The effects of hypo- and hyperthyroidism on fibre composition and mitochondrial enzyme activities in rat skeletal muscle

R. B. Lomax and W. R. Robertson
Department of Medicine, University of Manchester, Clinical Sciences Building, Hope Hospital, Eccles Old Road, Salford M6 8HD, U.K.

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ABSTRACT

Hypo- and hyperthyroidism have been associated with changes in the activities of mitochondrial enzymes in homogenates of skeletal muscles, but it is unclear whether such changes were due to changes in single fibre enzyme activities or to previously documented changes in relative numbers of fibres. In this study the activities of the mitochondrial enzymes α-glycerol phosphate dehydrogenase (m-αGPDH) and succinate dehydrogenase (SDH) were measured in single fibres of the soleus and gastrocnemius muscles of the rat by cytochemical assays.

In the soleus muscles of hypothyroid animals there was a decrease in the mean percentage (± s.d.) of type II fibres from 8.0 ± 6.0 to 0.8 ± 1.9% (P < 0.05) and decreases in SDH activities in all fibre types (P < 0.005). In the gastrocnemius muscles of these animals there were no changes in fibre composition but type IIB fibres had reduced (P < 0.05) m-αGPDH activities. In the hyperthyroid animals, in which body weight had increased relative to the euthyroid animals, there were increases in the percentages of type I and type II fibres in the soleus from 4.3 ± 1.7 to 13.1 ± 9.0% (P < 0.05) and from 9.6 ± 7.2 to 33.4 ± 9.6% (P < 0.005) respectively and an increase in the percentage of type IIA fibres in the gastrocnemius from 92.9 ± 2.3 to 97.0 ± 2.9% (P < 0.05). However, there were no increases in single fibre mitochondrial enzyme activities. It is therefore suggested that the administration of moderate, growth-promoting doses of thyroid hormones to euthyroid animals can cause changes in muscle fibre composition without stimulating the activities of mitochondrial enzymes.

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INTRODUCTION

Changes in the physiology and biochemistry of skeletal muscle have been well documented in both clinical hypo- and hyperthyroidism (Ramsay, 1966; McKeran, Slavin, Andrews et al. 1975; Khaleeli, Griffith & Edwards, 1983). In particular, Ramsay (1966) recorded that in hypothyroidism the proximal muscles involved in the maintenance of posture were most commonly affected, and these were all red muscles rather than white ones. Red muscles are characterized by having highly active Krebs' cycle metabolism and by containing a high proportion of slow-twitch, type I fibres whereas white muscles have high glycolytic capacities and a high proportion of fast-twitch, type II fibres (Kubista, Kubistova & Pette, 1971).

In the rat, changes in thyroid status have also been linked with changes in the metabolism of red muscles, namely in the soleus where hyperthyroidism is associated with a transformation of type I fibres into type II fibres and with increases in the activities of mitochondrial enzymes in homogenates of the whole muscle. Hypothyroidism, on the other hand, is associated with the reverse changes (Ianuzzo, Patel, Chen et al. 1977; Johnson, Mastaglia & Montgomery, 1980).

In white muscles, hyperthyroidism does not stimulate mitochondrial enzyme activities (Winder & Holloszy, 1977; Janssen, van Hardeveld & Kassenaar, 1978); such insensitivity may be due to the fact that they are composed of mainly type II fibres in which mitochondrial enzymes may already be maximally induced (Kubista et al. 1971). Other possible explanations for the differential sensitivities of red and white muscles to thyroid hormones include differences in innervation, blood supply and local thyroid hormone metabolism (Janssen et al. 1978).
In order to determine whether such changes in enzyme activities of the whole muscle homogenates are due wholly to changes in muscle fibre composition or reflect true changes in mitochondrial enzyme activities within single fibres, scanning microdensitometry was used to quantify reaction product in tissue sections (Lomax & Robertson, 1990). In this way cytochemical assays were used to measure the activities of succinate dehydrogenase (SDH) and mitochondrial α-glycerol phosphate dehydrogenase (m-αGPDH), good indicators of Krebs' cycle and glycolysis-associated activities respectively (Pette, 1966), in different fibre types in the gastrocnemius, a typical white muscle, and in the soleus, a typical red muscle.

