EDITORIAL

Hormone regulation of skeletal muscle

Muscles constitute privileged targets for hormones, which are known to control their differentiation, growth, and metabolism. This special *Hot BAM* issue contains a collection of 14 articles, devoted to reviews of recent results of the literature, and mainly to new data dealing with *Hormone regulation of skeletal muscle.*

Among the hormones, the influence of thyroid hormones has been the most extensively studied. This particular interest for thyroid hormones is further demonstrated in this issue, where more than half of the articles deal with the regulating effect of thyroid hormones. Thyroid hormones act by binding to specific nuclear receptors; here it is shown, that these receptors are found even in an amphibian, *Proteus anguinis*, which is apparently insensitive to these hormones. The thyroid hormone-receptor complex then activates or represses the genes encoding for the myofibrillar proteins. These proteins exist as different isoforms, which probably accounts, at least in part, for the different contraction velocities of the different muscle fibers. These velocities may be modified reversibly by hypothyroid treatment, as shown in the case of the rat soleus. It is now well known, that among the myofibrillar proteins myosin is particularly sensitive to the plasmatic level of thyroid hormones, which down- or up-regulate in specific ways, depending on the muscle, the expression of the various myosin isoforms; moreover in this issue it has been shown, that this regulation may be the result of an interaction between thyroid hormones and neural factors. Actin is an other main myofibrillar protein, which is affected by thyroid hormones, and it is shown here that the rat α-skeletal actin gene responds directly to them and that a potential thyroid hormone responsive element is present in the first intron of the gene. Thyroid hormones are also shown to act on cellular enzymes, such as carbonic anhydrase III and other cellular markers of muscle metabolism.

The second subject treated in this issue is the role of hormones on muscle metabolism and growth. A review reports how thyroid hormones act on the energetic state of the muscle cell, by controlling the protein synthesis, and two papers deal with the role of these hormones on the growth of L6 muscle cell cultures and striated muscles *in vivo*.

Two other types of hormones are also the object of studies. These are growth hormone and steroid hormones. The effects of growth hormone have been examined by hypophysectomy of rats, and both muscle fibrosis and change in muscle fiber size and type are observed. Steroid hormones are known to contribute in various ways to the regulation of muscle differentiation, growth, and metabolism. One article deals with the regulation by androgens of a sexually dimorphic muscle, the *levator ani* of the rat, and indicates that this dimorphism takes place at the fiber size and number levels at two critical periods (perinatal and puberty) of the male rat life. In the last two papers, evidence for interactions between steroid hormones and other hormones and their effects on muscle metabolism is demonstrated.

Not all, but many of the results which have been presented in this issue, deal with experiments performed in the rat. It is clear that no definitive conclusion can be drawn from results obtained mainly on one species. Further work will thus be needed before a complete view of the *Hormone regulation of skeletal muscle* will be attained.

I wish to end this short editorial by expressing many thanks to Professor Ugo Carraro for giving us the opportunity to bring together in a single issue of *BAM* the contributions of many laboratories working in the field of *Hormone regulation of skeletal muscle.*

Anne d'Albis

The Hormonal Control of Myosin Isoform Expression in Skeletal Muscle of Mammals: a Review

Anne d'Albis and Gillian Butler-Browne[(1)]

Laboratoire de Biologie Physicochimique, Université Paris-Sud, Orsay, France. (1) Physiologie et Biologie de la Motricité, UFR Biomédicale des Saints-Pères, Paris, France.

Abstract

After a rapid review of the different myosin isoforms present in skeletal muscles of mammals, a bibliographic survey of their regulation by hormones is made. Of the circulating hormones, it is the thyroid hormones which have been the most extensively studied. From all of the results which have been obtained so far, one can conclude that in striated muscles their role is to activate the expression of myosin isoforms with highest ATPase activity. Other hormones, such as the sexual hormones, are more muscle-specific and further work is needed before we can begin to understand their mechanism of action.

Key words: myosin isoforms, thyroid hormones, hormones

BAM 3 (1): 7-16, 1993

Commitment, differentiation, and diversification of skeletal muscle fibers

Skeletal muscle fibers are formed by the fusion of mononuclear myoblasts into multinuclear myotubes. With the exception of β-enolase [66], carbonic anhydrase [74], H36 integrin and desmin [65], which are expressed by myoblasts, all other characteristic muscle specific genes are only expressed once cells have entered differentiation. Although myoblasts do not express a full complement of contractile proteins, in particular myosin, these cells are nevertheless committed to differentiate and form muscle fibers. The most likely candidates for this process of commitment to the myogenic lineage are the helix-loop-helix family of muscle regulatory factors (myoD/myf 3, myogenin/myf 4, myf5 and myf-6/MRF4/ herculin). Their mode of action has been described previously [117, 103, 41] and is beyond the scope of this review.

