Acta Biochimica Polonica

Vol. 46 No. 3/1999

823–835

QUARTERLY

Effect of thyroid hormone on the myosin heavy chain isoforms in slow and fast muscles of the rat

Anna Jakubiec-Puka[1], Iwona Ciechomska[1], Urszula Mackiewicz[1], Józef Langford[2] and Hanna Chomontowska[1]

[1]*Department of Cellular Biochemistry, Nencki Institute of Experimental Biology, L. Pasteura 3, 02-093 Warszawa, Poland*

[2]*Department of Applied Physiology, Medical Research Centre, Polish Academy of Sciences, A. Pawińskiego 5, 02-106 Warszawa, Poland*

Received: 04 May, 1999

Key words: myosin heavy chains, skeletal muscle, hyperthyreosis, hypothyreosis, contractile apparatus, electrophoresis, rat, SDS/PAGE

The myosin heavy chain (MHC) was studied by biochemical methods in the slow-twitch (soleus) and two fast-twitch leg muscles of the triiodothyronine treated (hyperthyroid), thyreoidectomized (hypothyroid) and euthyroid (control) rats. The changes in the contents of individual MHC isoforms (MHC-1, MHC-2A, MHC-2B and MHC-2X) were evaluated in relation to the muscle mass and the total MHC content. The MHC-1 content decreased in hyperthyreosis, while it increased in hypothyreosis in the soleus and in the fast muscles. The MHC-2A content increased in hyperthyreosis and it decreased in hypothyreosis in the soleus muscle. In the fast muscles hyperthyreosis did not affect the MHC-2A content, whereas hypothyreosis caused an increase in this MHC isoform content. The MHC-2X, present only in traces or undetected in the control soleus muscle, was synthesised in considerable amount in hyperthyreosis; in hypothyreosis the MHC-2X was not detected in the soleus. In the fast muscles the content of MHC-2X was not affected by any changes in the thyroid hormone level. The MHC-2B seemed to be not influenced by hyperthyreosis in the fast muscles, whereas the hypothyreosis caused a decrease of its content. In the soleus muscle the MHC-2B was not detected in any groups of rats.

The results suggest that the amount of each of the four MHC isoforms expressed in the mature rat leg muscles is influenced by the thyroid hormone in a different way. The MHC-2A and the MHC-2X are differently regulated in the soleus and in the fast muscles; thyroid hormone seems to be necessary for expression of those isoforms in the soleus muscle.

✉Correspondence to: Anna Jakubiec-Puka, M. Nencki Institute of Experimental Biology, L. Pasteura 3, 02-093 Warszawa, Poland, tel.: (48 22) 659 8571; fax: (48 22) 822 5342.

Abbreviations: EDL, *extensor digitorum longus* muscle, GAST, *gastrocnemius* muscle; MHC, myosin heavy chain; SOL, *soleus* muscle; TRE, thyroid response element.

It is well known that the skeletal muscle is a very plastic tissue. The velocity of shortening and power produced during contraction can be changed following alterations of muscle function, innervation or other factors, among which the thyroid hormone is known to be very important. The thyroid hormone can exert some powerful influence over the phenotypic properties of the skeletal muscle. It causes increased velocity and power of muscle contraction [1]. However, the influence of thyroid hormone on the regulation mechanism(s) of protein expression in the muscle tissue is still poorly understood. Abnormal level of the thyroid hormone causes pathological changes in the muscle tissue; both the hyperthyroidism and the hypothyroidism induce muscle atrophy and can be manifested as myopathy [2, 3].

Myosin is the major muscle-specific protein responsible for muscle activity. It is composed of six subunits: two heavy chains and four light chains. The myosin heavy chain (MHC), (the protein of about 200 kDa) has many isoforms which are specific for different muscle or fibre types [4, 5]. Maximum velocity and power of muscle contraction are correlated with the MHC type [6, 7]. In the leg muscle of adult rats four isoforms of MHC were identified so far: the slow type, MHC-1 (β-cardiac), and three fast types: MHC-2A, MHC-2B and MHC-2X [5]. According to the general opinion, the slow muscle is more sensitive to thyroid hormone than the fast one and the thyroid hormone down-regulates the MHC-1 isoform and up-regulates the "fast" isoforms of MHCs [8–12]. However, the findings by various authors vary and are controversial [13–15]. Reasons for the controversies are unclear, as they are possibly following some different responses to thyroid hormone among the types of muscles [13] and, perhaps, among the animal species used in research. Differences among the results could be, as well, due to co-migration of the isoform MHC-2X and MHC-2A. However, the controversies may be also due to the examination exclusively of the relative changes in amount of the MHC isoforms. In the present work not only the relative, but also the total net changes in the content of individual MHC isoforms were evaluated.

In the present work the slow and the fast leg muscles of the hyperthyroid, hypothyroid and euthyroid rats of the same population were examined. Differences in the total net muscle content of the individual MHC isoform (MHC-1, MHC-2A, MHC-2B and MHC-2X), as well as differences in the relative amount of particular MHC isoform, in the muscle mass and in the total MHC muscle content between the hyperthyroid or hypothyroid and euthyroid rats were evaluated. The preliminary results have been published [16, 17].

MATERIALS AND METHODS

Experiments on rats. Female albino Wistar rats (WAG inbred strain, bred in the Nencki Institute of Experimental Biology in Warsaw), about 3 months old were used. Animals were fed on the standard Altromin diet; they were allowed access to food and water *ad libitum*. Animals of the same population were randomly assigned to one of the three groups: hyperthyroid, hypothyroid and euthyroid controls. Hyperthyreosis was induced by subcutaneous injections of the thyroid hormone – 3,3',5-triiodothyronine (T3) (Sigma), at a dose of 100 μg daily for 14 days. Hypothyreosis was induced by surgical thyroidectomy (under ether anaesthesia) followed by applying propylthiouracyl (Propycil 50, Solvay Arzneimittel, Kali-Chemie Pharma, Hannover, F.R.G.) in drinking water (0.04%) for 50 days. The hyperthyroidism and hypothyreoidism were induced as described by Dubaniewicz *et al.* [18], but our experiments took longer than those.

