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Body, Heart, Thyroid Gland and Skeletal Muscle Weight Changes in Rats with Altered Thyroid Status

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Summary

In the present paper we describe changes of anatomical parameters in inbred Lewis strain rats, namely their body weight, body weight gain per week, absolute and relative heart, thyroid gland and skeletal muscle weights, that are assumed to reflect experimentally altered thyroid status. The hyperthyroid state was induced by DL-thyroxine or Na 3,3',5-triiodo-L-thyronine, while methimazole was employed for inducing hypothyroidism. We have found that when compared to euthyroid rats, hypothyroidism resulted in a significantly lower body weight gain, absolute and relative heart weight and, in contrast, in a significant increase of absolute and relative thyroid gland weight. On the other hand, hyperthyroidism led to a significant increase of absolute and relative heart weight and to a significant reduction of absolute and relative thyroid gland weight. However, the body mass was not significantly altered in hyperthyroidism as compared with euthyroid rats. We conclude that our protocol leads to chronic hyper- or hypothyroidism as demonstrated by body, heart and thyroid gland weight changes. These anatomical data can thus be utilized as supplemental criteria for the assessment of the thyroid state of experimental rats.

Key words

Body weight • Organ weights • Hypothyroidism • Hyperthyroidism • Thyroid state • Skeletal muscles

Introduction

In order to study the regeneration of intrafusal and extrafusal muscle fibers, we have introduced a model of so-called heterochronous (donor and host animals are of different age) isotransplantation (both donor and host are inbred animals of the same strain), when slow or fast muscles from young rats were intramuscularly transplanted into a fast or a slow host muscle of adult recipients (Jirmanová and Soukup 1995). Early changes and long-term effects of heterochronous isotransplantation on the ultrastructure of regenerated muscle fibers (Soukup and Novotová 2000, Jirmanová and Soukup 2001) and on the intrafusal fiber type composition (Soukup and Thornell 1997) were described previously.

At present, we use the model of heterochronous isotransplantation for the analysis of regulation of myosin heavy chain (MyHC) expression in eu-, hyper- and hypothyroid inbred rats (Soukup *et al.* 1999, Zachařová *et al.* 1999, Ladecký *et al.* 2000, 2001, for review see Soukup and Jirmanová 2000). This method enables to analyze the contribution of genetic factors given by the fast or slow cell line of a donor muscle, fast or slow impulse frequency of the host axons and of

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experimentally altered levels of thyroid hormones in a single model. To evaluate our protocol used to alter the thyroid hormone status, we have measured total triiodothyronine and total thyroxine levels and the activity of α-glycerol-3-phosphate cytochrome c oxidoreductase (in preparation). The goal of the present study was to describe additional changes of anatomical parameters reflecting the alteration of thyroid status. We have therefore compared body weight, body weight gain per week, absolute and relative heart, thyroid gland and soleus and EDL muscle weights in hyperthyroid and hypothyroid rats with those of euthyroid rats. The present results have shown that our protocol leads to chronic hyper- or hypothyroidism, which induce changes of the body, heart and thyroid gland weight.

Methods

Animals

The experiments were performed on inbred Lewis strain female rats obtained from an authorized laboratory rat-breeding unit of the Institute of Physiology (Accreditation No. 1020/491/A/00). The Expert Committee of the Physiological Institute of the Academy of Sciences, Prague, Czech Republic, approved the investigation. The Ethical Principles and Guidelines for scientific experiments on animals were respected during the studies. The maintenance and handling of experimental animals followed the recommendations of the EU and the animals were treated in accordance with principles of the Care and Use of Animals approved for the Institute of Physiology CAS by NIH (Bethesda, USA) under No. A5228-01.

Thyroid status alteration

The hypothyroid status (HY) was induced and maintained by chemical "thyroidectomy" using 0.05% solution of methimazole (methyl-mercapto-imidazol, Sigma) administered in the drinking water for 2 to 12 months. In order to compare possible differences in the effect of T_3 and T_4, the hyperthyroid status (TH) was induced and maintained by intraperitoneal injections of 15 μg per 100 g body weight of either Na 3, 3'5 triido-L-thyronine (T_3; Sigma) or DL-thyroxine sodium (T_4; Sigma) 3 times a week for 2 to 6 months. As we found no significant difference in any of the investigated anatomical parameter between rats injected by T_3 or T_4, we pooled the results together as one group. Euthyroid rats were age-matched littermates of the experimental animals. In order to avoid possible mortality due to prolonged hyperthyroidism (see Discussion), the longest experimental period for hyperthyroid rats was 6 months, whereas hypothyroid rats were analyzed up to 12 months after starting the experimental treatment, similarly as euthyroid animals.