It is now known that distinct types of myoblasts, which have been termed somitic, embryonic, fetal, and satellite cells, appear sequentially during development [120, 23, 84, 110, 87, 108]. This results in the formation of successive generations of muscle fibers, each generation being the product of the different populations of myoblasts. During the subsequent development and maturation of the skeletal muscle a distinct fiber type pattern evolves [92]. The maintenance and modulation of this muscle fiber phenotype is the result of the interaction of factors intrinsic to the myoblast lineage and of extrinsic factors, such as thyroid hormone status, innervation, contractile activity, growth factors and extracellular matrix [52, 58].

The influence of innervation and contractile activity on muscle phenotype has been the object of several reviews in recent years [64, 4, 93, 3, 94]. The role of hormones has also been reviewed [4, 111, 116], but the results concerned mainly the regulation of myosin isoform expression in cardiac muscle [72]. Other authors have dealt with the hormonal control of muscle growth [48, 49]. The object of this review is to present the recent results on the effects of hormones on the expression of myosin isoforms in skeletal muscle fibers.

Types of myosin isoforms present in skeletal muscles

The identification of the different myosin isoforms present in skeletal muscles is one of the criteria, which has been extensively used to distinguish the different types of muscle fibers.

Myosin is a large molecule made up of two heavy chains (Mw about 200 000 D) and of two pairs of light chains (Mw about 20 000 D).

In mammals, 10 distinct myosin heavy chains have been described :

- One slow-twitch heavy chain (I) is present in the histochemically-defined type I fibers of adult mammals; these fibers are slow-contracting, have a low ATPase activity at alkaline pH, and oxidative properties. Myosin heavy chain I is encoded by the same gene as the β myosin heavy chain found in cardiac muscle [77].
- Three fast-twitch heavy chains (IIA, IID/IIX, and IIB). The IIA heavy chain is found in the histochemically-defined type IIA fibers of adult mammals. These fibers are fast-contracting, have a high ATPase activity at alkaline pH, and oxidative properties. The IIB heavy chain is found in the fast contracting histochemically-defined type IIB fibers, which have a high ATPase activity at alkaline pH, and glycolytic properties [see 92 for references]. The IID/IIX heavy chain has been described only recently in rat, mouse, and rabbit [5, 107, 88, 69, 1]; it is found in fibers, with an intermediate oxidative capacity [107, 70, 39].
- One embryonic E and one perinatal P heavy chains have also been identified. They are found in developing and regenerating muscle fibers, and occasionally in some of the head muscles of adult mammals, such as the masseter muscle of rat, mouse, and man [29, 16, 109], the bovine and pig tensor tympani muscle [105] or the rat extraocular musculature [121].
- Two "superfast" heavy chains have also been described, one in the superfast-contracting fibers of cat and dog jaw-closing muscles, myosin IIM [97, 78], and the other in the superfast-contracting fibers of the extraocular muscles, myosin EOM [121, 102], respectively.
- One slow-tonic heavy chain is found in the slow-tonic fibers of rat extraocular and jaw-closing muscles [78, 79]; these fibers are rare in mammals and are mainly observed in the polyinnervated muscle fibers of birds and amphibians.
- One "cardiac-type" heavy chain, the α cardiac, has been identified in masticatory muscles of rabbit and man [12, 31, 89, 24] and in muscle spindle fibres [90].

These last four heavy chains are quantitatively much less important than the first five heavy chains. However, more variants of heavy chains have been recently described, such as slow-type myosins in jaw muscles of the cat [55] and in fetal rat [38] and fast type myosins in the Syrian hamster [80] and in the rat [104].

The difference between the amino acid sequences of these heavy chains does not exceed 30%, and may be less than 2% [83].

Each heavy chain (HC) is associated with two light chains (LC), of two types, the so-called "essential" and "regulatory" light chains [see 111 and 92 for references]. There are five major essential light chain isoforms and two regulatory light chain isoforms; variants of these light chains have however been described in specific cases [97]. The way they are associated with the heavy chains results in the formation of the various myosin isoforms, which are indicated in Table 1.

All these myosin isoforms display different ATPase activities and are thus responsible, at least in part, for the different contractile speeds of the skeletal muscle fibers, as first proposed by Barany [6]. As stated above, their expression in muscles is under the control of both genetic and epigenetic factors and the contraction velocity of muscles may be modulated to meet the various physiological or pathological requirements imposed on the muscle. Modifications in the concentration of circulating hormones is one of the chief factors which may modify the expression of the myosin isoforms and thus induce changes in the muscle properties.

Effect of thyroid hormones

It is well known that striated muscles are privileged targets for thyroid hormones, which act by repressing or activating the genes coding for the different myosin isoforms [62].

In mammals, perinatal development is associated with a change in the plasmatic concentration of the thyroid hormones T3 and T4. The active hormone is the 3,5,3'triiodo-L-thyronine, T3, which will be considered here. Its concentration, which is barely detectable in embryonic and newborn rats, increases a few days after birth, reaches a peak at about 2 weeks, then slightly decreases to reach a plateau in the adult rat [40]. A similar but more precocious variation is observed for the rabbit [37].

