Ramsay ID. Muscle dysfunction in hyperthyroidism. *Lancet* 1966;2: 931–4.
93. Fraichard A. The T3R alpha gene encoding a thyroid hormone receptor is essential for post-natal development and thyroid hormone production. *EMBO J* 1997;16:4412–20.
94. Wikstrom L. Abnormal heart rate and body temperature in mice lacking thyroid hormone receptor alpha 1. *EMBO J* 1998;17:455–61.
95. Bochukova E, Schoenmakers N, Agostini M, Schoenmakers E, Rajanayagam O, Keogh JM, et al. A mutation in the thyroid hormone receptor alpha gene. *N Engl J Med* 2012;366:243–9.
96. Moran C, Agostini M, Visser WE, Schoenmakers E, Schoenmakers N, Offiah AC, et al. Resistance to thyroid hormone caused by a mutation in thyroid hormone receptor (TR)alpha1 and TRalpha2: clinical, biochemical, and genetic analyses of three related patients. *Lancet Diabetes Endocrinol* 2014;2:619–26.
97. Di Cicco E, Moran C, Visser WE, Nappi A, Schoenmakers E, Todd P, et al. Germ line mutations in the thyroid hormone receptor alpha gene predispose to cutaneous tags and melanocytic nevi. *Thyroid* 2021;31: 1114–26.
98. Gauthier K. Different functions for the thyroid hormone receptors TRalpha and TRbeta in the control of thyroid hormone production and post-natal development. *EMBO J* 1999;18:623–31.
99. Forrest D, Hanebuth E, Smeyne RJ, Everts N, Stewart CL, Wehner JM, et al. Recessive resistance to thyroid hormone in mice lacking thyroid hormone receptor beta: evidence for tissue-specific modulation of receptor function. *EMBO J* 1996;15:3006–15.
100. Yu F, Göthe S, Wikström L, Forrest D, Vennström B, Larsson L. Effects of thyroid hormone receptor gene disruption on myosin isoform expression in mouse skeletal muscles. *Am J Physiol Regul Integr Comp Physiol* 2000;278:R1545–54.
101. Haddad F, Roy RR, Zhong H, Edgerton VR, Baldwin KM. Atrophy responses to muscle inactivity. II. Molecular markers of protein deficits. *J Appl Physiol* 2003 95, 791–802.
102. Lecker SH, Jagoe RT, Gilbert A, Gomes M, Baracos V, Bailey J, et al. Multiple types of skeletal muscle atrophy involve a common program of changes in gene expression. *FASEB J* 2004;18:39–51.
103. Bodine SC, Latres E, Baumhueter S, Lai VKM, Nunez L, Clarke BA, et al. Identification of ubiquitin ligases required for skeletal muscle atrophy. *Science* 2001;294:1704–8.
104. Gomes MD, Lecker SH, Jagoe RT, Navon A, Goldberg AL. Atrogin-1, a muscle-specific F-box protein highly expressed during muscle atrophy. *Proc Natl Acad Sci U S A* 2001;98:14440–5.
105. Bilodeau PA, Coyne ES, Wing SS. The ubiquitin proteasome system in atrophying skeletal muscle: roles and regulation. *Am J Physiol Cell Physiol* 2016;311:C392–403.
106. Tawa NE Jr., Odessey R, Goldberg AL. Inhibitors of the proteasome reduce the accelerated proteolysis in atrophying rat skeletal muscles. *J Clin Invest* 1997;100:197–203.
107. Klein I, Ojamaa K. Thyroid (neuro)myopathy. *Lancet* 2000;356:614.
108. Riis AL, Jørgensen JOL, Gjedde S, Nørrelund H, Jurik AG, Nair KS, et al. Whole body and forearm substrate metabolism in hyperthyroidism: evidence of increased basal muscle protein breakdown. *Am J Physiol Endocrinol Metab* 2005;288:E1067–73.
109. Brennan MD, Coenen-Schimke JM, Bigelow ML, Nair KS. Changes in skeletal muscle protein metabolism and myosin heavy chain isoform messenger ribonucleic acid abundance after treatment of hyperthyroidism. *J Clin Endocrinol Metab* 2006;91:4650–6.