Surgical procedures

The present study was performed on animals that were used for further analysis of thyroid hormone influence on the MyHC isoform expression in regenerated and adult rat muscles (cf. Soukup *et al*. 1999, Zachařová *et al*. 1999, Ladecký *et al*. 2000, 2001, for review see Soukup and Jirmanová 2000). In these, 2- to 3-month-old rats, unilateral heterochronous isotrans-plantation was performed on animals anesthetized with Nembutal (40 mg/kg). The surgical manipulation did not cause any long-lasting deterioration in their well being (for a detailed description of the isotransplantation procedure see Jirmanová and Soukup 1995).

Measurement

Two to 12 months after start of the experiment, the operated rats were anesthetized as previously described and immediately thereafter, a 2 to 3 ml blood sample was obtained from the left ventricle for determination of tT_3 and tT_4 concentrations. The rats were killed by an overdose of Nembutal and the livers for the determination of α-glycerolphosphate cytochrome c oxidoreductase activity and the EDL muscles containing the grafts for histochemical and immunocytochemical analysis were excised. The results of these measurements are being currently prepared for publication.

For the present study, the body weight was determined at the beginning and at the end of the experiment (2 to 12 months after starting the experimental treatment). Furthermore, the heart, thyroid gland and control soleus and EDL muscles from the contralateral intact limb were excised and weighed. The relative organ weight was determined as the ratio of the organ weight to the body weight of individual animals (organ weight/body weight ratio). All data are expressed as the mean ± S.D. Statistical differences were evaluated by Student's t test.

Results

Body, heart, thyroid gland and skeletal muscle weight changes

The mean body and the mean absolute and relative organ weights were compared among euthyroid,

hypothyroid and hyperthyroid rats. As the maximal duration of the hyperthyroid treatment was only six months, the anatomical parameters of hyperthyroid animals (TH-6, n=22) were compared either with all euthyroid (EU-12, n=32) and all hypothyroid (HY-12, n=25) animals, or with those of euthyroid and hypothyroid animals that were sacrificed within the period of 2-6 months after the start of the experimental treatment (EU-6, n=18 and HY-6, n=13, respectively). EU-6 and HY-6 rats thus corresponded to the TH-6 group by age range and average age. Comparison of TH animals treated with either T_4 (nine rats) or T_3 (13 rats) showed no significant difference in any of the investigated anatomical parameters. In the following analysis all TH rats are therefore considered as one group.

The initial mean body weight did not significantly differ either between EU-12 and HY-12 groups, ranging from 166 g to 173 g, or among EU-6, HY-6 subgroups and TH-6 group, ranging from 173 g to 177 g (Table 1). In hyperthyroid animals, the weekly weight gain remained unchanged in comparison with euthyroid animals, whereas in hypothyroid rats, the body growth was strongly restricted and the weight gain of both HY-12 and HY-6 was about six times lower than the weight gain of euthyroid rats (5.4x and 6.3x, $p<0.001$, compared to EU-12 and EU-6 animals, respectively; Table 1, Fig. 1a).

Table 1. Mean values of body (BW) and organ weights of euthyroid (EU), hypothyroid (HY) and hyperthyroid (TH) female rats measured after 2-6 months (EU-6, HY-6, TH-6) and after 2-12 months (EU-12, HY-12) from the start of the experiment.