MATERIALS AND METHODS

All chemicals were obtained from Sigma Chemicals, Poole, Dorset, U.K. unless otherwise stated.

Animals and tissue preparation

Male adult Sprague–Dawley rats were housed at 21 ± 2 °C with a 12 h light:12 h darkness cycle and with standard chow (Labshore CRM, Cambridge, U.K.) available ad libitum. Hypothyroidism was induced in six rats by the inclusion in the drinking water of n-propylthiouracil (PTU; 0·05%; BDH Ltd, Poole, Dorset, U.K.) for 8 weeks (Ladensen, Kieffer, Farwall & Ridgway, 1986). Hyperthyroidism was induced in five rats by the presence in the drinking water of L-thyroxine (4 mg/l, made from a stock 4 mg/ml solution) for 6 weeks (Argov, Renshaw, Boden et al. 1988). Control rats (6) were given normal tap water, and all animals were killed at the age of 16 weeks by decapitation after being anaesthetized with ether.

Blood was collected into lithium-heparinized tubes and the plasma was snap-frozen in solid carbon dioxide and stored at −70 °C. Total triiodothyronine (T3) and total thyroxine (T4) were measured by standard radioimmunoassay of duplicate plasma samples (50 μl) using antisera raised in sheep (T3 antisera from Withington Hospital, Manchester, U.K.; T4 antisera from Scottish Antibody Production Unit, Carluke, Lanarkshire, U.K.) and 125I-labelled hormones (Amersham International plc, Amersham, Berks, U.K.). Intra- and interassay coefficients of variation were <7% and <10% over the ranges 0·5–8·0 nmol/l for T3 and 10–300 nmol/l for T4. Thyroid-stimulating hormone (TSH) was measured by radioimmunoassay using materials from the National Hormone and Pituitary Program, Bethesda, MD, U.S.A., including lyophilized rat pituitary standard (NIADDK-rTSH-RP-2), rat TSH antigen (NIADDK-rTSH-I-8) for iodination and rat TSH antiserum (NIADDK-anti-rTSH-S-5). Intra- and interassay coefficients of variation were <8% and 12% respectively over the range 3–30 μg/l.

Calf muscles were removed, coated in Dulbecco's phosphate-buffered saline containing 5% polyvinyl alcohol (grade G18/140 from Wacker Chemie, Munich, Germany), chilled to −70 °C in n-hexane ('free from aromatic hydrocarbons grade' from BDH) and stored for less than 2 weeks before sectioning. Serial transverse sections were cut in a Bright cryostat with a cabinet temperature of −30 °C and a knife temperature of −70 °C as described by Chayen, Bitskys & Butcher (1973).

Fibre classification and measurement of fibre diameter

Muscle fibres were classified in sections reacted for myofibrillar ATPase after formaldehyde fixation (Hayashi & Freiman, 1966). Sections were thawed, left for 1·5 h at 4 °C in methanol-free formaldehyde fixative and rinsed in distilled water. They were then incubated for 1 h at 37 °C in Tris–HCl buffer (0·1 mol/l; pH 9·4) containing CaCl2 (0·018 mol/l) and Na2ATP (1·5 mg/ml), and subsequently washed twice in 1% CaCl2 solution, once in 2% Co(NO3)2 solution and three times each in tap water and distilled water. Finally the sections were stained in 1% ammonium polysulphide solution for 1 min, washed again and mounted in Farrant's medium (Raymond Lamb Ltd, North Acton, London, U.K.). Comparison with serial sections reacted for myofibrillar ATPase after pre-incubation in acid and in alkali as detailed by Staron & Pette (1987) has shown that after the above protocol type I fibres in the soleus were unreacted, type II fibres were heavily reacted and type IC fibres had intermediate reaction. In the lateral region of the gastrocnemius muscle type IIA fibres were poorly reacted and type IIB fibres were heavily reacted (Lomax, 1990).