The slow soleus (SOL) and two fast muscles, *extensor digitorum longus* (EDL) and *gastrocnemius* (GAST) were examined. Muscles from two or three animals were used in each experi-

ment. Experiments on muscles taken from the hyperthyroid, hypothyroid and euthyroid animals were performed in parallel and the results were compared. Some populations of rats were examined at various times to eliminate some possible seasonal or accidental factors.

Preparation of myofibrils. Animals were killed by decapitation (under ether anaesthesia) and the entire muscles were immediately removed, their tendons were discarded and the muscles were transferred to ice. Pooled muscles from 2 or 3 animals were weighed, washed with buffer and homogenised. Crude myofibrils were separated quantitatively, as previously described [19]. Myofibrils from the muscles of both the hyperthyroid and the hypothyroid rats, as well as from the muscles of euthyroid controls, were obtained under exactly the same conditions, carefully monitoring volume of solutions during the procedure. Then they were studied in parallel and compared within the same experiment. All procedures were described in detail previously [19–21]. The total content of protein in myofibrils was estimated by the Lowry *et al.* [22] method.

Analysis of MHC. The total MHC content in the muscle was evaluated by estimating the total MHC in crude myofibrils. Myofibrillar proteins were separated by SDS/PAGE as described previously in detail [21, 23, 24]. Gels from the same electrophoresis run, stained and destained together, were used for comparison. The data from each experiment was derived from analysis of at least 8 gels running in at least 4 electrophoresis runs. For evaluation of the absolute muscle MHC concentration, the content of the total MHC was referred to bovine serum albumin (BSA, Serva) running in the same electrophoresis run.

Isoforms of MHC (1, 2A, 2B and 2X) were separated from myofibrils by SDS/PAGE (8% gel) with high glycerol concentration, according to the method of Talmadge and Roy for separation of the skeletal muscle myosin heavy chains [25]. Examples of separation of the MHC isoforms from the control SOL and EDL muscles are presented in Fig. 1.

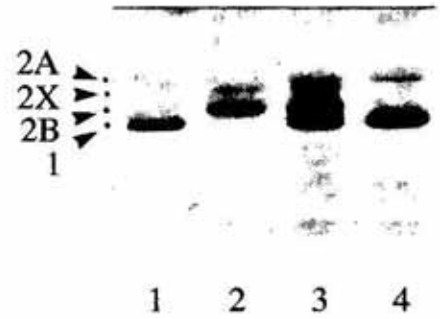

Figure 1. SDS/PAGE of MHC-isoforms from the control muscles.

Electrophoresis was performed as described in Materials and Methods. Myofibrilar protein was subjected to gels: lines 1 and 2, 0.5 μg; lines 3 and 4, 1 μg. Lines 1 and 4, SOL; line 2, EDL; line 3, mixture of myofibrils from SOL and EDL. Silver stained.

The relative amounts of MHC isoforms were determined by comparing the degree of intensity of staining with Coomassie Blue of the electrophoretic bands, as previously described [26]. A linear response for the individual MHC bands was obtained when 0.5 μg to 9 μg of myofibrillar protein was analysed [26]. For comparison the data were taken exclusively from the samples running in the same electrophoresis run. The data of each experiment were derived from 2 to 6 independent electrophoresis runs, and from 4 to 9 measurements. The data of individual experiments, or the collected data of some experiments on particular muscle, are presented.

Changes in the net content of the individual MHCs in the experimental muscles were calculated from the data concerning the MHC-total content in the muscle, and relative contribution of the MHC-isoforms, as previously described [21, 23]. The changes of the particular MHC isoforms were related to the muscle mass unit or to the entire muscle.

Statistical evaluation. The results were evaluated statistically by the *t*-test for related samples [27].

RESULTS

Weight of animals, mass of muscles and the MHC-total muscle content

The mass of the euthyroid rats was 168 ± (S.E.M.) 4 g. The mass of the hyperthyroid rats was in the range of euthyroid controls of the same population, 99 ± 2%. The mass of the hypothyroid rats decreased to 84 ± 5% of that of the euthyroid controls.

The mass of control muscles (euthyroid) was 74 ± (S.E.M.) 2 mg, 82 ± 2 mg and 973 ± 40 mg for the SOL, EDL and GAST, respectively. As mentioned in Materials and Methods, pooled muscles of 2 or 3 animals were weight in each experiment. The results presented are the means from some groups of pooled muscles. The S.E.M. values show that differences among the groups of animals examined were not high. The mass of muscles of the experimental animals (both the hyperthyroid and hypothyroid), decreased by about 10–20% as compared to the corresponding euthyroid controls (Table 1).

The absolute content of the total MHC in the control (intact) muscles was 37 ± 2, 37 ± 1, 50 ± 2 mg/g for SOL, EDL and GAST, respectively (when referred to the albumin standard).

The total content of the MHCs decreased in the experimental muscles by about 20–30%, with exception of the hyperthyroid EDL and the hypothyroid SOL, in which it remained at the range of control values (Table 1).

Pattern of the MHC isoforms

The MHC isoforms were separated by SDS/PAGE according to Talmadge and Roy [25] as described in Materials and Methods. We obtained identical separation of MHCs

Table 1. Changes in the MHC pattern in the muscles of the hyperthyroid and hypothyroid rats.

