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## INFLUENCE OF THYROID HORMONES ON EXERCISE TOLERANCE AND LACTATE THRESHOLD IN RATS

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Effects of thyroid hormone deficit, and triiodothyronine ( $T_3$ ) treatment on exercise performance, blood lactate (LA) concentrations and LA threshold ( $T_{LA}$ ) were studied in trained and untrained rats. Fourteen rats were thyroidectomized and then treated with propylthiouracil for 30 days (THY + PTU group). Fourteen sham operated rats served as controls. In each group there were 7 sedentary and 7 endurance-trained animals. Six weeks after thyroidectomy or sham operation the rats were subjected to a multistage running test with speed increasing from 13 m/min at 10° treadmill inclination till maximum. Blood samples for LA were taken from the rats' tail after each 3-min exercise stage. During 3 days following this test rats from all groups were injected (i. p.) with 75  $\mu$ g/100 g of triiodothyronine ( $T_3$ ), and 24 hrs afterwards the second exercise test was performed. In THY + PTU rats maximal running speed (RSmax) and the speed at which  $T_{LA}$  occurred were markedly decreased in comparison with control group. The level of LA at the maximal speed (LAMax) and that corresponding to  $T_{LA}$  were higher in THY + PTU rats than in controls.  $T_3$  injection to control animals diminished their RSmax and  $T_{LA}$ , whereas in THY + PTU rats it increased RSmax and shifted  $T_{LA}$  to a higher speed. Both in THY + PTU and control animals  $T_3$  elevated LAMax and the threshold LA concentration. Endurance training in control and THY + PTU animals markedly enhanced RSmax and  $T_{LA}$ . This was accompanied by increases in LAMax and concentration of LA at  $T_{LA}$  only in control group. After  $T_3$  injection to control trained rats RSmax and  $T_{LA}$  were diminished, whereas in THY + PTU trained group RSmax was unchanged and  $T_{LA}$  was elevated. Maximal blood LA increased only in THY + PTU trained rats whilst the threshold blood LA was elevated in both groups. It is concluded, that both the  $T_3$  deficiency and its excess reduce maximal exercise performance and shift  $T_{LA}$  to lower workloads. Endurance training or administration of  $T_3$  to hypothyroid rats markedly improve their exercise performance and elevate  $T_{LA}$ , however,  $T_3$  treatment markedly increases maximal and submaximal LA levels.

**Key words:** *thyroidectomy, triiodothyronine, graded exercise, blood lactate, endurance training.*

### INTRODUCTION

Thyroid hormones play an important role in the control of carbohydrate, lipid and protein metabolism. Little is known, however, about the effects of thyroid hormone deficit or excess on exercise metabolism. Marked reduction of exercise performance and a decrease in maximal oxygen uptake ( $VO_{2max}$ ) after

thyroidectomy were reported by Therminarias and Lucas (1) in dogs and by Baldwin et al. (2, 3) in rats. Oxydative potential of skeletal muscles and free fatty acid (FFA) availability were found to be diminished in hypothyroidism (4—8). Moreover, Kaciuba-Uściłko et al. (9) demonstrated that in thyroidectomized dogs muscle ATP and creatine phosphate concentrations were decreasing at low exercise intensity, in spite of the increased rate of glycogen breakdown. This was accompanied by large accumulation of lactate (LA) in working muscles. After exercise at high intensity high energy phosphates were reduced to very low values, whilst the glycogen depletion and muscle LA concentrations were similar to the values found in control — euthyroid animals. These data indicate that hypothyroidism in addition to the well known impairment of FFA mobilization and oxidation leads to inability of adequate resynthesis of ATP via glycolysis at high work load.

On the other hand, impaired exercise tolerance was demonstrated by some authors in hyperthyroid patients (10) as well as in human subjects (11—13) or in animals treated with thyroid hormones (14—16). This was attributed to enhanced body temperature, diminished skeletal muscle mass and impaired muscle metabolism. A part of these changes may be explained by potentiation by thyroid hormones of catecholamine action via beta adrenergic receptors (12, 17—19).