Lesser fibre diameter (Dubowitz, 1985) was measured using an Olympus BH-2 microscope linked to a Kontron MOP-Videoplan computer and a TV monitor (the ×10 objective was used and the 'zoom' facility set to 0·55). Individual fibres (at least 200 per muscle; one section from each animal) were outlined by pen on a pressure-sensitive tablet.

Measurement of mitochondrial enzyme activities in single muscle fibres

Sections (7·5 or 5 μm) of calf muscles were reacted for m-αGPDH or SDH respectively at 37 °C for 2 min. During this period initial rates of formazan production persisted (Lomax, 1990). The reaction medium for m-αGPDH was as validated previously (Lomax & Robertson, 1990) except for the replacement of intracellular electron acceptor (phenazine methosulphate;
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PMS) by methoxy-PMS and the inclusion of sodium azide, namely glycyglycine (0·1 mol/l) buffer at pH 7-4 containing G18 grade polyvinyl alcohol (2-5%), L-a-glycerophosphate (0 or 10 mmol/l), terminal electron acceptor (nitro-blue-tetrazolium; NBT; 1-2 mmol/l), CaCl₂ (1 mmol/l), methoxy-PMS (1 mmol/l from Serva Fine Biochemicals, Heidelberg, Germany) and sodium azide (10 μmol/l). The reaction medium for SDH was as validated by Blanco, Sieck & Edgerton (1988) except for the inclusion of polyvinyl alcohol, namely phosphate (0·1 mmol/l) buffer at pH 7-6 containing G18 polyvinyl alcohol (2-5%), sodium succinate (0 or 4·8 mmol/l), NBT (1·5 mmol/l), EDTA (5 mmol/l), methoxy-PMS (1 mmol/l) and sodium azide (0·75 mmol/l). The reactions were stopped by immersion in ice-cold acetate (0·05 mol/l) buffer at pH 6, and sections were washed in tap water and mounted with Farrant’s medium.

Enzyme activities were measured in single fibres of the soleus or the lateral region of the gastrocnemius by quantitation of the formazan reaction product with a M85A Scanning and Integrating Microdensitometer (Vickers Instruments, York, Yorks, U.K.). An A5 scanning mask was placed over the interfibrillar area of each fibre using the × 40 objective, and the absorbance at 585 nm was measured using a slit width of 20 nm, a size 1 spot (diameter 0·5 μm) and a 4 s scan time. In each section ten fibres of each fibre type which had been classified in a serial section reacted for myofibrillar ATPase were scanned and the mean was calculated. Four sections were incubated for 2 min in the presence of substrate, and from the mean absorbance of these sections (derived from the four means for each section) was subtracted the mean absorbance of four sections incubated for 2 min in substrate-free medium. Enzyme activities were expressed as mean change in absorbance/min. Data are expressed as mean ± S.D. and were compared by unpaired r-test.

**RESULTS**

Effect of thyroid state on body weight and serum T₃, T₄ and TSH concentrations

At death (age 16 weeks) mean body weight for the hypothyroid animals (308 ± 21 g, n = 6) was reduced (P < 0·005) compared with mean weights of the euthyroid (500 ± 35 g, n = 6) and hyperthyroid (535 ± 30 g, n = 5) animals. In serum from the euthyroid animals the mean concentrations of total T₃, total T₄ and TSH were 1·3 ± 0·2 nmol/l, 67·7 ± 21·6 nmol/l and 2·1 ± 0·3 μg/l respectively. In hypothyroidism these parameters were altered (P < 0·001) to 0·6 ± 0·1 nmol/l, 20·9 ± 1·6 nmol/l and 32·0 ± 5·7 μg/l respectively, and in hyperthyroidism total T₃ concentration was elevated (P < 0·01) to 47·7 ± 2·5 nmol/l, total T₄ concentr-

**Effect of thyroid state on fibre diameter and composition**

In the hypothyroid soleus muscles there were reductions in the mean diameters of types I and IC fibres from 58 ± 6 to 46 ± 4 μm (P < 0·005) and from 47 ± 6 to 37 ± 5 μm (P < 0·05) respectively, but mean type II diameter was unchanged (51 ± 10 μm). In the hypothyroid gastrocnemius the mean diameters of types IIA and IIB fibres were reduced (P < 0·005) from 65 ± 7 to 51 ± 3 μm and from 52 ± 5 to 41 ± 2 μm respectively. Hyperthyroidism was associated with no changes in fibre diameter in either muscle.