Coincident with this increase in T3, embryonic and perinatal myosin isoforms are progressively repressed and adult fast myosin isoforms are accumulated [4 for review]. This scheme is rather general since it has been observed in the limb muscles of all the mammals which have been investigated: man, sheep, cat, rabbit, rat, mouse, guinea-pig, and hamster [56, 118, 75, 47, 32, 54, 28, 68, 10, 15, 30, 45].

A correlation between the increase in T3 concentration and the activation of the expression of the adult fast myosin isoforms has been proposed and confirmed by hyperthyroidism and hypothyroidism experiments. Rats made hyperthyroid during development display an earlier switch from the embryonic and perinatal isoforms to adult-type fast isoforms, while the contrary holds true for rats made hypothyroid [50, 17, 62, 98, 32, 34, 25]. The hypothyroid *dwarf* mouse mutant, originally described in 1927 by Snell, provided an excellent model system in which one could investigate the direct influence of thyroid hormone on the isoform transitions in the absence of a possible secondary effect due to a stimulation of growth hormone production. In this mutant the developmental isoform transitions are substantially delayed in the skeletal muscle and completely blocked in the heart. A normal adult myosin phenotype can be restored by multiple injections of thyroxine [119, 18, 96].

It appears therefore that the T3 thyroid hormone represses the expression of embryonic and perinatal myosin

Table 1. Myosin types in skeletal muscles.

myosin isoform	heavy chain HC	"essential" LC	regulatory" LC
slow SM1	slow I (or β)	LC 1S	LC 2S
slow SM2	slow I (or β)	LC 1S and LC1'S	LC 2S
slow SM3	slow I (or β)	LC1'S	LC2S
fast AM1	fast IIA	LC 3F	LC 2F
fast AM2	fast IIA	LC 1F and LC 3F	LC 2F
fast AM3	fast IIA	LC 1F	LC 2F
fast BM1	fast IIB	LC 3F	LC 2F
fast BM2	fast IIB	LC1F and LC 3F	LC 2F
fast BM3	fast IIB	LC 1F	LC 2F
fast DM1/ XM1	fast IID/IIX	LC 3F	LC 2F
fast DM2/XM2	fast IID/IIX	LC1F and LC 3F	LC 2F
fast DM3/XM3	fast IID/IIX	LC 1F	LC 2F
embryonic EM	embryonic E	LC 1emb	LC 2F
perinatal PM1	perinatal P	LC 3F	LC 2F
perinatal PM2	perinatal P	LC1F and LC 3F	LC 2F
perinatal PM3	perinatal P	LC 1F	LC 2F
superfast M	superfast IIM	LC 1 variant	LC2variant
superfast EOM1	superfast EOM	LC 1F	LC 2F
superfast EOM2	superfast EOM	LC1F and LC 3F	LC 2F
superfast EOM3	superfast EOM	LC 3F	LC 2F
slow-tonic	slow-tonic	nd	nd
cardiac V1	α	LC 1S	LC 2S

isoforms, while it activates that of adult fast isoforms. Brozanski et al [14] have shown that the disappearance of perinatal myosin isoforms during postnatal development is delayed in the diaphragm of undernourished rat pups compared to controls; this undernutrition is also accompanied by a decrease in the serum T3 levels, which led the authors to suggest that the alterations in myosin isoform transitions are induced by the hypothyroidism which is associated with the undernutrition. In man, excessive levels of thyroid hormone during fetal development has also been shown to produce a precocious accumulation of the adult myosins as well as a precocious maturation of the muscle [15].

All the muscles of the same animal respond to an endogenous increase in thyroid hormone concentration or to its experimental change, but their response has been shown to vary depending on the muscle [28, 25, 91]. In the rat, the diaphragm displays the most precocious switch to adult fast myosin isoforms, while the masseter displays the latest one; in the rabbit, it is the tongue musculature, which displays the most precocious switch [30]. From these results, it appears that different skeletal muscles do not contain the same number of thyroid hormone receptors, which would result in their different sensitivities to the hormone.

This is also suggested by the effect of experimental hyperthyroidism induced in developing rats and by the comparison of the myosin switches in eight muscles of the treated-rats with that in euthyroid rats. Hyperthyroidism barely modifies the myosin switch in the diaphragm, which indicates that the endogenous thyroid hormone concentration is almost optimal for inducing the myosin switch in this muscle. On the contrary, hyperthyroidism accelerates the switch in the other muscles investigated, which become similar to the diaphragm; this result is indicative of a different sensitivity of the individual skeletal muscles to thyroid hormone [25]. In the rat masseter, where there is a persistence of the perinatal myosin isoforms in the young adults, the hyperthyroid treatment induces their total disappearance, whereas hypothyroidism induces their reappearance [77].

