Reversal of Hypothyroid Rat Soleus Myosin Isoform and Shortening Velocity Changes

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Abstract

The present study was designed establish the reversibility and time course of the changes in maximum isotonic shortening velocity (V_{MAX}) and myosin isoforms which occur in the slow-twitch soleus muscles of rats with altered thyroid status.

Rat thyroidectomy slowed maximum rate of isotonic shortening (V_{MAX}), isometric twitch contraction time (T_C), half relaxation time ($T_{1/2R}$) and maximum rate of tetanic force development (MRR) and intermediate myosin (IM) the faster moving, higher ATPase activity myosin isoform, usually found in adult rat soleus, disappeared. There were concomitant shifts in histochemical profile. After replacement triiodothyronine (T3) changes in maximum shortening velocity, histochemical fibre type proportions and percentages of myosin isoforms were reversed. The alterations in muscle contractility observed in patients suffering from altered thyroid status and their reversal after replacement T3 has been given, could certainly be explained on the basis of the changes outlined above.

Key words: Thyroid Hormone, Myosin, Soleus Muscle.

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The effects of altered levels of circulating thyroid hormones on the mechanical properties of human muscle have been known for some time [55, 83]. Higher than normal levels of circulating thyroid hormones induced a speeding of isometric muscle contractions, lower than normal levels produced slowing. In both hypothyroid and hyperthyroid situations there were no statistically significant changes in motor nerve conduction velocity when compared to measurements on euthyroid individuals. Hypothyroid patients showed an increase in the proportions of histochemically identified type I slow-twitch, low myosin ATPase, high oxidative enzyme muscle fibres and a decrease in type IIA, fast-twitch, high myosin ATPase, oxidative-glycolytic enzyme fibres [58]. These findings were confirmed and opposite changes reported in patients with higher than normal levels of circulating thyroid hormones [90]. Changes in histochemical and biochemical profiles of slow-twitch soleus muscles from rats with altered thyroid status have been reported [48, 69] in addition a respective slowing and speeding of slow twitch soleus muscle isometric contractile properties from hypo- and hyperthyroid animals has been reported [38, 43, 50, 51, 52], with no change in the fast twitch Extensor digitorum longus muscle [60]. The slowing and speeding of the isometric contractile properties of muscles from animals with altered thyroid status T_C, $T_{1/2R}$, and MRR has been correlated with their histochemical profile [12, 31, 51, 52, 66, 67] and altered isotonic mechanical properties have been also reported [13, 14, 21, 61]. Thus maximum velocity of isotonic shortening V_{MAX} was respectively increased and decreased in hyperthyroid and hypothyroid animals but V_{MAX} has also been reported as unchanged [31]. These alterations in biochemical, histochemical and contractile properties are known to occur in the absence of a speeding or slowing of the motor nerve conduction velocity [1, 2, 64].

The correlation between the maximum shortening velocity V_{MAX} of skeletal muscle and the specific activity of actomyosin ATPase activity has been known for some time [4] and this correlation has also been observed for single muscle fibres [29]. Myosin ATPase activity is known to be located in the myosin heavy chain and the underlying basis of the changes in myosin ATPase activity brought about

by cross-reinnervation of mammalian muscles was the induction or suppression of different myosin isoforms [47]. This has also been confirmed at the level of the single fibre thus as the proportion of fast-type heavy chains increased V_{MAX} of rabbit soleus fibres also increased [73]. Respective increases and decreases in Ca^{2+} activated myosin ATPase have been reported from the soleus muscles of hyperthyroid and hypothyroid rats together with a respective increase and decrease in Mg^{2+}-activated myofibrillar ATPase activity [13, 14, 48, 69]. Other workers have been unable to demonstrate such changes [31, 33]. However the activity of the actin activated myosin ATPase [4, 5] from hypothyroid soleus muscles have been shown to decrease [51, 52]. Analysis of myosin light chains by several groups [13, 14, 33, 51, 52, 69] has shown a respective increase and decrease in the percentage of fast light chains in hyper- and hypothyroid soleus muscles.

A maximum of 3 isoenzymic forms of slow myosin can be found in the soleus muscles of young adult euthyroid Wistar and Sprague-Dawley rats together with a faster moving form called intermediate myosin IM [3, 13, 14, 41, 42, 56, 57, 85]. Thyroid status has been found to have a profound influence on the isoforms of myosin expressed in rat skeletal and cardiac muscles [59], soleus muscles from hypothyroid animals showed only slow myosin whereas muscles from hyperthyroid animals showed a reduction in the proportion of slow myosin with complementary increases in the proportions of intermediate myosin IM [13, 14, 33].

The present study was designed to establish the changes in myosin isoforms and maximum isotonic shortening velocity V_{MAX} which occur in the slow-twitch soleus muscles of hypothyroid rats given replacement thyroid hormone and to obtain information about the time course of any such changes.

Materials and Methods

Experimental design.

The experimental design involved the allocation of a number of euthyroid 8 week old male Wistar rats into initially three groups on a random basis. These would become the age matched euthyroid, chronic hypothyroid and chronic hyperthyroid groups. Bilateral surgical thyroidectomy produced the hypothyroid group and low levels of thyroid hormones in a blood sample taken twelve weeks later confirmed their chronic hypothyroid status. After confirmation, some hypothyroid animals were placed in two further group and were given T3 for varying lengths of time whilst the remainder acted as a chronic hypothyroid group. In a terminal series of observations under general anaesthesia, the *in vivo* mechanical properties of the left limb muscles were measured at 37°C, the right limb muscles were also removed and weighed. The thyroid status of each of animal was established by direct measurement of T4 or T3 in a blood sample. Contractile, histochemical and biochemical results from hypothyroid animals given T3 were then compared with those obtained from chronic hypo', chronic hyper' and age matched euthyroid animals.