In the soleus muscles hyperthyroidism was associated with a reduced (P < 0·005) mean percentage of type I fibres and increased percentages of type IC (P < 0·05) and type II (P < 0·005) fibres. However, hypothyroidism was associated with a reduction (P < 0·05) in the percentage of type II fibres in the soleus (Plate and Text-fig. 1). In the gastrocnemius muscles of the hyperthyroid animals there was a mean increase (P < 0·05) in the percentage of type IIA fibres and a corresponding decrease in the percentage of IIB fibres (Text-fig. 2). Hypothyroidism was associated with no changes in gastrocnemius fibre composition.

**TEXT-Figure 1.** Soleus fibre composition in hypothyroid (hatched bars), euthyroid (open bars) and hyperthyroid (shaded bars) states. Data are expressed as mean percentage composition ± s.d. of six hypo- or euthyroid or five hyperthyroid animals. *P < 0·05, **P < 0·005 compared with euthyroid group (unpaired r-test).

Effect of thyroid state on mitochondrial enzyme activities

SDH activity was decreased (P < 0·005) in all three fibre types of the soleus in hypothyroidism, but there
were no changes in SDH activity within the soleus in hyperthyroidism (Text-fig. 3). In the hyperthyroid gastrocnemius SDH activity decreased in type IIB fibres \( (P < 0.05) \) but no other changes were apparent (Text-fig. 4).

M-\( \alpha \)GPDH activity in type IIB fibres of the gastrocnemius fibres was reduced \( (P < 0.05) \) in hypothyroidism but in hyperthyroidism there were no changes in the activity of this enzyme in any fibres in the gastrocnemius (Text-fig. 5). M-\( \alpha \)GPDH activity was undetectable in any fibres of the soleus in all three thyroid states.

DISCUSSION

Animals treated with \( T_4 \) and with PTU were shown by serum hormone measurements to be hyperthyroid and hypothyroid respectively. Body weight was decreased
in the hypothyroid animals relative to the euthyroid animals and was slightly elevated in the hyperthyroid animals. These results are in accordance with the observation that when the adult euthyroid rat is given moderate supplementary doses of thyroid hormones growth is stimulated (Gustafsson, Tata, Lindberg & Ernser, 1965; Argov et al. 1988).

In this study, hypothyroidism was associated with decreases in fibre diameter in both the soleus and gastrocnemius. It has previously been shown in these muscles of the rat that fibre size increases as a function of muscle weight (Capo & Sillau, 1983). Therefore, when the changes in body weight described above are borne in mind it seems likely that such reduced fibre diameters are at least partly due to an inhibition of muscle growth in the hypothyroid rat. It is unclear how these changes relate to clinical hypothyroidism where type I fibre enlargement and type II fibre atrophy have been observed in the vastus lateralis muscle (McKeran et al. 1975; Khalaeli et al. 1983).

This study confirmed previous reports of increases in the percentages of type II fibres in the soleus going from hypo- to euthyroidism and from eu- to hyperthyroidism (Johnson et al. 1980; Capo & Sillau, 1983). However, a novel finding was that in hyperthyroidism the percentage of type IC fibres increased as the percentage of type I fibres decreased. This observation was possible because the formaldehyde fixation method of myofibrillar ATPase reaction used in this study allowed sub-classification of fibres from the main class I. Since type IC fibres contain both type I and type II forms of heavy chain myosin (Staron & Pette, 1987) it seems possible that they are in transition between these two classes.