The myosin switch from the embryonic and perinatal myosin isoforms to the adult fast myosin isoforms occurs with the same chronology in both male and female rats. In two muscles, differences are however observed. In the masseter of the female and the male rat the half-transition time is 21 and 22 days, respectively, and the perinatal

isoforms are observed to persist longer in the male than in the female [29]. A more striking difference is observed in the case of the levator ani of the rat, where the half-transition time is 20 days for the male and 32 days for the female [26]. This large difference may be attributed, mainly, to a difference in sensitivity of the male and female muscle to thyroid hormone, since it is almost abolished by a hyperthyroid treatment [33]; to our knowledge, there are no other muscles which display different responses to thyroid hormone in the male and female.

In the adult rat, thyroid hormone represses the slow isoform, which is clearly observed in the soleus and diaphragm of the rat [60, 62]. On the other hand, it generally favours the accumulation of the fast isoforms. Hyperthyroidism, induced in rats by injections of T3 every other day for 20 weeks, produces in the soleus a significant decrease in the proportion (93 → 69 %) of slow myosin, and a parallel increase in the amount of fast myosin, presumably the type IIA isoform [19]. This myosin transformation in the soleus is accompanied by an increase in the contractile velocity of the muscle, which is in good agreement with the concept that the rate of cross-bridge cycling is directly linked to the type of myosin isoform present in the muscle fiber. In contrast, no significant change, either in the myosin isoform content, or in the contraction velocity, is observed in the plantaris, which contains almost only type II myosin isoforms. Experimental hypothyroidism on the contrary has an overall slowing effect on the muscle, which is correlated with an increase in the amount of slow myosin in fast-twitch muscles [71, 20].

Similar results were obtained by Fitzsimons et al [46] when studying the effect of hyperthyroidism on the red and white regions of the rat medial gastrocnemius. The red region showed an increase in the fast isoform and a corresponding decrease in the slow isoform, whereas there were no changes in the white region of the muscle. However, differences may be observed depending on the muscle [53, 62]. For example, expression of the IIA heavy chain is activated by thyroid hormone in the soleus, but repressed in the masseter; in that case, it is the IIB heavy chain, which is activated [62].

Thus it seems that thyroid hormone acts to induce the expression of a myosin isoform of higher ATPase activity. In the case of the soleus, where the predominant isoform is the slow isoform, the expression of IIA myosin will increase together with the contraction velocity of the muscle; in the case of the masseter, which contains IIA and IIB myosins, the IIB myosin, which has a higher ATPase activity, is favoured. The effect of thyroid hormone on the expression of the IID/IIX isoform has not yet been studied. However, if one believes that there is a preferential sequence for the transformation of myosin isoforms: I ↔ IIA ↔ IID/IIX ↔ IIB [106, 112, 81], then one can guess that muscles containing mainly type I and IIA isoforms will express the IID/IIX isoform in hyperthyroid rats, whereas muscles containing predominantly the IID/IIX isoform will express the IIB isoform in hyperthyroid rats (Table 2).

Table 2. Scheme of the shift in myosin heavy chains produced by hyperthyroidism (+T3) and by hypothyroidism (-T3).

myosin heavy chain	+ T3	- T3
slow I	IIA	I
fast IIA	IID/IIX, IIB	I
fast IID/IIX	IIB	IIA
fast IIB	IIB	IID/IIX

The cardiac V1 isoform, when present in the masticatory muscles of the rabbit, is not sensitive to thyroid hormone [27], whereas it is well known that it is activated by thyroid hormone in the cardiac ventricle of mammals [73].

The mechanism of action of thyroid hormones has been the subject of many studies. Abundant evidence now indicates that the responses of the muscle to thyroid hormone are mediated by cellular receptors localized in the cell nucleus [86, 101, 13 for reviews]. A debate has risen on the question as to whether the hormone acts directly, or by the intermediary of the nerve. This question has not yet been resolved, since contradictory results were obtained after denervation of the muscle [63, 122, 85, 98]. It is however clear that thyroid hormone binds to specific nuclear protein receptors [86, 43, 101, 13 for reviews], which in turn either activate or repress the different myosin genes. The results obtained in the cardiac ventricle suggest that modulation in the expression of the α and β myosin heavy chain genes by thyroid hormones is regulated at the transcriptional level [73, 22].

The recent report that thyroid hormone positively regulates MyoD1 gene transcription also opens a new way to investigate the mechanisms that orchestrate myosin expression [21].

Effect of sexual hormones

Androgens are known to contribute to the difference in size of male and female muscles and androgen-regulated gene expression is currently under investigation [9, 7 for reviews]. The effect of sexual hormones on the expression of myosin isoforms has been less well investigated. In adult mammals, one does not in general observe differences between the myosin content of male and female muscles, which indicates the absence of an effect of the sexual hormones on the expression of the myosin isoforms. Thus androgenic hormone treatment does not alter the myosin expression in female rat soleus and plantaris muscles [113] and its withdrawal does not affect the myosin phenotype of the male rat plantaris muscle [11]. On the other hand, chronic administration of an anabolic steroid hormone, nandrolone phenylpropionate, to female rats for 6 weeks does result in a small increase in the number of type IIA

fibres in the *extensor digitorum longus* [42], whereas a similar hormone, nandrolone decanoate, induces no significant effect in the same muscle of the rabbit [100].