The first group (A) constituted an age matched control euthyroid group, the second (B) a chronic hypothyroid and the third (C) a chronic hyperthyroid group. Hyperthyroidism was induced in the animals allocated to group C by giving them T3 in their drinking water, this was changed daily to avoid loss of potency as a result of the oxidation of iodine. Animals on average drank 10 ml of water per 100 g body weight per twenty four hours, the concentration of T3 in their drinking water was 0.1 µg/ml, therefore they would thus obtain a T3 dose of 1 µg/kg body weight / 24 hours; these animals were examined when they were 26 weeks old. Group B animals were anaesthetized at 8 weeks of age with Brietal Sodium (55 mg/kg) and Midazolam (5 mg/kg) I.P. and bilaterally thyroidectomized under aseptic conditions, twelve weeks after bilateral thyroidectomy the hypothyroid status of each animal was established and two thirds of the animals in group B were allocated to two further groups D and E, the remaining animals in group B constituted the chronic hypothyroid group. Hyperthyroidism was induced in the hypothyroid animals allocated to group D by giving them T3 in their drinking water using the same regime for group C animals; these animals were examined when they were 26 weeks old. When animals in group E were twenty weeks of age some were given daily intra peritoneal injections of T3 (50 µg/kg). Serum total T4, free T3 and calcium measurements were made from terminal blood samples from the majority of animals in each of the groups, measurements of serum calcium were made because surgical bilateral thyroidectomy involved the inevitable removal of the majority of the parathyroid gland.

Recording of mechanical properties.

Animals were anaesthetized with Urethane (1.25 g/kg body weight) and *in vivo* recordings of left limb soleus isometric and isotonic properties made at 37°C. These included isometric twitch half-relaxation time $T_{1/2R}$ measured from the peak of contraction to a point were force had declined to 50% of its peak value as a measure of the time course of relaxation [20, 36, 37], isometric twitch contraction time (T_C), maximum tetanic tension (Po), obtained by stimulating at 150 Hz for 500 ms and maximum rate of development of tetanic tension development (MRR) obtained by stimulating the muscle for 100 ms at increasing frequencies and normalising the rate as $\%Po.ms^{-1}$. A shortening velocity in millimeters per second was obtained for each load (P) applied up to a maximum of P = 0.8Po by stimulating the muscles tetanically for durations of between 50 ms at the lower loads and 200 ms at the highest loads. The stimulation frequency used was that which produced a maximum shortening velocity. The force and velocity data obtained from each muscle was fitted to Hill's equation [45] using a non linear curve fitting programme (Blackwell Scientific Software, Oxford, U.K.) with the measured Po from each muscle as one of the constraints, i.e. the velocity of shortening at maxi-

mum tetanic tension was zero. Constants a, b and maximum velocity of unloaded shortening (V_{MAX}) were obtained and used to calculate at each normalised load from 0.02Po to 0.8Po for each muscle its respective shortening velocity. At each time point a mean velocity for each of the normalised loads was calculated from several muscles and used to construct each of the curves shown in figure 2. The lengths of eight single muscle fibres were measured *in situ* using a dissecting microscope prior to a terminal anaesthetic overdose being given. The muscle was then removed together with its contralateral and the tendons trimmed, both muscles were weighed then prepared for histochemical and biochemical analysis. From measurements of fibre length in (cm) and weight in (g) and assuming a density of 1.06 g/cm^3, a value for the cross sectional area of each muscle could be calculated using the formula: - cross sectional area (cm^2) = volume/length and thus values for twitch force per unit area (Pt kg.cm^{-2}) and tetanic force per unit area (Po kg.cm^{-2}) could be calculated.

V_{MAX} extrapolated from forcevelocity data of the whole muscle results from the sum of the forcevelocity properties of all the fibres that make up that muscle. In a whole muscle composed of a mixture of fibre types it is possible that V_{MAX} is an underestimate of the maximum shortening velocity of the fastest contracting fibres. The slack test used by [28] provided a means whereby the velocity of shortening of single muscle fibres could be reliably measured under unloaded conditions. In single muscle fibres little difference between V_{MAX} and V_0 was found however when applied to whole muscles significant differences were found [3, 13, 14, 16, 17] it is thought that V_0 represents the maximum shortening velocity of the fastest contracting fibres.

Histochemical fibre typing

Muscles were terminally removed and mid-muscle transverse blocks were cut and placed on cryostat chucks and frozen in isopentane cooled with liquid nitrogen. 10 µm serial sections were cut at 20°C and stained for NADH-tetrazolium Reductase and Myosin ATPase after acid pH 4.3 [25, 68] or alkaline pH 10.3 preincubation. Photographic montages were made of the complete muscle cross sections from which the total fibre number and histochemical fibre type proportions could be calculated using a classification scheme [10, 11].

Myosin isoform preparation

The remaining muscles portions were cut and frozen in liquid nitrogen for myosin extraction. Myosin isoform separation was performed at 2^0 C on 4% polyacrylamide gels prepared with 20 mM sodium pyrophosphate buffer at pH 8.8 with 10% glycerol [22, 46] and densitometrically scanned at 550 nm.