Alterations in skeletal muscle metabolism caused e. g. by physical training, dietary modifications (see: 20), prolonged stimulation of beta adrenergic receptors (21) are reflected in changes of lactate threshold ( $T_{LA}$ ), that is a work load at which lactate starts to accumulate in blood during incremental exercise test. There are no data in the available literature on the influence of thyroid hormone deficit or excess on  $T_{LA}$ .

Our previous study demonstrated that  $T_{LA}$  can be detected in rats during multistage exercise test, with frequent determinations of blood LA levels in capillary blood taken from the tail (22). This animal model offers a possibility to evaluate  $T_{LA}$  after experimentally-induced endocrine changes. Thus, the purpose of this investigation was to examine an effect of thyroid hormone deficit or excess on exercise performance,  $T_{LA}$ , and exercise-induced blood LA changes in untrained and trained rats.

## MATERIAL AND METHODS

Male Wistar rats of the initial body mass  $200 \pm 7.0$  g were used. They were divided into 2 main groups: group I (THY + PTU) consisted of animals thyroidectomized surgically, and then treated for 30 days with propylthiouracil (Prosst, Quebec, Canada) dissolved in drinking water (0.04%), whereas group II (C) included euthyroid, sham-operated rats. In each group there were 7 sedentary (S), and 7 endurance-trained (T) animals. The rats were housed in the wire mesh cages at 20—22°C, with the light on from 6 a. m. till 6 p. m. The animals had free access to water and the commercial laboratory chow (Murigram, Poland). The experimental protocol was approved by the Ethical Committee at Medical Research Centre of the Polish Academy of Sciences.

## *Experimental procedure*

Six weeks after thyroidectomy or sham-operation both the sedentary and trained rats were exposed to a multistage running test on a treadmill to estimate their  $T_{LA}$  and the maximal running speed. They started the run at a speed of 13 m/min, at 10° treadmill inclination. Afterwards, they performed several 3-min exercise bouts with the treadmill speed increased by 4 m/min until 37 m/min was attained. The exercise bouts were separated by 3 min rest periods during which blood samples (0.05 ml) for LA determinations were taken from previously cut and heparinized tail. After achieving a speed of 37 m/min the treadmill velocity was further increased until the individual maximum speed was reached. More detailed description of the above procedure was reported previously (22).

Sedentary rats were exposed to the exercise-test after being familiarized for a few days with handling and the treadmill running, whilst the trained animals performed the exercise-test 24 h after the last training session. The training program consisted of 40–60 min endurance exercise (5 days per week for 5 weeks) on the treadmill at 10° inclination with speed increasing from 16 m/m in the 1st week to 28 m/m in the 4th and 5th week. More details of the endurance training program are given in the paper by Langfort et al. (23).

The next day following the multistage running test rats of all groups started to be treated with L-sodium BP triiodothyronine- $T_3$  (Glaxo Laboratories, Greenford, Middlesex, U.S.A.). They were injected intraperitoneally with 75  $\mu$ g/100 g b. m. of the hormone daily for 3 consecutive days, and 24 h after the last injection they repeated the exercise test according to the protocol described above.

Blood LA concentration was measured enzymatically using commercial kits (Boehringer Diagnostica, Mannheim, Germany).

## *Calculations*

The lactate threshold ( $T_{LA}$ ) was calculated as the exercise speed corresponding to the individual breaking point of LA curve, using the two-segmental linear regression (log LA vs log running speed), according to Breaver et al. (24).

Results obtained were evaluated statistically using the two-way analysis of variance followed by t-Student test for paired or unpaired data. The null hypothesis was rejected when  $p < 0.05$ . The data are expressed as means  $\pm$  SE throughout the paper.

## RESULTS

### *Influence of thyroid hormone deficit and $T_3$ -treatment on the maximal running speed, the maximal blood LA level, and lactate threshold in sedentary rats*

The maximal running speed (RSmax) was markedly reduced in THY+PTU rats in comparison with that in control (C) group. Three-day treatment of hypothyroid rats with  $T_3$  increased their RSmax. On the other hand,  $T_3$  injections to control animals slightly, but significantly diminished their working ability. Blood LA concentrations after the maximal exercise load was significantly higher in THY+PTU rats than in controls.  $T_3$ -treatment increased maximal blood LA both in THY+PTU and C animals (*Fig. 1*).