In the case of the guinea-pig temporalis, which belongs to a small group of sexually dimorphic muscles, Lyons et al. [76] have shown that the male muscle contains IIA myosin isoforms, whereas the female muscle contains IIB myosin isoforms; moreover, a treatment of females by testosterone induces the synthesis of the IIA isoforms, whereas castration of the males induces the synthesis of the IIB isoforms. The levator ani of the rat is another sexually dimorphic muscle, which grows normally in the male, but remains atrophied in the female; in this muscle, no difference is observed between the myosin isoform content of the male and female muscles [26]. Whereas as reported above the chronological difference between the myosin transitions in the male (t1/2 = 20 days) and in the female (t1/2 = 32 days) is abolished in hyperthyroid rats, testosterone-treated females display the same myosin transition as the control females [33] and castrated males display the same myosin transition as the control males [26].

In the rabbit masseter, the developmental expression of the cardiac V1 isoform is the same in males and females until 2 months; from 2 months onwards, the curves start to diverge, at the time corresponding to the onset of spermatogenesis [8]; the inhibiting effect of androgenic hormones on the expression of V1 is further demonstrated by the effect of male castration [27].

Parallel mechanisms are currently invoked for the actions of both steroid and thyroid hormone receptors, *i.e.* that these receptor proteins, upon association with the specific hormones, undergo a transformation to a state where they are able to interact with chromatin and regulate the transcription of specific genes [43, 113, 115]. However recent studies have suggested that certain members of the steroid hormone receptor superfamily can be activated in a hormone-independent manner, which provides new insights into the activation of the steroid hormone receptors [94].

Effect of other hormones

Other hormones, such as somatomedines, insulin, glucocorticoids and cathecolamines, have been shown to have an effect on myogenesis [116, 49 for reviews]. However, the effect of these hormones on the myosin isoform expression has been the subject of only a few studies. Insulin has been shown to influence myosin isoform expression in fast-twitch muscle, since experimental diabetes induces a decrease in fast and an increase in slow isoforms, whereas no effect is observed in slow-twitch muscle [99]. On the other hand glucocorticoid treatment has been shown to induce a fiber shift, corresponding to a slight modification in the proportions of type IIA and IIB fibers [44].

Combined effects

In a few cases, the resulting effect of two factors on myosin expression have been studied. Administration of testosterone to female rats dramatically increases insulin resistance and induces changes in slow muscle morphology, with an increase in the number of type II fibers [57]. On the other hand, steroid treatment of suspended rats does not modify the myosin isoform pattern induced by mechanical unloading [114], while hypothyroidism predominates over mechanical unloading in the regulation of myosin isoforms in slow muscle [38]. Finally, the treatment of hypophysectomized rats by human growth hormone increases the proportion of slow-type myosin, in skeletal muscle [2].

The antagonistic effects of chronic low frequency stimulation and thyroid hormone on myosin expression have been observed in rat fast-twitch muscles [67] and a dominant effect of hyperthyroidism over hypertrophy has been observed in all rat muscles [59]. The means by which thyroid hormone predominates over other potential influences on myosin expression is unknown at the present time and does not allow one to discrimination between common or separate signal pathways.

Concluding remarks

Skeletal muscles are known to be privileged targets for hormones, which induce changes in their weight, metabolism, and fiber type profile. In this review, we have concentrated on describing the action of hormones on the myosin phenotype of skeletal muscles. Whereas much work has been carried out on the action of thyroid hormone, which allows one to conclude that thyroid hormone activates the genes coding for the myosins of highest ATPase activities, the precise action of other hormones remains to be defined. The cloning of various hormone receptors [61, 35, 82, 51] should allow one to obtain a greater understanding of the mechanism of action of these hormones.

Address correspondence to:

Anne d'Albis, Laboratoire de Biologie Physicochimique, Université Paris-Sud, Bâtiment 433, 91405 - Orsay, France. Tel: 33 1 69 41 64 28. Fax: 33 1 69 85 37 15.

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Thyroid Hormones and Muscle Bioenergetics

Pierre Kaminsky, Marc Klein and Michel Duc

Service de Médecine Interne J., Hôpital de Brabois, Nancy, Hôpitaux de Brabois, Vandoeuvre, France

Abstract

By controlling the protein synthesis, thyroid hormones act on the energetic state in muscle cell. They regulate the DNA expression of myosin, Ca-ATPase, and Na-K pump isoforms, as well as the activity of several key-enzymes of the main energetic pathways (glycolysis and mitochondrial metabolism). A lack or an excess of thyroid hormones therefore induce a new equilibrium between the energetic production and consumption. Hyperthyroidism results in a transition to fast myosin isoforms and in an increased metabolism dependence on glycolysis. A rise in the mitochondrial enzyme activity is theoretically observed *in-vitro*, although the hypercatabolism of protein probably counterbalances this effect *in-vivo*. Thyroid hormone defect results in a transition to slow myosin isoforms and in an inhibited mitochondrial metabolism. This is compensed by an increased glycogen consumption during exercise, despite the observed decreased enzyme activity found *in-vitro* in glycolysis. Hypothyroidism possibly affects the H^+ metabolism. Both hyper and hypothyroidism therefore induce a higher acidosis and a lower muscle performance during exercise, which could explain the exercise intolerance in patients with dysthyroidism.