Electrophoregrams of the MHC isoforms were performed and scanned as describe in the Materials and Methods. The relative amounts of the individual MHC isoforms were estimated. The sum of all MHCs in the sample was taken as 100 %. The results of some experiments (groups of pooled animals) were gathered and presented as means ± S.E.M. The S.E.M. values show differences in proportion of individual MHC isoforms among groups of rats. Differences in proportion of MHC isoforms between experimental and control muscles were statistically evaluated within individual experiments by the *t*-test for related samples. $^{*}P < 0.05$ statistically significant; $^{**}P < 0.01$ statistically significant; $^{***}P < 0.001$ statistically significant.

Muscle	Experiment	Muscle mass	MHC-total	MHC isoforms (percentage of total MHC)			
		% of control	% of control	1	2A	2X	2B
Hyperthyroid rats							
SOL	Control			83±0.7	17±0.8	0.3	0
	Hyperthyr.	82±2.6 **	73±3.2 ***	59±1.6 ***	22±0.9 ***	19±1.0 ***	0
EDL	Control			0	9.6±1.1	21±1.3	69±2.3
	Hyperthyr.	93±1.7 *	97±4.0	0	8.2±1.2 *	19±1.4	72±1.9
GAST	Control			8.6±1.3	5.6±1.3	20.5±0.4	68±2.0
	Hyperthyr.	82±3.2 *	85±2.7 ***	6±1.9	5.3±0.3	22±2.8	68±4.2
Hypothyroid rats							
SOL	Control			89±1.9	9.8±1.4	0.9±0.6	0
	Hypothyr.	85±3.4 *	98±2.3	99±0.5 ***	0.8±0.5 ***	0	0
EDL	Control			5.7±1.2	8.3±1.0	27±1.0	63±1.8
	Hypothyr.	89±1.2 *	79±2.0 ***	9.2±0.7	15±1.3 ***	32±0.3 **	50±1.6 ***
GAST	Control			3.3±0.7	7.8±1.3	27±0.8	62±1.4
	Hypothyr.	82±2.0 *	80±2.6 ***	7.6±1.5 **	10±1.1 **	27±0.6	56±1.5 *

from the rat SOL and GAST as the authors of the method (Figs. 1, 2).

SOL muscle. The MHCs of the SOL muscle from euthyroid animals contained about 83% of the type 1 isoform and about 17% of the type 2A. The MHC-2X was not detected at all, or traces of it were present in the SOL of some animals (Table 1). The differences among the rat groups were not high.

In the SOL muscle of the hyperthyroid rats some considerable changes in the MHC pattern took place: proportion of the MHC-1 decreased, while that of the MHC-2A increased. Additionally, the MHC-2X appeared, heaving reached 14–21% of the MHCs present in the muscle (some induction of the MHC-2X expression in the SOL by hyperthyroidism was observed recently by other authors, as well [14, 15]). All changes in the MHC isoform proportion in the hyperthyroid muscles were highly evident and significant (Figs. 2, 3, Table 1).

In the SOL of the hypothyroid rats the MHC pattern shifts in an opposite direction to that of the hyperthyroid rats. Namely, the proportion of the MHC-1 increased, while that of the MHC-2A decreased significantly; in some experiments the MHC-2A was undetectable. The MHC-2X was not detected in the SOL muscle of any group of the hypothyroid rat studied (Figs. 2, 3, Table 1 and results not shown).

The MHC-2B isoform was not detected in any of the SOL muscles studied.

EDL muscle. The MHCs of the control EDL muscle contained about 9%, 21–27% and 63–69% of the isoform 2A, 2X and 2B, respectively; in the muscles of individual animals MHC-1 isoform was present (Table 1), as observed also previously [21]. In the EDL muscle of hyperthyroid rats the proportion of the MHC-2A and MHC-2X decreased, while that of the MHC-2B increased. In the EDL muscle of the hypothyroid rats the pattern of MHCs shifted in the opposite direction: the proportion of the MHC-2A and MHC-2X increased, and that of the MHC-2B decreased significantly; the proportion of the MHC-1 (if at all present in the EDL of the rat population studied) increased (Table 1, Fig. 2).

GAST muscle. The MHCs of the control GAST muscle contained about 3–8%, 5–8%, 20–27% and 62–68% of the isoform type 1, 2A, 2X and 2B, respectively. The usually observed (in this and in the previous papers [21, 26]) small percentage of the MHC-1 in the GAST muscle corresponds to the well known presence of some type-1 fibres in this muscle. In the GAST of hyperthyroid rats the proportion

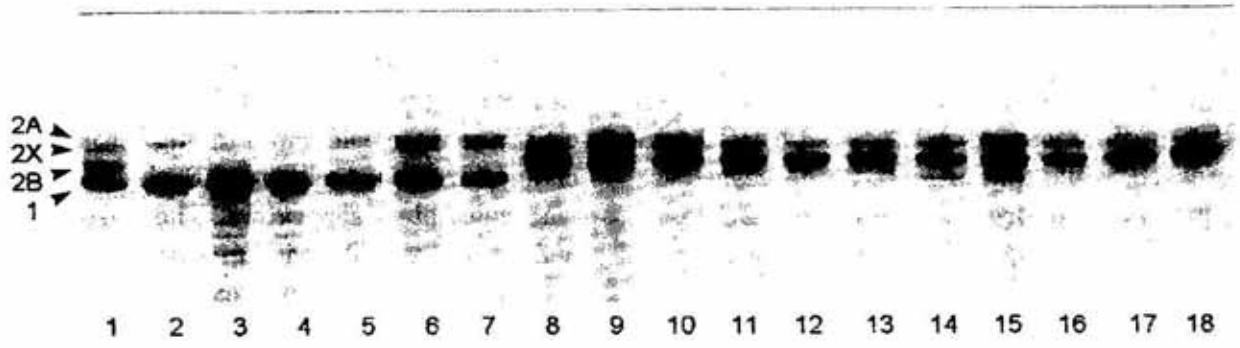

Figure 2. SDS/PAGE of MHC-isoforms from the hyperthyroid, hypothyroid and euthyroid muscles.