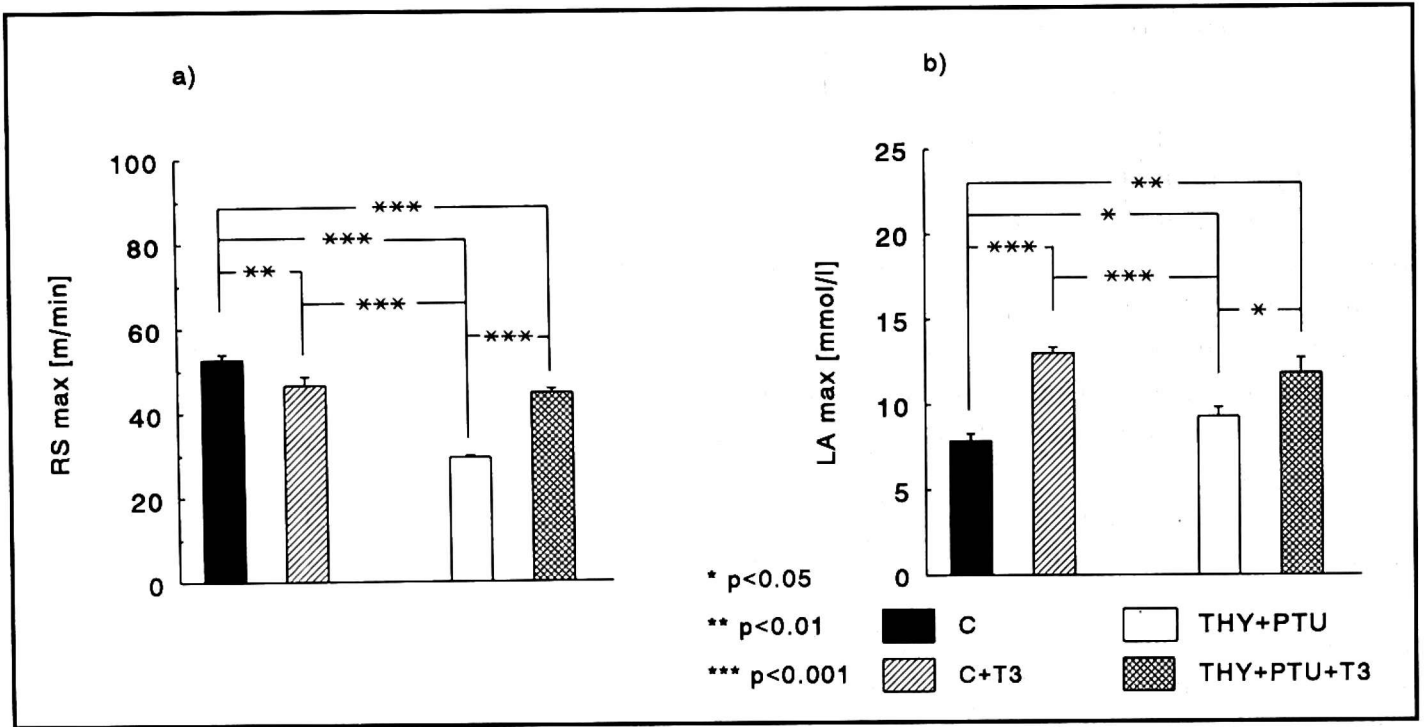


Fig. 1. The maximal running speed — RSmax (a) and the maximal blood lactate (LA) concentrations (b) in untrained rats; C — control euthyroid rats, C+T<sub>3</sub> — control rats injected for 3 days with triiodothyronine. Values are means  $\pm$  SE; asterisks indicate significance of differences.