	EU-12		**HY-12**		**EU-6**		**HY-6**		**TH-6**	
BWS (g)	166±27	(32)	173±24	(25)	173±27	(18)	174±21	(13)	177±21	(22)
BWF (g)	247± 57	(32)	192± 27***	(25)	218± 32	(18)	181±20***	(13)	231±22	(22)
BW/w (g)	3.75±1.83	(31)	0.70±0.62***	(25)	4.52±1.90	(17)	0.72±0.55***	(13)	4.81±2.47	(22)
HW (mg)	799±104	(23)	568± 94***	(19)	756±103	(10)	530±76***	(9)	979±147***	(24)
HW/BW (mg/g)	3.25±0.53	(23)	2.99±0.52	(19)	3.47±0.25	(10)	2.85±0.30***	(9)	4.27±0.76***	(24)
ThW (mg)	30.7±11.1	(9)	170.8±66.4***	(19)	26.4±7.3	(8)	119.8±15.9***	(9)	15.8±13.9*	(19)
ThW/BW (mg/g)	0.13± 0.04	(9)	0.88±0.31***	(19)	0.13±0.04	(8)	0.65±0.09***	(9)	0.07±0.07*	(19)
SW (mg)	97.4±21.3	(14)	87.4±16.2	(13)	98.8±17.1	(8)	89.4±10.2	(7)	94.8±8.6	(14)
SW/BW (mg/g)	0.41±0.10	(14)	0.46±0.09	(13)	0.44±0.05	(8)	0.47±0.05	(7)	0.42±0.05	(14)
EW (mg)	104.6±12.8	(7)	83.8±10.5**	(10)	105.3±13.6	(6)	86.1±9.8*	(5)	101.3±11.6	(13)
EW/BW (mg/g)	0.49± 0.09	(7)	0.45±0.06	(10)	0.46±0.06	(6)	0.48±0.06	(5)	0.44±0.03	(13)

*BWS - starting body weight, BWF - final body weight, BW/w - BW increase per week, HW - heart weight, ThW - thyroid gland weight, SW - soleus muscle weight, EW - EDL muscle weight . Data are expressed as mean ± S.D., number of animals (in parentheses). Significance: HY-12 vs. EU-12; HY-6 or TH-6 vs. EU-6. *p<0.05, **p<0.01, ***p<0.001.*

The absolute heart weight significantly ($p<0.001$) increased in the TH-6 group by 29.5 % compared to EU-6 rats and even by 22.5 % in comparison with EU-12 rats (Table 1, Fig. 1b). On the contrary, the absolute heart weight in hypothyroid rats significantly ($p<0.001$) decreased in HY-6 compared to EU-6 rats by 30 % and in HY-12 compared to EU-12 by 29 %. The relative heart weight significantly ($p<0.001$) increased by 23 % and 31 % in TH-6 group in comparison with EU-6 and EU-12 rats, respectively. On the other hand, the relative heart weight in hypothyroid rats significantly ($p<0.001$) decreased in HY-6 compared to EU-6 by 18 %, whereas the decrease by 8 % in HY-12 compared to EU-12 was not significant (Table 1, Fig. 1b).

The weight of the thyroid gland dramatically increased in hypothyroid rats by about 400 % (354 % and 456 % between HY-6 and EU-6 subgroups and between HY-12 and EU-12 groups, respectively) and by even more relatively to the body weight (400 % and 577 %, respectively). In TH-6 rats, the absolute and relative weights of the thyroid gland significantly decreased ($p<0.05$), the absolute weight by 40 % and 48.5 % in comparison with EU-6 and EU-12, respectively and the relative weight by 46 %, in comparison with both EU-6 or EU-12 rats (Table 1, Fig. 1c).

The absolute weight of control soleus and the relative weight of both control soleus and EDL muscles did not differ among the rats with a different thyroid status, while the absolute weight of control EDL muscles significantly decreased by 18 % and 20 % in HY-6 and HY-12 rats, compared to EU-6 and EU-12 rats, respectively (Table 1, Fig. 1d).