Electrophoresis was performed as described in Materials and Methods: 3 μg of myofibrilar protein was subjected to gels. Line 1, mixture of SOL and EDL. Lines 2–7, SOL: line 2, euthyroid; lines 3 and 4, hypothyroid; line 5, euthyroid; lines 6 and 7, hyperthyroid. Lines 8–13, EDL: line 8, euthyroid; lines 9 and 10, hypothyroid; line 11, euthyroid; lines 12 and 13, hyperthyroid. Lines 14–18, GAST: lines 14 and 15, hypothyroid; line 16, euthyroid; lines 17 and 18, hyperthyroid. Coomassie Blue stained.

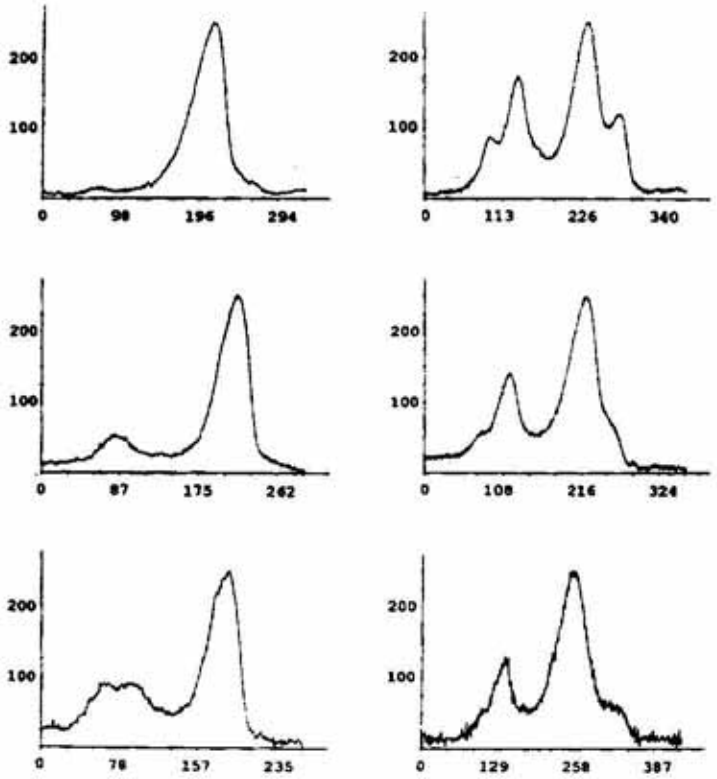

Figure 3. Scanning curves of SDS/PAGE gels of the MHC-isoforms from the hyperthyroid, hypothyroid and euthyroid muscles.

On the left – SOL; on the right – GAST; upper pictures, hypothyroid muscles; central pictures, euthyroid muscles; lower pictures, hypertyroid muscles. Axes x – distance, axes y – arbitral image density units.

of the MHC isoforms was not changed significantly in comparison with control animals. In the GAST of hypothyroid animals the proportion of the isoforms MHC-1 and MHC-2A increased, while that of MHC-2B decreased significantly; the proportion of the MHC-2X remained unchanged (Figs. 2, 3, Table 1).

Muscle contents of the individual MHC-isoforms

For evaluation of changes in the net content of particular MHC isoforms from changes in their proportion, the values presented in Table 1 were recalculated in relation to the content of the MHC-total in the control muscle. To simplify the calculation they were related to 100 mg of the MHC-total in the control muscle and, correspondingly, expressed in micrograms in Table 2. The content of a particular MHC isoform in experimental muscle was calculated from its relative content and from the changes in MHC-total in this muscle. Changes in the concentration of MHC isoforms in muscle tissue were calculated taking into account changes in the muscle mass. Each experiment was calculated separately. The results presented in Table 2 and Fig. 4 are the means ± S.E.M. from individual experiments.

MHC-1. Both the muscle content of the MHC-1 and its tissue concentration decreased in the SOL, EDL and GAST muscles of the hyperthyroid rats, while they increased in the muscles of the hypothyroid rats (Table 2, Fig. 4).

MHC-2A. The concentration of the MHC-2A increased in the hyperthyroid SOL muscle, while decreased in the hypothyroid one. Contrary to that, in the fast muscles the content and the concentration of the MHC-2A increased in the hypothyroid muscles. In the hyperthyroid muscles some decrease of the

Table 2. Content of the particular MHC isoforms in the muscles of the hyperthyroid and hypothyroid rats.

The gathered data presented in Table 1 were re-calculated and expressed relative to 100 mg of the total MHC content of the control muscle. Values are means ± S.E.M. Differences between experimental and control were statistically evaluated. $^{*}P < 0.05$ statistically significant; $^{**}P < 0.01$ statistically significant; $^{***}P < 0.001$ statistically significant.