Statistical analysis

Statistical analysis of all results was carried out using a one way analysis of variance, if F was significant, Duncan's Multiple range test for unequal numbers of observations [27] was applied using a statistical analysis programme (Unistat for Windows, London, U.K.) P = 0.05 was the probability level used throughout this study.

Results

Thyroid hormone levels

The mean serum total T4 of the 26 week age matched euthyroid control group A was 56.9 nmol/l; standard error of the mean (SEM) = 0.5; n = 12. Chronic hypothyroid group (B) animals had T4 values at the lowest level of measurement resolution i.e. < 2.0 nmol/l. Chronic hyperthyroid group (C) and the experimental group (E) animals with replacement T3 showed reduced serum total T4 levels, this latter finding is consistent with feedback inhibition of T4 output resulting from high serum T3. The mean value of free T3 for euthyroid animals was 4.08pmol/l, standard error of the mean (SEM) = ± 0.31, number of observations = 12. All animals in the chronic hypothyroid group showed serum levels of free T3 below 0.9pmol/l (the lower limit of resolution of the analytical method) and the serum free T3 level of animals in the chronic hyperthyroid group (C) and T3 replacement group (E) was found to exceed 36pmol/l (the upper limit of resolution of the analytical method).

Serum calcium levels

No evidence of tetany was ever seen in any animal within this study.

The mean serum calcium level of each of the groups examined in this study was not significantly different from the 26 week age matched control group (A) mean value of 2.58 mmol/l (SEM) = ± 0.17 number of observations = 12.

Myosin Isoforms

The percentage composition of myosin isoforms are shown in table 2, and representative scans are shown in figure 1. Only two bands were ever seen in the Wistar soleus muscles used here and these corresponded to the two slowest moving bands numbered respectively 5 and 4 by [47]. When myosins from soleus and fast twitch extensor digitorum longus (E.D.L.) muscles from the same animals were co-electrophoresed the two soleus bands comigrated with the two slowest moving E.D.L. bands.

Refinements [15, 84] of the electrophoretic techniques used to separate isomyosins from Wistar and Sprague-Dawley strain slow-twitch rat soleus muscles have in the hands of various workers [3, 13, 14, 41, 42, 56, 57, 85] revealed a maximum of three slow myosin isoforms. Two bands of slow myosin which did not coelectrophorese with the fast isozymes were identified by [41, 42, 57], the most prominent and slowest moving of three bands was labelled SM_2, a faster moving and less prominent SM_1 was present plus the fastest moving, least prominent and most infrequently found band which was labelled SM_1'. Further work by [3] with Wistar strain rats and [33] using Sprague-Dawley rats has revealed a total of two slow myosins which were respectively labelled SM_2 or SM2 (these were the

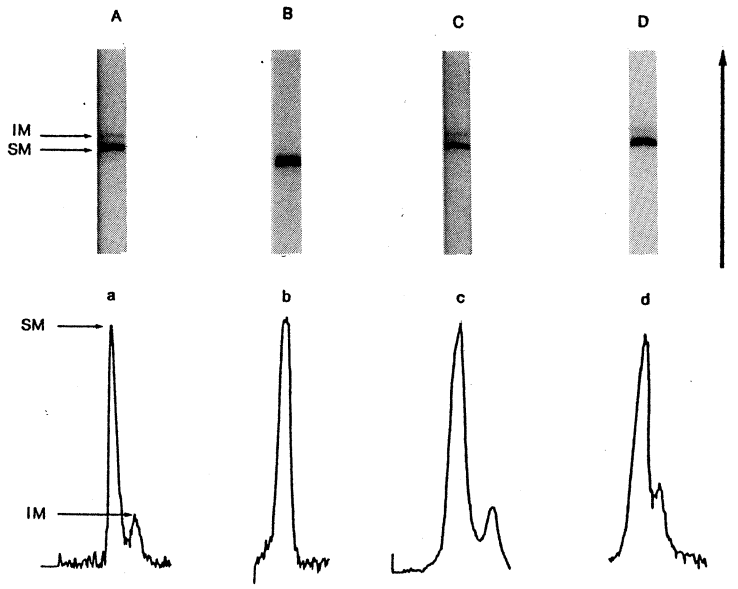

Figure 1. Electrophoretic gels and scans of native myosin in nondissociating conditions from the four groups. (The arrow shows the direction of migration) (A) Soleus muscle from a 26 week old euthyroid animal. (B) Soleus muscle from a chronic hypothyroid animal. (C) Soleus muscle from a chronic hyperthyroid animal. (D) Soleus muscle from a hypothyroid animals given T3 for 42 days.

slowest moving and most prominent bands), in addition SM_1 or SM1 was identified as a faster moving but less plentiful band and both groups identified an intermediate myosin labelled IM. Three slow myosins from Wistar strain slow-twitch soleus muscles have been identified by [85], the least prominent but fastest moving they called SM1 a light chain LC1sa homodimer; SM2 a light chain LC1sa/LC1sb heterodimer and the most prominent and slowest moving form identified as SM3 which was found to be a light chain LC1sb homodimer. It would seem reasonable to conclude that the band of slow myosin identified as isomyosin 5 in the work reported here is a composite band possibly including one or more of the slow myosins reported by [33, 56, 57, 85] and therefore will be referred to as slow myosin or SM.