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The bioenergetic state in cells is dependent on the balance between the energetic production and consumption. To a first approximation, this can be resumed as an equilibrium between synthesis and hydrolysis of ATP, which is considered as the main energetic vector in cell.

The glycolysis pathway partially ensures the ATP production in muscle. However, as in all tissues highly dependent on the energetic metabolism, ATP is essentially produced by the mitochondrial metabolism, which supplies more than 90% of the total energetic availability [3]. The ATP consumption site specific to muscle is the actin-myosin interaction, which induces the fibre contraction. The ATP hydrolysis level during the muscle contraction varies with the activity of the myofibrillar ATPase, which characterizes the fibre type. Moreover, ion exchanges through the muscle membrane are ATP dependent.

The thyroid hormones act on several steps of the energetic production and on the ATPase activity, mainly by controlling the protein synthesis.

1. Control of the energetic production by thyroid hormones

1.1. Energetic pathways

In experimentally thyroid-deprived animals, the activity of several key-enzymes in glycolysis, such as myophosphorylase, fructose 1-6 diphosphatase or lacticodehydrogenase has been found to be reduced *in vitro* in skeletal muscle [11, 28]. Conversely, these enzyme complexes appear to be activated in experimental hyperthyroidism. In fact, these modifications affect especially the type II muscle fibres, or the diaphragm (a mixed muscle), whilst the type I muscles are not or only slightly affected. These abnormalities could partially explain the decreased lactate level found after exercise in serum of hypothyroid subjects and the increased glycogen content observed in muscle biopsy in hypothyroidism [30].

A decreased free fatty acid (FFA) turnover is moreover well documented in hypothyroidism, despite an elevated FFA level in serum [30]. In addition to the frequent finding of lipid droplet accumulation in the muscle biopsy of hypothyroid subjects, this suggests an inhibition of the fat catabolism. Indeed, Baldwin and co-workers [2] have demonstrated a dramatic decrease of ^{14}C-labelled palmitate uptake into muscle homogenates. To our knowledge, similar studies have not been performed in hyperthyroid muscles, but an increased activity of ß hydroxybutyrate

tate uptake into muscle homogenates. To our knowledge, similar studies have not been performed in hyperthyroid muscles, but an increased activity of ß hydroxybutyrate dehydrogenase has been documented in hypothyroid rat muscles [39].

1.2. Mitochondrial metabolism

Several mitochondrial key-enzymes (tricarboxylic Krebs cycle, enzyme complexes of the respiratory chain) are inhibited by experimental thyroid hormone deficiency or activated by experimental thyroid excess [2, 11, 28, 29, 35, 39]. These modifications are especially observed in type I and type IIA muscle fibres, and for a few studies in heart muscle.

The *in-vitro* effects of thyroid administration have been reported in isolated mitochondria: thyroid hormones increase the amino-acid uptake, the synthesis of the mitochondrial inner membrane, the oxygen consumption, the cytochrome concentration and the cytochrome c oxydase activity [27]. Receptors specific to T_3 have been demonstrated on the mitochondrial membrane, suggesting a direct effect of this hormone on the mitochondrial metabolism [33].

Several mechanisms can be put forward to explain the control of the mitochondrial metabolism by the thyroid hormones. Firstly, thyroid hormones modulate the nuclear DNA genes encoding the majority of the mitochondrial proteins, but also the mitochondrial DNA genes encoding several subunits of the respiratory chain enzyme complexes. This provides an explanation for the rise in cytochrome content and in the activity of the cytochrome c oxidase [27]. Secondly, thyroid hormones regulate the substrate incorporation by mitochondria and their availability to their oxidation, implying modifications of the $NADH_2/NAD$ ratio [27]. In addition to the above mentioned effects on the FFA metabolism, it is probable that the thyroid hormones act on the pyruvate dehydrogenase complex, since the incorporation of ^{14}C-labelled pyruvate into muscle homogenates has been found to be strongly suppressed in experimental hypothyroidism [2]. Thyroid hormones also act on the membrane synthesis, by modifying the ratio of unsaturated FFA/ saturated FFA. Since the enzyme complexes of the respiratory chain are located in the mitochondrial inner membrane, thyroid hormones therefore control indirectly their total concentrations, by modifying the functional area of the mitochondrial membrane [27].

All these modifications can be compared with the ultrastructural abnormalities of mitochondria reported in hypothyroid muscle biopsy, such as honey moon aspect, abnormal inclusions or cristae [19]. Conversely, an abnormally increased number of mitochondria has been reported in hyperthyroid heart muscle.