	Muscle	MHC-total (mg)	Content of MHC isoforms							
			MHC-1		MHC-2A		MHC-2X		MHC-2B	
			mg	% of control	mg	% of control	mg	% of control	mg	% of control
Hyperthyroid rats										
SOL	Control	100	83±0.7		17±0.8		0.3		0	
	Hyperthyr.	73±3.2	45±2.3 ***	54±2.7	17±1.2	103±8.4	14.7±1.7 **	4900	0	0
EDL	Control	100	0		9.6±1.1		21±1.3		69±2.3	
	Hyperthyr.	97±4.0	0	0	7.9±1.2 *	82 ±6.6	19±1.3	90±9.9	70±4.7	102±6.3
GAST	Control	100	8.6±1.3		5.6±1.3		20.5±0.4		68±2.0	
	Hyperthyr.	85±2.7	5.2±1.8	58 ±14	4.5±0.1	87 ±15	18.9±2.4	92±11	58±3.3 *	85±2.4
Hypothyroid rats										
SOL	Control	100	89±1.9		9.8±1.4		0.9±0.6		0	
	Hypothyr.	98±2.3	98±2.8	110±6.5	0.8±0.8 *	8.1	0	0	0	0
EDL	Control	100	5.7±1.2		8.3±1.0		27±1.0		63±1.8	
	Hypothyr.	79±2.0	8.3	146	12±0.1 *	141±13	26±1.7	96±11	40±1.3 **	63±2.1
GAST	Control	100	3.3±0.7		7.8±1.3		27±0.8		62±1.4	
	Hypothyr.	80±2.6	5.9±2.1	179 ±6	8.4±1.5	112±12	22±2.0	81±8.5	45±2.7 *	72±5.1

MHC-2A content, but not its concentration was observed (Table 2, Fig. 4).

MHC-2X. The MHC-2X muscle content, as well as its tissue concentration, increased considerably in the SOL of the hyperthyroid rats. In the SOL of hypothyroid rats the MHC-2X isoform was not detected (Table 2, Fig. 4).

In the fast muscles of both the hyperthyroid and the hypothyroid rats, the content and the concentration of the MHC-2X were like those in the muscles of euthyroid rats (Table 2, Fig. 4).

MHC-2B. In the fast muscles of the hyperthyroid rats the MHC-2B content and its concentration were like those in the euthyroid animals. In the hypothyroid animals both the content and the concentration of the MHC-2B were decreased (Table 2, Fig. 4).

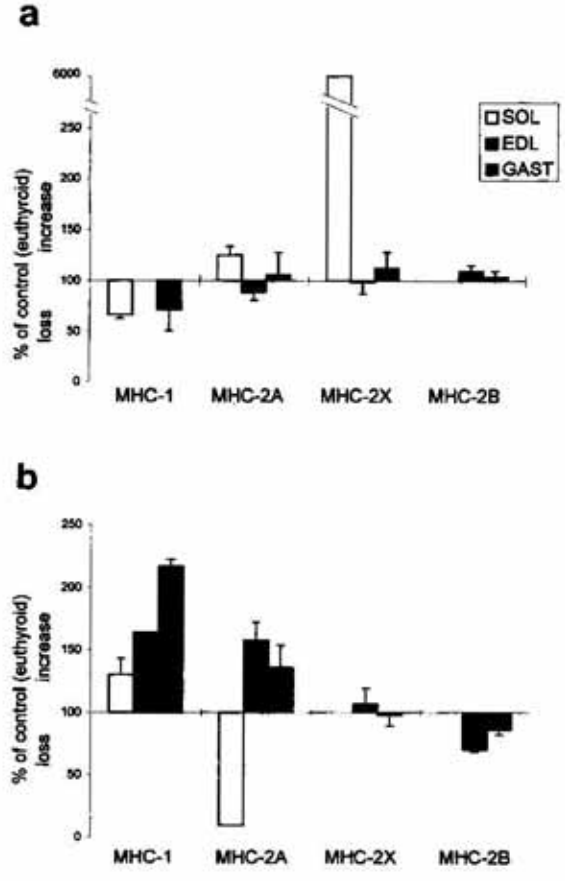

Figure 4. Changes in concentration of the MHC-isoforms in muscle tissue (expresses relatively to the muscle mass).

Muscles of the hyperthyroid (a) and hypothyroid (b) rats.

Summing up, the obtained information suggests the following effects of thyroid hormone on the particular MHC isoforms in the rat leg muscles:

1. In all studied muscle (SOL, EDL and GAST) the relative contribution of each MHC isoform to the total MHC changed inversely in the hyperthyreosis to that in the hypothyreosis.

2. Changes in the MHC-1 content, induced by the hyperthyreosis or hypothyreosis, were similar in the SOL and in the fast muscles, whereas changes in the amount of the "fast" isoforms were different in the SOL and in the fast muscles.

3. Changes in the amount of particular MHC isoforms, induced by hyperthyreosis, as well as by hypothyreosis, were similar in both fast muscles (EDL and GAST) of the same animals.

4. Each MHC isoform under study responded differently to alterations in the thyroid hormone availability. The net amount of particular MHC isoforms changed as follows.

a. The MHC-1 decreased following hyperthyreosis, while it increased following hypothyreosis in all muscles studied.

b. The MHC-2A increased in hyperthyreosis in the SOL; it was not changed in the fast muscles. In hypothyreosis the MHC-2A decreased in the SOL while increased in the fast muscles.

c. The MHC-2X content increased considerably in the SOL muscle following the thyroid hormone treatment; in the SOL of some rats the expression of that isoform seemed to be switched on *de novo*. In hypothyreosis the MHC-2X was not detected in the SOL muscle. Contrary to the SOL, in the EDL and GAST muscles the amount of MHC-2X was not affected by any changes in the thyroid hormone level.

d. The MHC-2B seemed to remain unaffected by the hyperthyreosis, whereas the hypothyreosis caused its decrease in both fast mus-

cles. Expression of the MHC-2B was not induced in the SOL muscle either by thyroid hormone excess or its deficiency.