Slow and fast-twitch muscles of small mammals contain varying proportions of histochemically defined type IIA fibres, from these muscles it is possible to extract an isomyosin which has been called intermediate myosin (IM) or FM3a [3, 13, 14, 24, 33, 34, 41, 42, 56, 57] or FM3a [85]. Light chain analysis of IM or FM3a from rat soleus muscles has shown the presence of light chains LC1f, LC2f and LC1s indicating that IM or FM3a could be LC1s homodimer a LC1f homodimer or an LC1s/LC1f heterodimer [85]. The most likely heavy chain composition of this type of myosin is that of an HCIIa homodimer but the possibility of heavy chain heterodimers has been raised [47, 71, 78, 87] though not demonstrated.

It has been pointed out by [47] that in rat soleus the proportion of total myosin identified as band 4 matched the proportion of histochemical type IIA fibres, the proportion of total muscle cross sectional area and total force output contributed by these fibres. This isomyosin was probably located in the mechanically intermediate motor units identified by [19] and which were confirmed as being histochemically type IIA fibres by [53, 54]. The results presented here show the percentage of total myosin represented by band 4 increased and decreased directly as did the percentage of type IIA fibres, in addition the percentage of mechanically defined intermediate motor units was altered as a result of altered thyroid status [1, 2, 64]. On the basis of this we have called band 4 intermediate myosin IM.

IM and SM were present in the soleus muscles of euthyroid control and chronic hyperthyroid animals whereas IM was absent from chronic hypothyroid soleus muscles, this finding agrees with those of [13, 14, 33]. The mean percentages of SM and IM myosins found in euthyroid muscles were respectively 88.5% ± 0.67 (SEM) n = 4 and 11.5% ± 0.67; these were similar to those reported by [3, 13, 14, 33, 41, 42, 56, 57, 85]. A larger amount of IM myosin (mean 17.2% ± 2.9 n = 4) and a smaller amount of SM (mean 82.8% ± 2.9 n = 4), when compared to euthyroid, was found in the soleus muscles of chronic hyperthyroid animals confirming the findings of [14, 33]. After giving T3 to hypothyroid animals for 42 days the percentage of IM was higher and that of SM lower than the respective percentages in muscles from euthyroid animals, the mean percentage of IM present was 17.5% ± 3.3 n = 4. This was not significantly different from that found in euthyroid muscles although it was increased by 1.53 times (range 11.6% - 26.9%). However analysis of variance followed by multiple range test revealed no statistically significant differences between the respective percentage mean values of IM and SM from soleus muscles of age matched euthyroid, chronic hyperthyroid and experimen-

tal group D animals. IM myosin was also found in the muscles of hypothyroid animals given T3 for 10 days (group E) mean = 5.2% ± 1.05 n = 4, it is inferred that IM myosin was absent from the muscles of groups D and E during the period the animals were hypothyroid and that it was induced as a consequence of T3 being given.

Mechanical properties

The mean values plus standard errors of T_C, $T_{T1/2R}$, V_{MAX}, MRR, for each of the groups are shown in the separate rows of Table 1. Mean values in each row which have been given the same letter superscript were not statistical different from each other, all other combinations of differences between mean values in each row were significantly different. Curves describing the relationship between applied load as a percentage of maximum tetanic force and maximum velocity of shortening measured in fibre lengths per second are shown in figure 2. Mean values of isometric and isotonic muscle contraction parameters obtained from the euthyroid, hypothyroid and hyperthyroid animals used in this study were similar to those previously reported [13, 14, 21, 31, 38, 51, 52, 61, 62]. Differences between T_C, V_{MAX}, $T_{1/2R}$ and MRR of chronic hypothyroid, chronic hyperthyroid and age matched euthyroid controls were well defined and statistically significantly different. No statistically significant differences were found between any of the groups in respect of twitch:tetanus ratio i.e. the ratio of twitch force per unit muscle cross sectional area/maximum tetanic force per unit cross sectional area. Altered thyroid status had no effect on muscle fibre number and the total soleus muscle fibre numbers of each group were not statistically significantly different from each other.

Muscles from chronic hypothyroid and hyperthyroid animals were respectively slowed and speeded when compared to euthyroid and the differences were statistically significantly different at P = 0.05 level of probability. It is clear from table 1 that the muscles from experimental group D hypothyroid animals given T3 for 42 days and those from group E given 18 days intraperitoneal T3 have speeded to the extent that isometric T_C, $T_{1/2R}$, and MRR and isotonic V_{MAX} were not significantly different from those of chronic hyperthyroid muscles. Muscles from animals in group E given T3 for 10 days were not as fast as those in the above two groups but had clearly speeded from their hypothyroid state such that they resembled soleus muscles from euthyroid animals. The reasonable inference is made that during the period when the animals in groups D and E were hypothyroid they had slowed mechanical responses which were speeded as a consequence of the T3 given. The time course is such that it took 10 days of T3 to restore the animals to the euthyroid state and a further 7 days to induce a condition very close to that observed in the hyperthyroid animal.