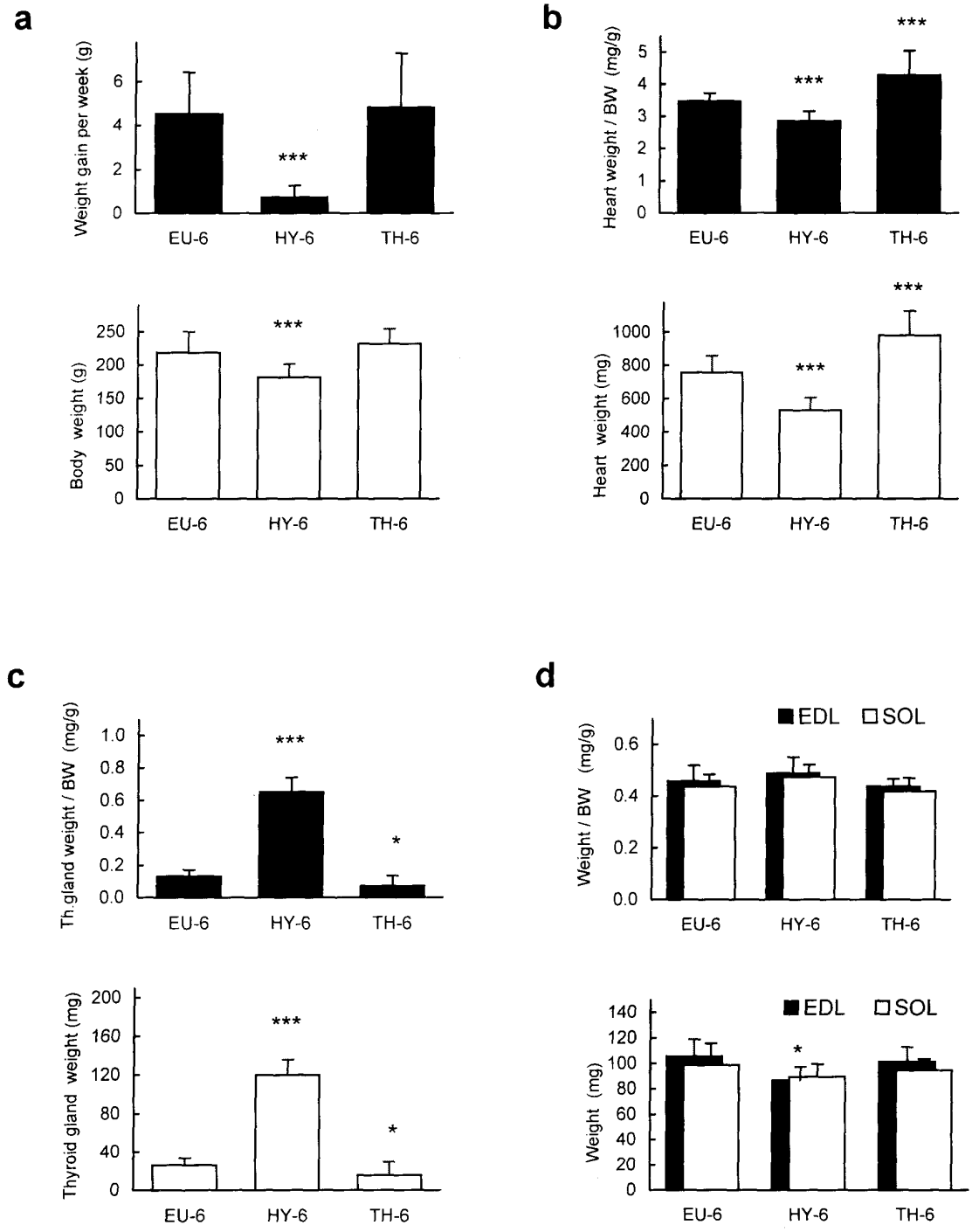

Fig. 1a-d. *Body and organ weights of hypothyroid (HY-6) and hyperthyroid (TH-6) female rats compared with age matched euthyroid control rats (EU-6). BW - body weight; EDL - extensor digitorum longus; SOL - soleus.*

Discussion

This study has investigated several anatomical parameters of rats analyzed in the ongoing study of the fiber type transformation and muscle myosin isoform gene expression in soleus and EDL muscles influenced by pharmacologically altered thyroid hormone levels. Body, heart and thyroid gland weights, total triiodothyronine (tT_3) and total thyroxine (tT_4) levels and the activity of α-glycerol-3-phosphate dehydrogenase are widely used for evaluating the hypo- and hyperthyroid status (e.g. Oppenheimer 1979). The analysis of the selected anatomical parameters has shown that our protocol results either in (i) profound hypothyroidism demonstrated by a significant decrease of body growth, absolute and relative heart weight and in a significant increase of absolute and relative thyroid gland weight or (ii) extensive hyperthyroidism leading to a significant increase of absolute and relative heart weight, atrophy of thyroid gland, but not to any change in the body mass in comparison with euthyroid rats. The ongoing measurements of tT_3 and tT_4 levels by a radioimmunoassay using commercial RIA kits (Immunotech S.A., Marseille) and of the mitochondrial α-glycerol-3-phosphate dehydrogenase (mGPDH, EC 1.1.99.5), measured as α-glycerol-3-phosphate cytochrome c oxidoreductase activity in liver mitochondria at room temperature by the method of Sottocasa *et al.* (1967), confirm the efficacy and reliability of our protocol for inducing hypo- and hyperthyroidism. These preliminary results (papers in preparation), similarly as the presented changes of selected anatomical parameters, show that our protocol leads to hypothyroidism that is characterized by significantly lower levels of tT_3 and especially of tT_4 (more than 10 times in comparison with euthyroid state) in blood serum and by reduced (by about half) enzyme activity of mGPDH in liver mitochondria. On the other hand, the hyperthyroid animals had significantly increased levels of tT_3 (and also tT_4 in case of T_4 administration) and a significantly increased mGPDH activity (about 4 times compared to euthyroid rats). The administration of either T_3 or T_4 had comparable effects, as we did not find any significant difference in analyzed anatomical parameters and fiber type transformations (paper in preparation) between rats treated with either T_3 or T_4. This is quite conceivable as only T_3 is the active hormone and it was significantly increased after application of either drug. However, the preliminary results show that the inductive effect of T_3 on the mGPDH activity is stronger than that of T_4 (paper in preparation).