2. Energetic consumption

2.1. Myosin isoforms

Myosin is composed by two heavy and four light chains. Several isoforms have been described and are encoded by a multiple gene family. The expression of this gene family is regulated by nervous and mechanical stimuli, and by hormonal systems [13]. In the heart muscle, three myosin isoforms have been reported; thyroid hormone deficiency induces a transition to the V_3 isoform (α_2 dimere), and a replacement therapy in a thyroid-deficient animal increases the proportion of V_1 isoforms ($ß_2$ dimere) [6].

Similar results have been observed in skeletal muscle, in which at least five different isoforms exist in rodents [36]. Histochemical studies of experimental hypothyroidism have shown an abnormally high proportion of type I fibres in muscle biopsies [12]. Similar results have been reported in hypothyroid man [31, 38]. Moreover, a transition from type I to type IIA fibres has been observed when thyroid hormones were administered in hypothyroid rats [11, 28]. These modifications appear to be caricatural when thyroid hormones are in excess.

Myosin transition is linked to the T_3 control of the genes encoding the SM (slow myosin), IN (intermediate myosin) and FM3 (type 3 fast myosin) isoforms, so that thyroid hormones preferentially affect the myosin distribution of slow type I fibres, when fast type II fibres seem to be only minimally concerned [5, 13]. These modifications are not related to the muscle innervation, since the myosin transition obtained by repetitive stimulations is inhibited in hypo or hyperthyroid animals [29].

This thyroid-related myosin isoform distribution theoretically results in a new energetic equilibrium: myosin isoforms which are present in type I fibres (or the V_3 isoform in heart) express a "slow" ATPase activity and are therefore "economical" in their energetic consumption. Conversely, the isoforms which characterize type II fibres consume more energy and this corresponds to a faster and higher developed twitch tension.

2.2. Sarcoplasmic Ca-ATPase dependent channels

The actin-myosin interaction is controlled by the intracellular level of Ca^{++}, the nerve impulse inducing the Ca^{++} extrusion from the sarcoplasmic reticulum. Moreover, the muscle relaxation is related to the Ca^{++} uptake by the sarcoplasm. This Ca^{++} uptake is faster in type II than type I fibres [4]. Hypothyroid rat soleus (slow type I muscle) contains essentially mRNA of the type I isoform of Ca-ATPase, whilst the mRNA level of the type II isoform is 150-fold greater in the presence of T3. In hyperthyroidism, this last isoform represents 50% of the total mRNA content [32]. Fast type II muscles appear to be less affected [32].

2.3. Na-K-ATPase dependent pumps

The Na-K-ATPase dependent pumps maintain the electrochemical gradient of sodium and potassium across the plasma membrane. They are composed by two subunits, three isoforms of the a subunits being known. The thyroid hormones modulate the gene expression encoding these isoforms [10]. Using labelled ouabain, which will bind sodium pumps, the pump abundance in hypothyroids has been found to be half the euthyroid values and twice in hyperthyroids [20]. In heart as well as in skeletal muscles,

physiological levels of thyroid hormones essentially control the gene expression encoding the α_2 et ß proteins, whilst thyroid hormone excess induces a rise in the α_1 protein [10].

These modifications of the Na-K-ATPase activity with the thyroid hormone level are known to be an important factor of energetic consumption in muscle and their higher (respectively lower) activity in hyperthyroidism (respectively hypothyroidism) is put forward to explain the increased (respectively decreased) thermogenesis [30].

3. In-vivo consequences of dysthyroidism

3.1. Hyperthyroidism

The causes of the cardiovascular disorders induced by thyrotoxicosis are multiple: hemodynamic modifications, sympathetic overactivity or direct alterations of the myocardium by the thyroid hormones. In thyrotoxicosis, the heart is hypertrophied and hypercontractile. The heart hypertrophy is probably due to the haemodynamic modifications. However, the transition in myosin and Ca-ATPase isoforms induced by thyroid excess explains the increased systolic cardiac volume and left ventricular systolic or diastolic functions at rest [26]. Moreover, the cardiac output and the left ventricular systolic volume increase during exercise by the same magnitude in volunteers before and after they received thyroid hormones [23, 24]. Thus, the fact that cardiac performance seems to be not compromised by thyroid excess suggests that the exercise intolerance in hyperthyroidism may be due to noncardiac mechanisms [26], which can be haemodynamic modifications or skeletal muscle alterations [23].

Martin and coworkers have recently demonstrated that the same exercise induced in subjects who voluntarily received supplementary thyroid hormones a rise in the frequency and the cardiac output, whilst the arterio-venous difference in oxygen was reduced and the serum lactate increased [23]. These modifications were attributed to a diminished oxidative capacity of hyperthyroid skeletal muscles. Taking into account the results obtained by *in-vitro* biochemical investigations, this assumption appears to be paradoxical. However, the increase per unit volume in oxidative enzyme activity reported in experimental studies may be counterbalanced by the protein hypercatabolism and subsequently the muscle atrophy documented in hyperthyroid myopathy [38]. In the same way, using phosphorus nuclear magnetic resonance spectroscopy (P.MRS), we have not found in hyperthyroid man an increased phosphocreatine synthesis rate after exercise [18], which was expected by the fact that phosphocreatine synthesis is strongly dependent on the rate of ATP synthesis by the mitochondrial metabolism. However, this result was previously reported in experimental hyperthyroidism by Argov and coworkers [1].