Histochemical Fibre Type Proportions

Differences between the proportions of type I, type IIA and IIC fibres of chronic hypothyroid, hyperthyroid and age matched euthyroid controls were also well defined and shown in table 2. Hypothyroid animals had soleus muscles in which the proportions of types IIA and IIC fibres was reduced to almost zero and the proportion of type I fibres increased whereas muscles from chronic hyperthyroid animals had an increased proportion of IIA and IIC fibres and a decreased proportion of type I fibres, this finding was in agreement with previous observations [12, 48, 51, 52, 62, 66, 67, 69]. Although type IIC fibres were seen in the study reported here the percentage was not as high as that reported by Fitts et al, the reason for this difference is unclear.

Soleus muscles from animals given T3 for 42 days and those from group E given T3 for 18 days when compared to group hypothyroid group B muscles showed statistically significant increases in the percentages of type IIA and IIC

Table. 1. Contractile properties.

Measured Parameter	Age Matched Euthyroid Group A	Chronic Hypothyroid Group B	Chronic Hyperthyroid Group C	Hypothyroid Plus 42 days T3 Group D	Hypothyroid With i.p. T3 Group E 10 Days	Hypothyroid With i.p. T3 Group E 18 days
T_C ms	34.2 ± 0.8^{b} n = 12	72.4 ± 2.7 n = 8	21.7 ± 0.7^{a} n = 8	22.7 ± 2.0^{a} n = 4	33.3 ± 1.1^{b} n = 8	25.5 ± 0.7^{a} n = 4
$T_{1/2R}$ ms	50.3 ± 1.9^{b} n = 12	125.9 ± 8.9 n = 8	28.4 ± 2.7^{a} n = 8	$33.9 \pm 4.2^{a\,c}$ n = 4	$44.9 \pm 1.8^{b\,c}$ n = 8	$32.8 \pm 2.3^{a\,c}$ n = 4
MRR $\%P_0 \cdot ns^{-1}$	2.5 ± 0.1 n = 12	1.7 ± 0.1 n = 8	$3.7 \pm 0.3^{a\,c}$ n = 8	$3.8 \pm 0.3^{a\,c}$ n = 4	3.1 ± 0.15^{b} n = 8	$3.4 \pm 0.2^{b\,c}$ n = 4
V_{MAX} $FL.s^{-1}$	9.0 ± 0.2^{b} n = 12	7.2 ± 0.2 n = 8	13.2 ± 1.2^{a} n = 7	12.7 ± 0.9^{a} n = 4	10.4 ± 0.4^{b} n = 7	12.5 ± 0.3^{a} n = 4

The mean ± Standard Error of the Mean (SEM) and n the number of observations are shown separately in each row of the table. Differences between means in each row with the same letter superscripts were not statistically significantly different at P = 0.05. All other combinations of differences between mean values in each row were statistically significant.

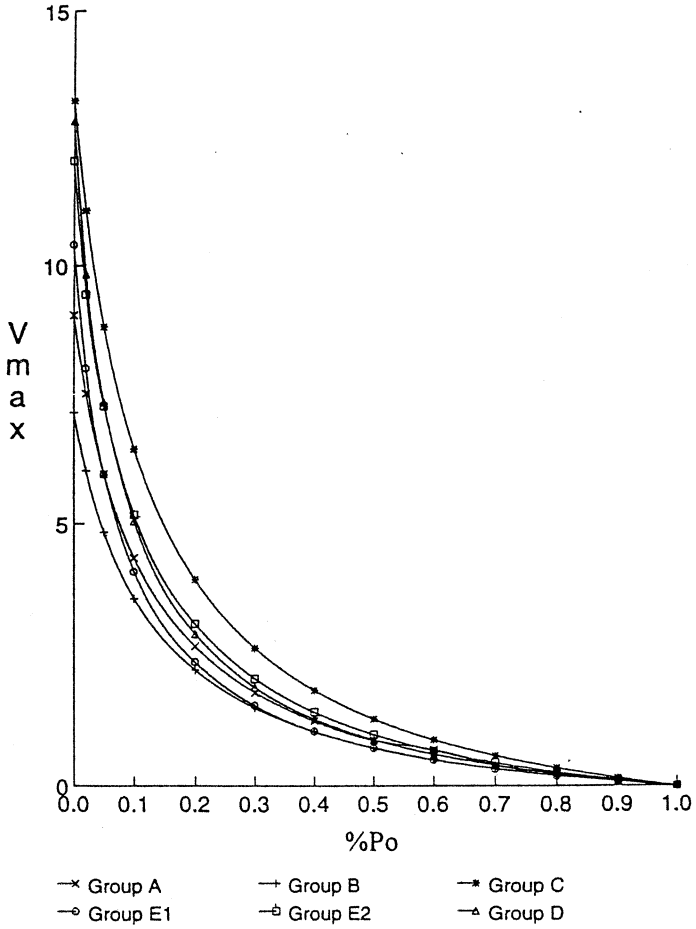

Figure 2. Shows the maximum shortening velocities in fibre lengths per second ($FL.s^{-1}$) on the ordinate, plotted against normalised load (P/Po) on the abscissa. A. Euthyroid group A (n = 12); B. Chronic hypothyroid group B (n = 8); C. Chronic hyperthyroid group C (n = 7); D. 42 days T3, group D (n = 4); E1 18 days i.p. T3 (n = 4) group E; E2. 10 days (group E) (n the number of observations is shown in parenthesis). The SEM bars at each load point on each of the curves have been omitted for reasons of visual clarity only.

fibres with concomitant decreases in the percentage type I fibres. Muscles from group D animals given T3 for 42 days could not be statistically distinguished from those in chronic hyperthyroid group C. Despite the obvious increase in the proportion of IIA fibres muscles in the 18 day T3 group E this group could not be distinguished statistically from the muscles of group A euthyroid animals. Those given T3 for 10 days also showed an increase in the percentage of IIA fibres but the mean value for this group was not statistically significantly different from hypothyroid group B muscles.