DISCUSSION

In this paper we have estimated the relative changes in the content of the individual MHC isoforms (i.e. in their relative contribution) induced in the rat leg muscles by the experimental hyperthyreosis and hypothyreosis (Table 1). Simultaneously, we also considered the true net changes in the amount of individual MHC isoforms in the entire muscle (total muscle content) and changes in their tissue concentration (Table 2, Fig. 4). Comparison of the results presented in Tables 1 and 2 and in Fig. 4 indicates that true net changes in the content of individual MHC isoforms are not the same as the relative ones. By the other words, the observed differences stress that the true changes of particular MHC isoforms are not identical as those showed by changes in their proportion. The true values of the MHC isoform contents were calculated taking into account loss of the muscle mass and alterations in the total MHC content within the experimental muscles, in relation to the euthyroid controls. This way of evaluation is surely better in recognition of the response of individual MHC isoforms to changed thyroid hormone level than the evaluation of the relative MHC changes only. The results obtained in this study show that each of the MHC isoforms under study responded differently to alterations in the thyroid hormone level, and some of them changed differently in the SOL and in the fast muscles.

It is a well-known fact, that the tissue content of each protein results from the equilibrium between its synthesis and degradation. The regulation of both of them in the muscle tissue is still poorly understood. Synthesis of proteins, specific for the skeletal muscle, is controlled by some families of the muscle-specific transcriptional factors [28–30]. The best known to date is the Myo-D family [10, 29], whose members, Myo-D and Myogenin, are expressed preferentially in the mature fast and slow muscles, respectively [31]. By means of factors of the Myo-D family the transcription of the "fast" MHC isoforms may be regulated [5], while the synthesis of the MHC-1 is independent of those factors [32]; it is perhaps regulated by the other group of transcriptional factors – the MEF2 family [28]. The regulatory mechanisms responsible for the muscle-type-specific expression of the MHC isoform genes have not been identified yet. Doubtless, however, those processes are substantially influenced by the thyroid hormone. It can affect the level of muscle proteins in many ways. In general, in the muscle tissue the thyroid hormone accelerates both the protein synthesis and degradation [33–35]. The thyroid hormone can influence several stages in the protein synthesis process. At the transcriptional level it acts by the T3 nuclear receptor, which reacts with the specific DNA sequence (TRE) in the regulatory region of the gene promoter. In the fast and slow muscles the distinct T3 receptors are preferentially expressed [1]. The T3 receptors can act as monomers, or in association with other nuclear proteins. Such complexity creates diversity in regulation of the gene transcription by means of thyroid hormone, which may have either a positive or a negative effect upon the RNA transcription [36–38]. Up to now, the TRE-sequence was identified within the promoter of MHC-1 gene [39]; it was not found in the MHC-2B gene, while as to the MHC-2A and MHC-2X genes no information is available so far [1, 10]. Some indirect regulation of the MHC gene expression by the thyroid hormone is also possible. It may be *via* Myo-D/Myogenin transcriptional factors (whose genes contain the TRE-sequence) [10], by some nongenomic actions [40], and also *via* changes in motoneurons or end-plates. All of them can be affected by either the hyperthyroidism or the hypothyroidism [2, 41, 42]. Thus, the influence by the thyroid hor-

mone upon the level of muscle proteins is surely complex.

The results of this paper show down-regulation of the MHC-1 expression by thyroid hormone excess and its up-regulation in hypothyreosis. These results confirmed, in general, the previous ones documenting the induced expression of the MHC-1 in the SOL muscle in hypothyroidism [8, 11, 14, 43]; the developing muscle was the exception [44]. Comparing the true changes in the content of MHC-1, we found similar effect of the hyperthyreosis or hypothyreosis on the MHC-1 isoform in the SOL and in the fast muscles (Fig. 4). This suggests the same way of action by the thyroid hormone on the MHC-1 in the slow and in the fast muscles. Such similarity, and also the inverse effects of the hyperthyreosis and hypothyreosis (Table 2, Figs. 2–4), strongly point out the main influence of the thyroid hormone on the MHC-1 expression by direct transcriptional regulation. It must be stressed here that muscle denervation influences similarly the MHC-1 content as hyperthyreosis [21].

The MHC-2A content changed inversely in the hyperthyroid and in the hypothyroid SOL. On the other hand, in hypothyreosis it changed inversely in the SOL and in the fast muscles (Table 2, Fig. 4), similarly as it was observed in the denervated muscles [21]. Thus, those results suggest that the thyroid hormone up-regulates the MHC-2A in the SOL, while in the fast muscles the situation is quite different. The MHC-2A isoform is up-regulated by the lack of the thyroid hormone, whereas the excess of the hormone seems to remain without influence on this isoform. An influence of thyroid hormone on the MHC-2A has been controversial up to now. Our results, together with the results of other researchers [11, 43], strongly suggest the existence of the direct transcriptional regulation of the MHC-2A synthesis by the thyroid hormone in the SOL muscle. The observed type-dependent differences may be caused by different T3 receptors, and additionally, perhaps by some other, muscle fiber type-dependent factors.

The MHC-2X isoform responded quite differently to the T3 treatment in the SOL and in the fast muscles (Table 2, Fig. 4). In the SOL the MHC-2X was strongly up-regulated in hyperthyreosis, or even perhaps its expression was switched on *de novo*. Contrary to that, in the fast muscles, the MHC-2X seems to be insensitive to any changes in the thyroid hormone level (Table 2, Fig. 4); the similar observation was made by Li and Larsson [14]. However, controversial results concerning that question have been also published [15]. The response of the MHC-2X to the T3 treatment, different in the slow and in the fast muscle may depend on different T3 receptors, or possibly on some indirect action by the thyroid hormone upon the MHC-2X synthesis. It is possible, as well, that in the SOL muscle the hyperthyrosis causes de-suppression of the MHC-2X synthesis. Similar effect on the MHC-2X to that induced by hyperthyreosis was observed after muscle denervation [21].