With regard to the measurement of mitochondrial enzyme activities in single fibres, in this study the scanning microdensitometer sampled only the inter-fibrillar mitochondria in the core region of muscle fibres, and not the subsarcolemmal mitochondria located in the fibre periphery. Mitochondria from these two subcellular locations might possibly respond to thyroid hormones in different ways. However, in hypothyroidism the reductions in SDH activity in all fibres of the soleus and the lack of change in SDH activity in either fibre type of the gastrocnemius mirror changes previously described in homogenates (Ianuzzo et al. 1977; Fasshauer et al. 1978). Hyperthyroidism, however, did not lead to an increase in single fibre enzyme activity in either muscle. Increases in SDH activity described in the soleus homogenate might be due to increases in the percentages of ‘SDH-rich’ type IC and II fibres in this muscle, so that only relative numbers of fibres, and not their individual enzyme activities, could be responsible for the increase. An alternative explanation for the lack of change in single fibre enzyme activities while serum hormone concentrations and muscle fibre compositions have changed markedly is that these may be the effects of severe and mild hyperthyroid states respectively. Since the model of hyperthyroidism used in this study caused an increase in rat body weight rather than a reduction as seen with more severe models, this implies that skeletal muscle responds to thyroid hormones in a biphasic manner. It is interesting to note that while the effects of thyroid hormones on soleus fibre composition require a nervous input to the muscle (Johnson et al. 1980), Hall-Craggs, Wines & Max (1983) found that thyroid hormone-induced changes in SDH activity were unaffected by denervation, supporting the hypothesis that these are two separate effects of thyroid hormones mediated by different mechanisms.

Although m-αGPDH activity was demonstrated in both types of fibre in the gastrocnemius, in none of the thyroid states studied was it possible to detect m-αGPDH activity in the soleus. However, in homogenates of rat soleus this enzyme has been shown to be sensitive to changes in thyroid status (Ianuzzo et al. 1977; Winder & Holloszy, 1977). This discrepancy could be due to contamination of soleus homogenate by extraneous m-αGPDH-rich tissues or to insensitivity of the cytochemical assay, although the latter explanation seems unlikely since cytochemical assays are usually more sensitive than conventional homogenate assays (Chayen et al. 1973). However, another possible explanation is that the presence of phosphate buffer, a potent mitochondrial swelling agent (Tata, Ernser, Lindberg et al. 1963) in the homogenate m-αGPDH assay, may have deformed the inner mitochondrial membrane and expressed latent enzyme activity not revealed by the cytochemical assay which uses glycol-glycine buffer. Such a phenomenon has been described with the cytochemical assay of glutamate dehydrogenase, an intramitochondrial enzyme, the activity of which may be increased by tapping the tissue prior to chilling or by the inclusion of phosphate buffer in the reaction medium (Altman, 1972). Since Donnelan, Barker, Wood & Beechey (1970) have shown that m-αGPDH, unlike glutamate dehydrogenase and SDH, is located on the outer (cytoplasmic) face of the inner mitochondrial membrane it is unclear why mitochondrial disruption might increase the activity of this enzyme. A possible explanation is that in this muscle m-αGPDH may be normally ‘hidden’ from the cytoplasm, for example in pinched-off membrane vesicles. In fact Brdiczka & Reith (1986) have suggested that the inner mitochondrial membrane is a highly convoluted, heterogeneous structure with m-αGPDH and SDH being localized in different regions.

To conclude, in-vivo treatment of adult rats with PTU and T4 induced biochemical hypo- and hyperthyroidism respectively, and fibre composition and

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diameter in both red and white muscles were changed in hypo- and hyperthyroidism. Significant changes in mitochondrial enzyme activities of single fibres were seen in hypothyroidism but not in hyperthyroidism. It is suggested that while moderate growth-promoting doses of thyroid hormones may alter the expression of myosin and cause changes in fibre composition, as seen in this study, higher growth-inhibiting doses are required to increase the activities of mitochondrial enzymes.

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REFERENCES


DESCRIPTION OF PLATE

Sections of rat soleus muscles reacted for myofibrillar ATPase after formaldehyde fixation showing type I (unreacted), type IC (intermediate reaction) and type II (heavily reacted) fibres. All × 80.

FIGURE 1. Soleus muscle from a hypothyroid animal showing the presence of only type I fibres.

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FIGURE 2. Soleus muscle from a euthyroid animal.

FIGURE 3. Soleus muscle from a hyperthyroid animal showing increased proportions of type IC and type II muscles.