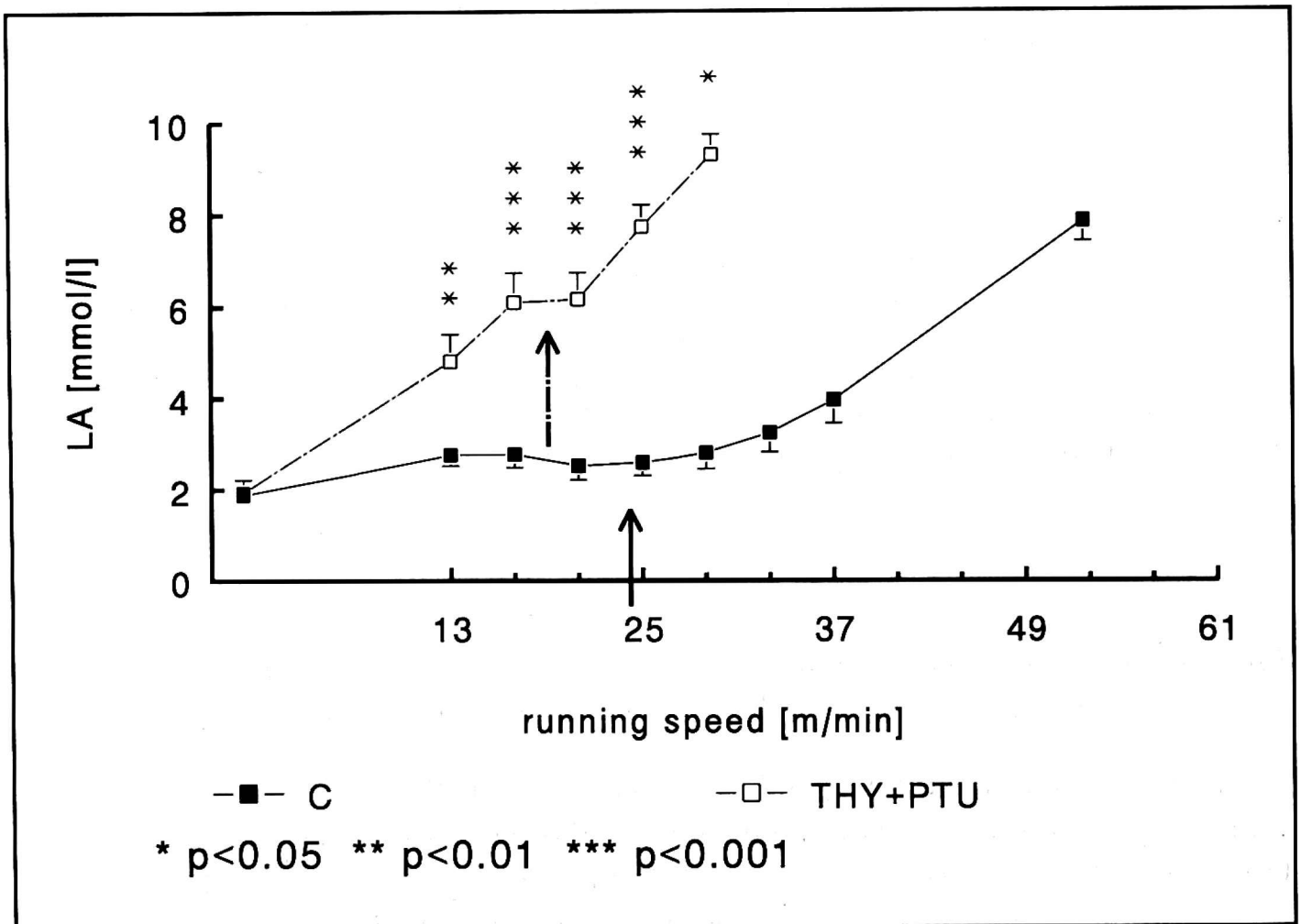


Fig. 2. Blood lactate (LA) concentrations in relation to running speed in untrained control (C) and hypothyroid (THY + PTU) rats. Values are means  $\pm$  SE; asterisks denote significance of differences between C and THY + PTU groups; Arrows indicate the anaerobic threshold.

The pattern of changes in blood LA concentrations in relation to treadmill speed in sedentary C and THY+PTU rats is presented in Fig. 2. The  $T_{LA}$  occurred at lower running speed in THY+PTU rats than in controls.  $T_3$ -treatment of hypothyroid rats resulted in an increase of the speed at which their  $T_{LA}$  was detected, whilst in controls  $T_3$ -injections caused a decrease in  $T_{LA}$ . Blood LA concentrations found at the threshold intensities was much higher in THY+PTU than in C group.  $T_3$ -injections resulted in an increase of this value in THY+PTU rats and in control animals (Fig. 3).