Contrary to the other isoforms examined, the net content of MHC-2B was practically unaffected by the hyperthyreosis in any of the muscles examined (Table 2, Fig. 4). In this matter there are also controversies, our results are similar to those by Hudges *et al.* [31] and Li & Larsson [14] but differ from the results by some others [13, 15]. Our results, obtained by evaluation of the net content of the MHC-2B, suggest that excess of the T3 does not up-regulate the MHC-2B synthesis in the rat leg muscle. It can be easy explained by lack of the TRE sequence in the MHC-2B gene promoter. However, the loss of the MHC-2B content in the fast muscles of the hypothyroid rats (Fig. 4) could point to some secondary effect(s) of thyroid hormone deficiency on the synthesis of MHC-2B, or on the rate of its degradation.

In conclusion, the presented results show that each of the four MHC isoforms, expressed in the mature rat leg muscles, is influenced by the thyroid hormone in a different

way. The MHC-2A and MHC-2X isoforms are differently regulated by the thyroid hormone in the SOL than they are in the fast muscles. It seems that thyroid hormone is necessary for expression of the MHC-2A and MHC-2X in the SOL muscle.

The authors wish to thank Mrs A. Matusiak for skilful technical assistance.

REFERENCES

1. Caiozzo, V.J. & Haddad, F. (1996) Thyroid hormone: Modulation of muscle structure, function, and adaptive responses to mechanical loading; in *Exercise and Sport Sciences Reviews* (Holloszy, J.O., ed.) pp. 321–361, Williams & Wilkins, Baltimore.
2. Laureno, R. (1996) Neurologic manifestations of thyroid disease. *Endocrinologist* **6**, 467–473.
3. Nobrega, A.C.L., Vaisman, M. & de Araujo, C.G.S. (1997) Skeletal muscle function and body composition of patients with hyperthyroidism. *Med. Sci. Sports Exerc.* **29**, 175–180.
4. Pette, D. & Staron, R.S. (1990) Cellular and molecular diversities of mammalian skeletal muscle fibers. *Rev. Physiol. Biochem. Pharmacol.* **116**, 1–76.
5. Schiaffino, S. & Reggiani, C. (1996) Molecular diversity of myofibrillar proteins: Gene regulation and functional significance. *Physiol. Rev.* **76**, 371–423.
6. Reiser, P.J., Moss, R.L., Giulian, G.G. & Greaser, M.L. (1985) Shortening velocity in single fibers from adult rabbit soleus muscles is correlated with myosin heavy chain composition. *J. Biol. Chem.* **260**, 9077–9080.
7. Wells, L., Edwards, K.A. & Bernstein, S.I. (1996) Myosin heavy chain isoforms regulate muscle function but not myofibril assembly. *EMBO J.* **15**, 4454–4459.
8. Caiozzo, V.J., Herrick, R.E. & Baldwin, K.M. (1992) Response of slow and fast muscle to hypothyroidism: Maximal shortening velocity and myosin isoforms. *Am. J. Physiol.* **263**, C86–C94.
9. Larsson, L., Li, X., Teresi, A. & Salviati, G. (1994) Effects of thyroid hormone on fast- and slow-twitch skeletal muscles in young and old rats. *J. Physiol.* **481**, 149–161.
10. Muscat, G.E.O., Downes, M. & Dowhan, D.H. (1995) Regulation of vertebrate muscle differentiation by thyroid hormone: The role of the myoD gene family. *BioEssays* **17**, 211–218.
11. Diffee, G.M., Haddad, F., Herrick, R.E. & Baldwin, K.M. (1991) Control of myosin heavy chain expression: Interaction of hypothyroidism and hindlimb suspension. *Am. J. Physiol.* **261**, C1099–C1106.
12. Ianuzzo, C.D., Hamilton, N. & Li, B. (1991) Competitive control of myosin expression: Hypertrophy *vs.* hyperthyroidism. *J. Appl. Physiol.* **70**, 2328–2330.
13. Izumo, S., Nadal-Ginard, B. & Mahdavi, V. (1986) All members of the MHC multigene family respond to thyroid hormone in a highly tissue-specific manner. *Science* **231**, 597–600.
14. Li, X. & Larsson, L. (1997) Contractility and myosin isoform compositions of skeletal muscles and muscle cells from rats treated with thyroid hormone for 0, 4 and 8 weeks. *J. Muscle Res. Cell Motil.* **18**, 335–344.
15. Haddad, F., Arnold, C., Zeng, M. & Baldwin, K. (1997) Interaction of thyroid state and denervation on skeletal myosin heavy chain expression. *Muscle Nerve* **20**, 1487–1496.
16. Jakubiec-Puka, A., Wieczorek, U., Langford, J. & Morga, J. (1996) Influence of thyroid hormone on the myosin heavy chains in the denervated and self-reinnervated skeletal muscle. *International Symposium, Exercise in prevention, diagnosis, and therapy of metabolic disorders*, 25–27 Sept. 1996, Warsaw, P-01.

17. Jakubiec-Puka, A., Ciechomska, I., Mackiewicz, U., Chomontowska, H. (1998) Effects of hyperthyreosis and hypothyreosis on the myosin heavy chain isoforms. *XXVII European Muscle Conference*, 11-16 Sept. 1998, Lund, Sweden, J-13.

18. Dubaniewicz, A., Kaciuba-Uscitko, H., Nazar, K. & Budohoski, L. (1989) Sensitivity of the soleus muscle to insulin in resting and exercising rats with experimental hypo- and hyper-thyroidism. *Biochem. J.* **263**, 243-247.