In summary these results show that slow-twitch soleus muscles from chronic hypothyroid animals contained almost 100% type I slow-twitch oxidative fibres, only one slow myosin isoenzyme band and slowed isometric and isotonic contractions. Hypothyroid animals given T3 showed speeded isometric and isotonic responses with increased percentages of type IIA and IIC fibres and intermediate myosin was found to be present in addition to slow myosin. Muscles from both groups given T3 for 10 and 18 days developed speeded isometric and isotonic responses although the histochemical and myosin isoform profiles of the former resembled the muscles of hypothyroid animals and those of the latter resembled muscles from euthyroid animals.

Discussion

The findings presented here demonstrate that biochemical, histochemical and contractile changes known to occur in the soleus muscles of chronic hypothyroid animals can be reversed by giving T3 for various lengths of time.

We are in agreement with [57] that considerable between muscle variation exists in respect of the proportions of slow and intermediate myosins in rat slow-twitch muscles and we have, like [85], seen older euthyroid Wistar animals with soleus muscles in which neither IM nor type IIA fibres were present. Further variation with respect to the differences in heavy chain composition of single muscle fibres and histochemical fibre types taken from Sprague-Dawley and Wistar strains has been pointed out [85, 87].

Adaptive transformation of rat soleus muscle during growth has been described [54] for albino rats (the strain was not stated) and this process resulted in a transformation of type IIA to type I fibres with slowing of motor unit mechanical properties as animals aged. There were no significant changes in motor unit innervation ratios thus a genuine transformation must have occurred. By 34 weeks of age adaptive transformation had taken place to such an extent that the percentage of type I fibres and slower contracting motor units had reached 90% of the total. It is not clear what underlies the process of adaptive transformation, it has been suggested [54] that such changes are unlikely to predetermined far into adult life and probably results from altered motorneurone activity which in turn alters the motor unit muscle fibre mechanical and histochemical profiles. Another possible explanation could be that the levels of circulating thyroid hormones in older animals have declined to such an extent that contractions become slower and type I fibres predominate. Certainly thyroid hormone levels are at their highest in the first 3 to 4 weeks of life and then decline to adult levels. A further explanation of adaptive transformation could be that type IIA fibres become less sensitive to thyroid hormone as the animals age but there is no evidence to support this idea. Clearly the percentage of fibre types and speed of contraction of motor units depend not only on thyroid status but also on strain and age of the animal. Any alterations in contractility, histochemical and myosin isoform profiles that would be brought about as a result of altered thyroid status must be set against the background of changes attributable to adaptive transformation.

The present work showed that IM is absent from the soleus muscles of hypothyroid animals and confirms previous findings [13, 14, 33]. After 10 daily doses of T3 IM

Table. 2. Histochemical and myosin isoform results.

	Age Matched Euthyroid Group A	Chronic Hypothyroid Group B	Chronic Hyperthyroid Group C	Hypothyroid Plus 42 days T3 Group D	Hypothyroid Plus i.p. T3 Group E 10 Days T3	18 daysT3
% Type I	84.3 ± 2.2^{b} n = 9	98.4 ± 0.9^{a} n = 8	59.0 ± 0.5 n = 8	67.9 ± 2.6 n = 9	94.2 ± 1.1^{a} n = 6	80.9 ± 2.3^{b} n = 4
% Type IIA +IIC	15.7 ± 2.2^{a} n = 9	1.6 ± 0.9^{b} n = 8	41.0 ± 0.5^{c} n = 8	32.1 ± 2.6^{c} n = 7	5.8 ± 0.1^{b} n = 6	19.1 ± 2.3^{a} n = 4
% SM Myosin isoform	88.5 ± 0.7^{a} n = 4	100^{b} n = 4	82.8 ± 2.9^{a} n = 4	82.5 ± 3.3^{a} n = 4	94.8 ± 1.1 n = 4	
% IM Myosin isoform	$11.5 \pm 0.7^{a\,c}$ n = 4	0^{b} n = 4	17.2 ± 2.9^{a} n = 4	17.5 ± 3.3^{a} n = 4	$5.2^{b\,c} \pm 1.1$ n = 4	

The mean ± Standard Error of the Mean (SEM) and n the number of observations are shown separately in each row of the table. Differences between means in each row with the same letter superscripts were not statistically significantly different at P = 0.05. All other combinations of differences between mean values in each row were statistically significant.

had reappeared and this time course is consistent with that described here for the changes in isometric and isotonic contractions and is in agreement with previous findings [62]. Evidence of the induction of adult fast myosin heavy chains with a similar time course to that described above has come from the work of [72, 89]. Working with dwarf mice they showed a modest change in skeletal myosin composition during the first six days of T3 replacement with an increased rate of appearance after this. Further evidence of the rapid induction of myosin heavy chains by thyroid hormone comes from the work of [49] who showed the induction of fast MHCIIa mRNA in rat soleus muscle after 8 daily doses of T3 (50 µg/Kg). A more rapid T3 induced increase in myosin heavy chain mRNA'S in hypothyroid slow-twitch muscle has been reported by [44], who injected hypothyroid rats with T3 (20 µg\Kg \ 24 hours), they found that fast IIA mRNA has increased by 75% 1 week after commencement of T3.