Moreover, we postulate that the exercise intolerance may be related to a higher recruitment of the glycolysis pathway: supporting this hypothesis, hyperthyroid subjects studied by P.MRS exhibited a higher acidosis during exercise than controls [18]. This assumption is coherent with the transition in myosin isoforms observed in thyrotoxicosis and with the increased enzyme activity in this pathway observed in thyrotoxicosis

3.2. Hypothyroidism

By symmetrical reasons as aboveexplained, the transition in myosin and Ca-ATPase isoforms induced by the lack of thyroid hormones can explain a decreased contraction and relaxation rate of muscle fibres. Indeed, hypothyroidism provokes a fall in the left cardiac output and in the left ventricular systolic fraction [9, 37].

P.MRS studies in experimental or human hypothyroidism have demonstrated in skeletal muscle a decreased phosphocreatine resynthesis rate after exercise, related to the functional inhibition of the oxidative phosphorylation [1, 17]. Moreover, measurements of the intracellular pH during exercise have shown a more pronounced acidosis in hypothyroid than in euthyroid muscles [16, 17]. These results contrast with those of biochemical investigations which have demonstrated a glycolysis inhibition *in-vitro*. In fact, according to this glycolysis inhibition, the pH decrease during exercise has been found to be delayed in hypothyroid in comparison with euthyroid muscles [34]. However, the importance of the functional inhibition of the mitochondrial metabolism implies an enhanced glycolysis recruitment in order to compensate the mitochondrial failure so, an increased glycogen consumption during exercise has been found in hypothyroid rats [2], as well as a lactate accumulation in hypothyroid dogs [14], suggesting an alteration of the pyruvate oxidation. In addition, the abnormal acidosis found in hypothyroid muscle is probably related to an abnormal H^+ metabolism, since the pH recovery after exercise appears abnormally slow [16, 34]. The H^+ exchanges across the cell membrane depend on the lactate expulsion and Na^+/H^+ antiports. The lactate transport through the muscle membrane is probably affected by the lack of thyroid hormones, since a decreased lactate level in serum after exercise is well documented, contrasting with the previously mentioned rise in intracellular lactate content [14, 21]. Moreover, the defect in sodium pumps tends to decrease the intracellular Na^+ level, which can interfere with the Na^+/H^+ antiport functioning [34].

4. Muscle efficiency in dysthyroidism

The twitch tension developed during a tetanic stimulation has been found to be similar in hypothyroid and in euthyroid muscles, whilst the ATP turnover appeared reduced [8, 22, 38]. Conversely, hyperthyroid muscles in rodents and humans exhibited a decreased twitch tension with an increased ATP turnover. The laws of thermodynamics therefore imply that in hypothyroidism (respectively in hyperthyroidism), the muscle efficiency is higher (respectively lower) than in euthyroidism [7, 22, 38], and that the calorific production by muscle must be lower (respectively higher). As these results are mainly related to the transition of the myosin isoforms, one can postulate that thermogenesis is dependent on this transition.

However these modifications of the muscle efficiency are not sufficient to improve the muscle performance. The treatment of the thyroid disease in man increases the muscular volume in hyperthyroids and decreases it in hypothyroids, whilst a rise in the muscle strength is observed in both cases [40]: this suggests an improvement of the muscle performance per unit volume, at least in hypothyroidism. In the same way, the energetic cost of an aerobic exercise, as estimated by the depletion of phosphocreatine, is higher in hypo and hyperthyroid than in euthyroid muscle [1, 17, 18]. As in both cases, an abnormal acidosis is demonstrated and implies a deplacement of the dissociation equilibrium of the inorganic phosphate, this provokes an increased monovalent ion $H_2PO_4^-$ [15], which had been implicated in alteration of the actin-myosin coupling [25].

Conclusion

The thyroid hormones modulate the energetic balance in muscle by modifying the energy production and the energy consumption. Theoretically, the transition to slow (respectively fast) myosin isoforms in hypothyroidism (respectively hyperthyroidism), characterized by a gain (respectively a loss) of energy is counterbalanced by a reduction (respectively an enhancement) of the ATP production in the glycolysis and especially the mitochondrial metabolism. Nevertheless, in both hypo and hyperthyroidism, the muscles are less performant, maybe due to a high recruitment of glycolysis, which induces acidosis, by disorders of the ion exchanges through the plasma membrane and by alteration of the actin-myosin coupling during contraction.

Address correspondence to:

P. Kaminsky, Service de Médecine Interne J., CHU de NANCY, Hôpitaux de Brabois, 54511 Vandoeuvre-Cedex, France.

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Edited by Ugo Carraro and Stanley Salmons
Published by UNIPRESS Padova (Italy)
Proceedings of a Conference held in Abano Terme,
Padova (Italy), May 30 - June 1, 1991

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