The arrested body growth in hypothyroid rats was associated with a considerable retardation of body and heart weights as well as of the weight of the fast EDL muscle and with marked thyroid gland hypertrophy. The body growth of hyperthyroid rats, was comparable to that of euthyroid rats. On the other hand, the absolute and relative heart weights significantly increased apparently as a consequence of increased heart activity due to excess of the thyroid hormone. The increased heart weight was observed in many physiological experiments analyzing the effect of increased thyroid hormone levels on cardiac performance. The cardiac hypertrophy was already found after 1-2 weeks of treatment (e.g. Lortet *et al.* 1989, Heckmann and Zimmer 1992, Craig *et al.* 1998).

Yu *et al.* (1998) have found the gender differences in rats treated with T_3 (30 μg/100 g); while in 3-6 months old female rats 4 week treatment had no significant effect on their body weight compared with untreated female rats, the development of the body mass of male rats was afflicted after the same treatment. Thyroid and sex hormones belong to the same nuclear receptor superfamily and it is therefore not surprising that T_3 and estrogen may interact in the regulation of gene expresion (Zhu *et al.* 1996, for review see Yu *et al.* 1999). Furthermore, rats treated with 30 μg/100 g of body weight of T_3 every second day for 4 weeks exhibited 50 % and those treated for 8 weeks even 78.5 % mortality, probably related to tachycardia and/or cardiac arrhythmias (Larsson *et al.* 1994). It was also found that cardiac arrhythmias were more pronounced in young male than female rats (Yu *et al.* 1998). In order to reduce the original mortality that occurred in our pilot experiments, we have used female rats and have reduced the amount of administered thyroid hormone from 30 μg/100 g of body weight by a half.

In hypothyroid rats, the body weight gain per week was very low (or even negative) during the whole experimental period and their body growth was practically arrested. These findings are in agreement with previously reported data on rats (Diffee *et al.* 1991, Escobar *et al.* 1997, Syed *et al.* 1999, Wang *et al.* 2000), but are partly contradictory to the data in human subjects with thyroid disorders, where more than half of the hypothyroid patients have problems with increasing body weight and, on the other hand, most of hyperthyroid subjects suffer from loss of body weight. The thyroid hormones are supposed to be involved in the regulation of

body and fat weight homeostasis, namely by reducing the body fat content (Burgi *et al.* 1990, Syed *et al.* 1999). The lack of thyroid hormones is supposed to reduce energy expenditure, increase the fat mass and, possibly, influence circulating leptin levels indirectly by regulation of adipose tissue mass (Syed *et al.* 1999). Methimazole administration for longer time periods was found to increase plasma leptin values (Leonhardt *et al.* 1999). The 'satiety factor' leptin suppresses food intake in hypothyroid rats (Cusin *et al.* 2000, Wang *et al.* 2000) and could reduce the level of metabolism and body growth gain (Wang *et al.* 2000). Low food intake can also be partly caused by reduced water intake (personal observation) due to the unpleasant taste of the methimazole solution, although it was found that the body growth of thyroidectomized rats was also slowed down (Diffee *et al.* 1991, Escobar *et al.* 1997, Wang *et al.* 2000).