Muscles from hypothyroid animals given T3 for 42 days and muscles from chronic hyperthyroid animals had both IM and SM present and were indistinguishable from each other in terms of isoenzyme, fibre type proportions and contractile properties. After giving T3 to hypothyroid animals for 10, 42 and 18 days soleus muscle contractile properties respectively resembled those of euthyroid and hyperthyroid animals however the mean percentage IM present after T3 replacement was not significantly different from euthyroid muscle although it was increased by 1.5 times in the 42 day T3 animals. We are presented with a situation in which the soleus muscles of animals that had been given T3 were mechanically and histochemically speeded to resemble those of respectively euthyroid and hyperthyroid animals but the proportion of IM and fibre types were those more characteristic of euthyroid muscles. The speeding of isometric and isotonic properties which preceded changes in histochemical fibre type proportions is similar to that previously described [66, 67]. Evidence indicating a stiffening of the elastic elements of the pre-existing type I fibre population of euthyroid animals given T3 has been put forward to provide an explanation [67] of the observation that early changes in isometric mechanical properties precede histochemical changes. A possible corollary of this could be that a proportion of the slowing of soleus muscles from hypothyroid animals could be attributable to a decrease in stiffness of the series elastic elements [18]. There is evidence to support the idea that all preexisting type I fibres are slowed in hypothyroid animals. If one considers the difficulty in explaining how T_C can increase from the euthyroid value of 34.2 ms to the hypothyroid value of 72.4 ms with the conversion of only 15.7 % type IIA and IIC fibres to type I fibres one is forced to the conclusion that unless all the preexisting type I fibres were also slowed the changes in T_C could not have come about. Slowing of all motor units has been shown to be the case for soleus muscles from hypothyroid animals [2] here it was observed that all motor units were slowed but there was still a range of contraction times with some units being faster than others. The ranges of contraction times obtained from euthyroid and hypothyroid animals did not overlap and a decrease in stiffness of series elastic elements could explain the whole or part of this. Giving T3 to euthyroid Wistar rats resulted in an increase of 30% in the number of faster contracting motor units, [63] unlike hypothyroid situation the range of motor unit T_C overlapped with those from euthyroid animals. It is therefore not clear what role a generalised increase in series elastic stiffness would play in this situation.

It has been shown [13, 14, 48, 51, 52, 69] that chronic hypothyroid soleus muscles, with only SM present, had lower actin and Mg^{2+}-activated myosin ATPase activity than age matched euthyroid muscles with both SM and IM present. V_{MAX} is correlated with actin and Mg^{2+}-activated

myosin ATPase activity, this means therefore that SM has a lower actin activated myosin ATPase activity than IM and this accompanied the lower V_{MAX} of chronic hypothyroid animals. The increased percentages of IM and type IIA fibres found in the muscles of those given T3 for 10, 18 and 42 days were accompanied by an increased V_{MAX}; muscles from chronic hyperthyroid animals also showed increased V_{MAX} and are known to have increased actin and Mg^{2+} activated myosin ATPase activity with a concomitant decrease in the percentage of SM and type I fibres. It is also known that myosin heavy chain HCIIA is deinduced in hypothyroid rat soleus muscles [13, 14, 44, 49] and the slow heavy chain HC1 is upregulated, in addition SM from hypothyroid soleus muscles contains light chain LC1s and LC2s in equimolar proportions [51, 52]. If we ignore possible LC1sa or LC1sb heterogeneity the subunit composition of SM from soleus muscles of hypothyroid animals would therefore consists of two slow heavy chains, two LC2s and two LC1s light chains $(MHC1)_2(LC1s)_2(LC2s)_2$. This SM has the lowest maximum isotonic shortening velocity and actin activated Mg^{2+} myosin ATPase activity and was found in type I fibres.

Soleus muscles from euthyroid Wistar rats contained about 20% type IIA fibres and have been shown to contain fast light chains LC1f, LC2f, [47, 51, 52] Sprague-Dawley contained fast light chains LC1f, LC2f, and a small amount of LC3f [13, 14, 85]. Soleus muscles from hypothyroid Wistar and Sprague-Dawley rats contained less than 2% type IIA fibres and fast light chains had almost disappeared [13, 14, 32, 51, 52, 69]. Since there were no significant changes in muscle fibre number in soleus muscles from hypothyroid animals we conclude that in the absence of T3 fast myosin light chains must have been replaced by slow light chains. Although we have not measured light chain composition in this study it seems reasonable to put forward the suggestion that giving T3 to hypothyroid animals induced fast light chains in their soleus muscles. This is supported by the observation that an increase in the percentage of fast light chains occurred in the soleus muscles of euthyroid animals given T3 [13, 14, 32, 69]. We might expect that soleus muscles from hypothyroid animals given T3 for 10, 18 and 42 days will, in addition to an increased proportion of the fast light chains LC1f and LC2f, show an increased proportion of fast MHCIIa heavy chains and increased actin and Mg^{2+} activated myosin ATPase activity. The sum of these changes resulted in soleus muscles that had speeded maximum shortening velocities, increased proportions of IIA fibres and IM. The observations with respect to the presence of LC1f and LC2f in IM confirms those of others about a possible subunit composition for IM [3, 23, 34, 42, 47, 85, 88]. Although our results do not allow us to be more specific about the subunit composition of IM if the faster light chains associate more readily with MHCIIa heavy chains then it is likely that T3 induced IM has the subunit composition $(MHCIIa)_2(LC1f)_2(LC2f)_2$ although the possibility that IM found could be hybrid of a slow-twitch and fast-twitch heavy chain with their appropriate slow and fast light chains has been explored [47, 82].