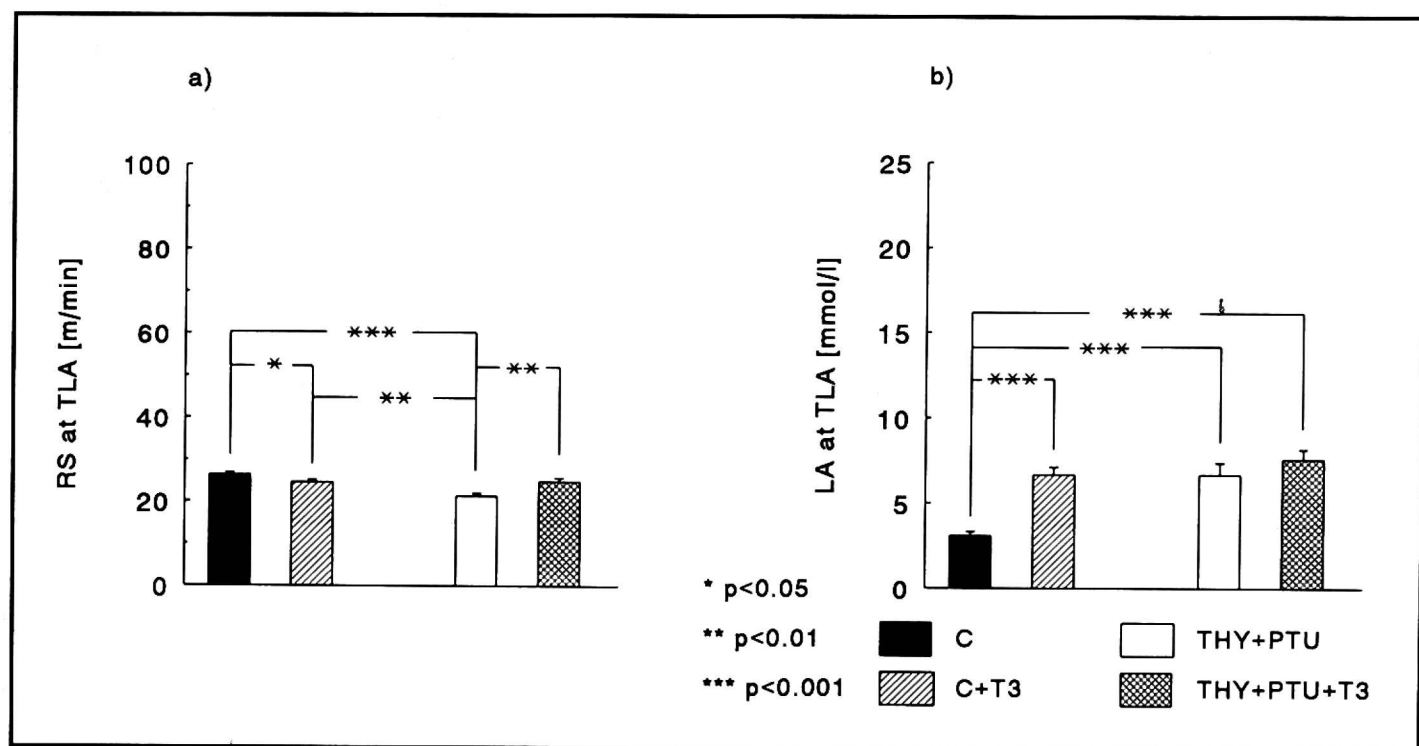


Fig. 3. The running speed at the lactate threshold —  $T_{LA}$  (a) and corresponding blood lactate (LA) concentrations (b) in untrained rats. Other denotations as in Fig. 1.

#### *Influence of thyroid hormone deficit and $T_3$ -treatment on the maximal running speed, the maximal blood LA level, and lactate threshold in endurance trained rats*

The maximal running speed was markedly enhanced after 5 weeks of endurance training both in C and THY+PTU animals ( $p < 0.001$ ). In the former it increased by approx. 23%, whereas in the latter by approx. 96%. Training induced a significant increase in maximal blood LA concentration only in control rats ( $p < 0.001$ ) (Fig. 4).