Rao-Rupanagudi *et al.* (1992) reported values of the weight of the thyroid gland in euthyroid Sprague-Dawley rats. These ranged from 17.2±4.5 mg to 41.7±27 mg in 2- to 24-month-old female rats, which fits well with our values.

Information concerning the possible long-term effects of altered thyroid hormone levels on rat skeletal muscle weight is rather sparse. Sillau (1985) found no change in PTU-treated hypothyroid rats, as the weight of the soleus and gastrocnemius-plantaris muscles decreased proportionally to the body weight. Diffee *et al.* (1991) found arrested body weight gain and substantially lower soleus and plantaris muscle weight in thyroidectomized rats, but no change in vastus intermedius muscle weight compared to normal rats. Relative weights were not determined, but the mean soleus muscle weight decreased by 27 %, which was more than the 18 % mean body weight decline. Capó and Sillau (1983) found significantly decreased soleus and gastrocnemius weight after 4 weeks of T_3 administration, whereas the decrease in body weight was not significant. Some reported data are difficult to compare as relative values are not given and/or the used high doses of T_3 apparently caused a deterioration of the physiological state or even a high mortality rate of treated animals (Larsson *et al.* 1994, Li and Larsson 1997, Yu *et al.* 1998). In our experiments, no substantial influence was found in the examined muscles, with the exception of a significant decrease of EDL muscle weight in hypothyroid animals. This may be due to the fact that the methimazole treatment reduces the proportion of the large diameter 2B fibers which are replaced by 2A and type 1 fibers with much smaller diameters (e.g. Ladecký *et al.* 2000, 2001).

We conclude that our protocol leads to chronic hyper- or hypothyroidism as demonstrated by body, heart and thyroid gland weight changes. These anatomical data can thus be used as supplementary criteria of the thyroid state of experimental animals, e.g. in the ongoing analysis of MyHC isoform expression in regenerated and adult muscles in rats with experimentally altered thyroid status.

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References

BURGI U, BURGI-SAVILLE ME, BURGHERR J, CLEMENT M, LAUBER K: T3 plus high doses of beta-blockers: effects on energy intake, body composition, bat and heart in rats. *Int J Obes* **14**: 1023-1038, 1990.

CAPÓ LA, SILLAU AH: The effect of hyperthyroidism on capillarity and oxidative capacity in rat soleus and gastrocnemius muscles. *J Physiol* **242:** 1-14, 1983.

CRAIG EE, CHESLEY A, HOOD DA: Thyroid hormone modifies mitochondrial phenotype by increasing protein import without altering degradation. *Am J Physiol* **275**: C1508-1515, 1998.

CUSIN I, ROURU J, VISSER T, BURGER AG, ROHNER-JEANRENAUD F: Involvement of thyroid hormones in the effect of intracerebroventricular leptin infusion on uncoupling protein-3 expression in rat muscle. *Diabetes* **49**: 1101-1105, 2000.

DIFFEE GM, HADDAD F, HERRICK RE, BALDWIN KM: Control of myosin heavy chain expression: interaction of hypothyroidism and hindlimb suspension. *Am J Physiol* **261**: C1099-C1106, 1991.

ESCOBAR-MORREALE HF, ESCOBAR DEL REY F, MORREALE DE ESCOBAR G: Thyroid hormones influence serum leptin concentrations in the rat. *Endocrinology* **138**: 4485-4488, 1997.

HECKMANN M, ZIMMER HG: Effects of triiodothyronine in spontaneously hypertensive rats. Studies on cardiac metabolism, function, and heart weight. *Basic Res Cardiol* **87**: 333-343, 1992.

JIRMANOVÁ I, SOUKUP T: Critical period in muscle spindle regeneration in grafts of developing rat muscles. *Anat Embryol* **192**: 283-291, 1995.

JIRMANOVÁ I, SOUKUP T: Early changes in extrafusal and intrafusal muscle fibres following heterochronous isotransplantation. *Acta Neuropathol* **102:** 473-484, 2001.

LADECKÝ R, JIRMANOVÁ I, ZACHAŘOVÁ G, MRÁČKOVÁ K, ERŽEN I, SMERDU V, SOUKUP T: Changes in the proportion of type 1, 2A, 2X/D and 2B muscle fibres in regenerating grafts in rats with experimentally changed thyroid status. *Physiol Res* **49**: P41, 2000.