Fast myosin light and heavy chains have been induced in soleus muscles of chronic hyperthyroid 10, 18, and 42 day T3 animals, would the induction of these faster myosin isoforms be likely to have brought about the observed speeding of the maximum rate of isotonic shortening V_{MAX}? There is general agreement that in a variety of developmental states and experimental conditions both in skeletal and cardiac whole muscles and single fibres a continuum of relationship exists between heavy chain composition and the isotonic shortening velocity. The larger the proportion of fast heavy chains the faster isotonic shortening velocity (V_{MAX} or V_0) will be and the smaller the proportion of fast heavy chains the slower isotonic shortening velocity will be. There is evidence to suggest that the ratio of the amounts of light chains LC1f:LC3f is important in determining the shortening velocity of single histochemically defined type IIB fibres from rabbit muscles [80, 81] [40, 65] and that the presence or absence of light chain LC2f also determines the velocity of shortening of single fibres.

It has been pointed out by [9, 65] that there was a continuum of V_0 amongst single fibres from rat and rabbit fast and slow twitch muscles and within this continuum only slow fibres could be unequivocally identified by their velocity of shortening. Further variation in V_0 despite uniform MHC1 heavy chain content in rabbit soleus fibres in the work of [73] has been referred to by [9], these authors put forward two hypothesis to explain the sources of variation. The first was that fibres shorten at different velocities because they contain different MHC isoforms, it is now clear that the suggestion that both fast and slow myosins can coexist within single muscle fibres [70] is correct as some adult single muscle fibres have been found to contain two or more types of heavy chains and light chains [6, 8, 39, 76, 77] [8, 78, 79, 86]. Developing muscles coexpress both fast and slow heavy myosins [35, 74] as do histochemically identified type C fibres [6, 7, 75]. The second hypothesis suggests that contractile proteins other than MHC's are at least partially responsible for the variability in maximum shortening velocity.

The mechanical, biochemical, histochemical and myosin heavy and light chain studies so far carried out on soleus muscles from hypothyroid animals provides a unique model for helping to unravel the interactions and contributions of the varies elements that go to make up the functional responses of this adaptive tissue and lend support to the second hypothesis of [9] that contractile proteins other than MHC's can be partially responsible for variations in maximum isotonic shortening velocity. V_0 of soleus muscles from hypothyroid animals is about 18% faster than V_{MAX} [13, 14] which indicates that despite the homogeneity of fibre types and light and heavy chains some fibres contract more quickly than others. Again despite this homogeneity all motor units in soleus muscles from hypothyroid animals were slowed [2] but to differering extents

since a range of T_C, $T_{1/2R}$, and MRR was still apparent; the ranges of these parameters did not overlap with those of euthyroid animals. What proteins might be responsible?

That sarcoplasmic reticulum (SR) has a role to play in reducing the free Ca^{2+} concentration of the cytosol and thus relaxation has long been accepted but whether or not the rate of Ca^{2+} accumulation is fast enough to bring about the *in vivo* observed rates of relaxation has been debated [20]. It has been pointed [36] out that it is probably the initial and transitory rate of Ca^{2+} uptake by the SR that is reflected in $T_{1/2R}$ as probably the best physiological measure of relaxation. Thyroid hormone induced changes in the rate of calcium handling by the SR have been reported, [30] here it was shown that the calcium accumulating ability of SR from thyroidectomized rat fast-twitch whole hind limb muscle and fast-twitch tibialis anterior and extensor digitorum longus muscles was reduced. [69] showed respective decreases and increases in calcium accumulation by isolated sarcoplasmic reticulum (SR) from soleus muscles of chronic hypo and hyperthyroid animals, these results confirmed those of [31] who also showed increases in the initial rate of Ca^{2+} uptake by SR and Ca^{2+} stimulated SR ATPase of chronic hyperthyroid muscles. Thyroid hormones have been shown to have an effect on one of the subunits of a regulatory protein involved in Ca^{2+} binding in rat soleus muscles. Thus hypothyroidism speeds the normal transformation of fast to slow isoforms of troponin I, the subunit responsible for the inhibition of the actin myosin interaction [26].

A hypothesis to explain the speeding effects of T3 on slow-twitch soleus muscle from hypothyroid animals could lie in the very early induction, in an increasing proportion of some soleus muscle fibres but not all, of an increase in the initial and transitory rate of Ca^{2+} uptake by the sarcoplasmic reticulum which was functionally linked to the changes in contractile proteins. Thus the proportion of fast isoforms of troponin I would also increase as would the proportion of fast MHCIIa heavy chains and fast light chains in some but not all fibres, these would have higher actin and Mg^{2+} activated ATP'ase activities. Initially T_C $T_{1/2R}$ and MRR would increase followed by V_{MAX} and V_0. Single fibre measurements of myosin ATPase activities and sarcoplasmic reticulum calcium handling in the early stages of T3 replacement correlated with information on myosin and troponin isoforms, would be particularly useful in this context.

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