19. Jakubiec-Puka, A., Kulesza-Lipka, D. & Krajewski, K. (1981) The contractile apparatus of striated muscle in the course of atrophy and regeneration. I. Myosin and actin filaments in the denervated rat soleus. *Cell Tissue Res.* **220**, 651-663.

20. Jakubiec-Puka, A., Kordowska, J., Catani, C. & Carraro, U. (1990) Myosin heavy chain isoform composition in striated muscle after denervation and self-reinnervation. *Eur. J. Biochem.* **193**, 623-628.

21. Jakubiec-Puka, A., Ciechomska, I., Morga, J., Matusiak, A. (1999) Contents of myosin heavy chains in denervated slow and fast rat leg muscles. *Comp. Biochem. Physiol. B*, **122**, 355-362.

22. Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265-275.

23. Jakubiec-Puka, A., Catani, C. & Carraro, U. (1992) Myosin heavy-chain composition in striated muscle after tenotomy. *Biochem. J.* **282**, 237-242.

24. Jakubiec-Puka, A., Szczepanowska, J. & Wieczorek, U. (1995) Myosin heavy chains in striated muscle immobilized in a shortened position. *Basic Appl. Myol.* **5**, 147-153.

25. Talmadge, R.J. & Roy, R.R. (1993) Electrophoretic separation of rat skeletal muscle myosin heavy-chain isoforms. *J. Appl. Physiol.* **75**, 2337-2340.

26. Szczepanowska, J. & Jakubiec-Puka, A. (1992) Myosin heavy chains in striated muscle after immobilization. *Basic Appl. Myol.* **2**, 97-105.

27. Motulsky, H. (1995) *Intuitive Biostatistics*. Oxford University Press, New York, Oxford.

28. Ludolph, D.C. & Konieczny, S.F. (1995) Transcription factor families: Muscling in on the myogenic program. *FASEB J.* **9**, 1595-1604.

29. Weintraub, H. (1993) The MyoD family and myogenesis: Redundancy, networks, and thresholds. *Cell* **75**, 1241-1244.

30. Leferovich, J.M., Lana, D.P., Sutrave, P., Hughes, S.H. & Kelly, A.M. (1995) Regulation of c-ski transgene expression in developing and mature mice. *J. Neurosci.* **15**, 596-603.

31. Hughes, S.M., Taylor, J.M., Tapscott, S.J., Gurley, C.M., Carter, W.J. & Peterson, C.A. (1993) Selective accumulation of MyoD and Myogenin mRNAs in fast and slow adult skeletal muscle is controlled by innervation and hormones. *Development* **118**, 1137-1147.

32. Thompson, W.R., Nadal-Ginard, B. & Mahdavi, V. (1991) A MyoD1-independent muscle-specific enhancer controls the expression of the β-myosin heavy chain gene in skeletal and cardiac muscle cells. *J. Biol. Chem.* **266**, 22678-22688.

33. DeMartino, G.N. & Goldberg, A.L. (1978) Thyroid hormones control lysosomal enzyme activities in liver and skeletal muscle. *Proc. Natl. Acad. Sci. U.S.A.* **75**, 1369-1373.

34. Brown, J.G., Bates, P.C., Holliday, M.A. & Millward, D.J. (1981) Thyroid hormones and muscle protein turnover. *Biochem. J.* **194**, 771-782.

35. Zeman, R.J., Bernstein, P.L., Ludemann, R. & Etlinger, J.D. (1986) Regulation of Ca^{2+}-dependent protein turnover in skeletal muscle by thyroxine. *Biochem. J.* **240**, 269-272.

36. Hollenberg, A.N., Monden, T., Madura, J.P., Lee, K. & Wondisford, F.E. (1996) Function of nuclear co-repressor protein on thyroid hor-

mone response elements is regulated by the receptor A/B domain. *J. Biol. Chem.* **271**, 28516–28520.

37. Zhang, X., Jeyakumar, M. & Bagchi, M.K. (1996) Ligand-dependent cross-talk between steroid and thyroid hormone receptors. *J. Biol. Chem.* **271**, 14825–14833.

38. Zhu, Y.-S., Yen, P.M., Chin, W.W. & Pfaff, D.W. (1996) Estrogen and thyroid hormone interaction on regulation of gene expression. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 12587–12592.

39. Edwards, J.G., Bahl, J.J., Flink, I.L., Cheng, S.-Y. & Morkin, E. (1994) Thyroid hormone influences beta myosin heavy chain (β-MHC) expression. *Biochem. Biophys. Res. Commun.* **199**, 1482–1488.

40. Davis, P.J. & Davis, F.B. (1996) Nongenomic actions of thyroid hormone. *Thyroid* **6**, 497–504.

41. Kazakov, V.M. (1992) Terminal intramuscular motor innervation and motor end-plates in thyrotoxic myopathy. *Neuromusc. Disorders* **2**, 343–349.

42. Prakash, Y.S., Gosselin, L.E., Zhan, W.Z. & Sieck, G.C. (1996) Alterations of diaphragm neuromuscular junctions with hypothyroidism. *J. Appl. Physiol.* **81**, 1240–1248.

43. Petrof, B.J., Kelly, A.M., Rubinstein, N.A. & Pack, A.I. (1992) Effect of hypothyroidism on myosin heavy chain expression in rat pharyngeal dilator muscles. *J. Appl. Physiol.* **73**, 179–187.

44. d'Albis, A., Chanoine, C., Janmot, C., Mira, J.-C. & Couteaux, R. (1990) Muscle-specific response to thyroid hormone of myosin isoform transitions during rat postnatal development. *Eur. J. Biochem.* **193**, 155–161.