LADECKÝ R, ZACHAŘOVÁ G, JIRMANOVÁ I, SMERDU V, SOUKUP T: Fibre types in the regenerated soleus and EDL isografted into EDL muscle in rats with altered thyroid status. *Physiol Res* **50**: P15, 2001.

LARSSON L, LI X, TERESI A, SALVIATI G: Effects of thyroid hormone on fast- and slow-twitch skeletal muscles in young and old rats. *J Physiol (London)* **481**: 149-161, 1994.

LEONHARDT U, GERDES E, RITZEL U, SCHAFER G, BECKER W, RAMADORI G: Immunoreactive leptin and leptin mRNA expression are increased in rat hypo- but not hyperthyroidism. *J Endocrinol* **163**: 115-121, 1999.

LI X, LARSSON L: 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, 1997.

LORTET S, ZIMMER HG, ROSSI A: Inotropic response of the rat heart during development and regression of triiodothyronine-induced hypertrophy. *J Cardiovasc Pharmacol* **14**: 707-712, 1989.

OPPENHEIMER JH: Thyroid hormone action at the cellular level. *Science* **203**: 971-979, 1979.

RAO-RUPANAGUDI S, HEYWOOD R, GOPINATH C: Age-related changes in thyroid structure and function in Sprague-Dawley rats. *Vet Pathol* **29**: 278-287, 1992.

SILLAU AH: Capillarity, oxidative capacity and fibre composition of the soleus and gastrocnemius muscles of rats in hypothyroidism. *J Physiol* **361:** 281-295, 1985.

SOTTOCASA GL, KUYLENSTIERA B, ERNSTER L, BERGSTRAND A: An electron-transport system associated with the outer membrane of liver mitochondria. *J Cell Biol* **32**: 415-438, 1967.

SOUKUP T, THORNELL L-E: Expression of myosin heavy chain isoforms in regenerated muscle spindle fibers after muscle grafting in young and adult rats – plasticity of intrafusal satellite cells. *Differentiation* **62**: 179-186, 1997.

SOUKUP T, ERŽEN I, JIRMANOVÁ I, ZACHAŘOVÁ G, RAUCHOVÁ H, PAVELKA S: Absence of thyroid hormones down regulates the expression of fast myosin heavy chain (MyHC) isoforms in regenerating soleus muscle isografted into fast extensor digitorum longus muscle. *Physiol Res* **48**: S44, 1999.

SOUKUP T, JIRMANOVÁ I: Regulation of myosin expression in developing and regenerating extrafusal and intrafusal muscle fibres with special emphasis on the role of thyroid hormones. *Physiol Res* **49**: 617-633, 2000.

SOUKUP T, NOVOTOVÁ M: Ultrastructure and innervation of regenerated intrafusal muscle fibers in heterochronous isografts of the fast rat muscle. *Acta Neuropathol* **100**: 435-444, 2000.

SYED MA, THOMPSON MP, PACHUCKI J, BURMEISTER LA: The effect of thyroid hormone on size of fat depots accounts for most of the changes in leptin mRNA and serum levels in the rat. *Thyroid* **9**: 503-512, 1999.

WANG JL, CHINOOKOSWONG N, YIN S, SHI ZQ: Calorigenic actions of leptin are additive to, but not dependent on, those of thyroid hormones. *Am J Physiol Endocrinol Metab* **279**: E1278-1285, 2000.

YU F, DEGENS H, LI X, LARSSON L: Gender- and age-related differences in the regulatory influence of thyroid hormone on the contractility and myosin composition of single rat soleus muscle fibres. *Pflűgers Arch* **437**: 21-30, 1998.

YU F, DEGENS H, LI X, LARSSON L: The influence of thyroid hormone on myosin isoform composition and shortening velocity of single skeletal muscle fibres with special reference to ageing and gender. *Acta Physiol Scand* **167**: 313-316, 1999.

ZACHAŘOVÁ G, MRÁČKOVÁ K, JIRMANOVÁ I, SOUKUP T: Stereological evaluation of the soleus muscle isografted into fast extensor digitorum longus (EDL) muscle in rats with different thyroid status. *Gen Physiol Biophys* **18**, Suppl 1: 84-86, 1999.

ZHU Y-S, YEN PM, CHIN WW, PFAFF DW: Estrogen and thyroid hormone interaction on regulation of gene expression. *Proc Natl Acad Sci USA* **93**:12587-12592, 1996.

Reprint requests

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