110. O’Neal P, Alamdari N, Smith I, Poylin V, Menconi M, Hasselgren PO. Experimental hyperthyroidism in rats increases the expression of the ubiquitin ligases atrogin-1 and MuRF1 and stimulates multiple

- proteolytic pathways in skeletal muscle. *J Cell Biochem* 2009;108: 963–73.
111. Nakashima K, Ohtsuka A, Hayashi K. Effects of thyroid hormones on myofibrillar proteolysis and activities of calpain, proteasome, and cathepsin in primary cultured chick muscle cells. *J Nutr Sci Vitaminol (Tokyo)* 1998;44:799–807.
 112. DeMartino GN, Goldberg AL. Thyroid hormones control lysosomal enzyme activities in liver and skeletal muscle. *Proc Natl Acad Sci U S A* 1978;75:1369–73.
 113. Lesmana R, Sinha RA, Singh BK, Zhou J, Ohba K, Wu Y, et al. Thyroid hormone stimulation of autophagy is essential for mitochondrial biogenesis and activity in skeletal muscle. *Endocrinology* 2016;157: 23–38.
 114. Sandri M. Autophagy in skeletal muscle. *FEBS Lett* 2010;584:1411–6.
 115. Sandri M, Sandri C, Gilbert A, Skurk C, Calabria E, Picard A, et al. Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. *Cell* 2004;117:399–412.
 116. Mavalli MD, DiGirolamo DJ, Fan Y, Riddle RC, Campbell KS, van Groen T, et al. Distinct growth hormone receptor signaling modes regulate skeletal muscle development and insulin sensitivity in mice. *J Clin Invest* 2010;120:4007–20.
 117. Musaro A, McCullagh K, Paul A, Houghton L, Dobrowolny G, Molinaro M, et al. Localized Igf-1 transgene expression sustains hypertrophy and regeneration in senescent skeletal muscle. *Nat Genet* 2001;27:195–200.
 118. Bodine SC, Stitt TN, Gonzalez M, Kline WO, Stover GL, Bauerlein R, et al. Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. *Nat Cell Biol* 2001;3:1014–9.
 119. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. *Cell* 2012;149:274–93.
 120. Risson V, Mazelin L, Roceri M, Sanchez H, Moncollin V, Corneloup C, et al. Muscle inactivation of mTOR causes metabolic and dystrophin defects leading to severe myopathy. *J Cell Biol* 2009;187:859–74.
 121. White JP, Gao S, Puppa MJ, Sato S, Welle SL, Carson JA. Testosterone regulation of Akt/mTORC1/FoxO3a signaling in skeletal muscle. *Mol Cell Endocrinol* 2013;365:174–86.
 122. Koopman R, Gehrig SM, Léger B, Trieu J, Walrand S, Murphy KT, et al. Cellular mechanisms underlying temporal changes in skeletal muscle protein synthesis and breakdown during chronic beta-adrenoceptor stimulation in mice. *J Physiol* 2010;588:4811–23.
 123. Cicatiello AG, Saggiocchi S, Nappi A, Di Cicco E, Miro C, Murolo M, et al. Thyroid hormone regulates glutamine metabolism and anaplerotic fluxes by inducing mitochondrial glutamate aminotransferase GPT2. *Cell Rep* 2022;38. <https://doi.org/10.1016/j.celrep.2022.110409>.
 124. Nappi A, Miro C. The intricate role of glutamine in pathophysiological contexts. *J Basic Clin Physiol Pharmacol* 2023;34:555–7.
 125. Sartori R, Milan G, Patron M, Mammucari C, Blaauw B, Abraham R, et al. Smad2 and 3 transcription factors control muscle mass in adulthood. *Am J Physiol Cell Physiol* 2009;296:C1248–57.
 126. Winbanks CE, Weeks KL, Thomson RE, Sepulveda PV, Beyer C, Qian H, et al. Follistatin-mediated skeletal muscle hypertrophy is regulated by Smad3 and mTOR independently of myostatin. *J Cell Biol* 2012;197: 997–1008.
 127. Trendelenburg AU, Meyer A, Rohner D, Boyle J, Hatakeyama S, Glass DJ. Myostatin reduces Akt/TORC1/p70S6K signaling, inhibiting myoblast differentiation and myotube size. *Am J Physiol Cell Physiol* 2009;296:C1258–70.