The pattern of changes in blood LA concentrations in relation to treadmill speed in trained C and THY+PTU rats is presented in Fig. 5. The running speed at which  $T_{LA}$  occurred was increased in trained C and THY+PTU rats. The blood LA concentrations at the  $T_{LA}$  increased only in control animals ( $p < 0.001$ ), so the threshold levels of LA were almost the same in trained C and THY+PTU rats (Fig. 6).

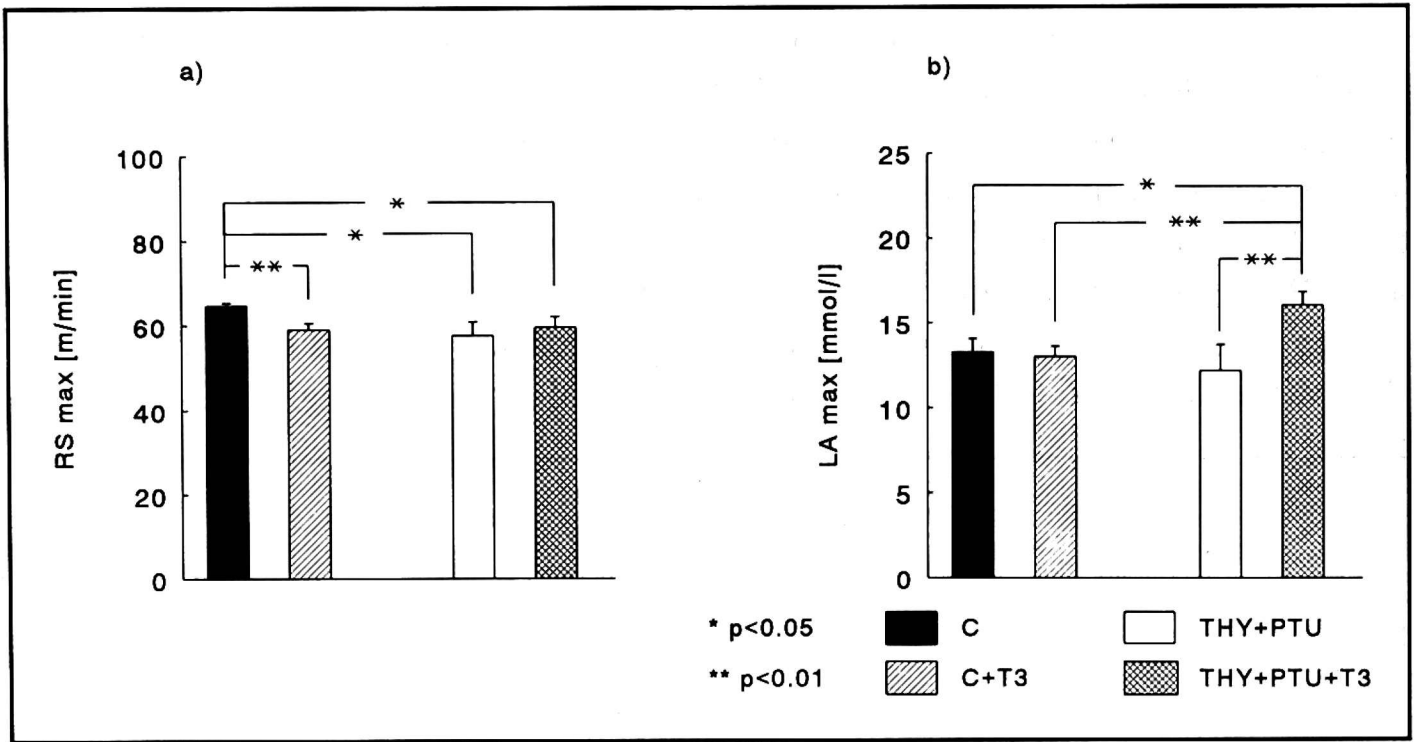


Fig. 4. The maximal running speed — RSmax (a) and the maximal blood lactate (LA) concentrations (b) in trained rats. Other denotations as in Fig. 1.

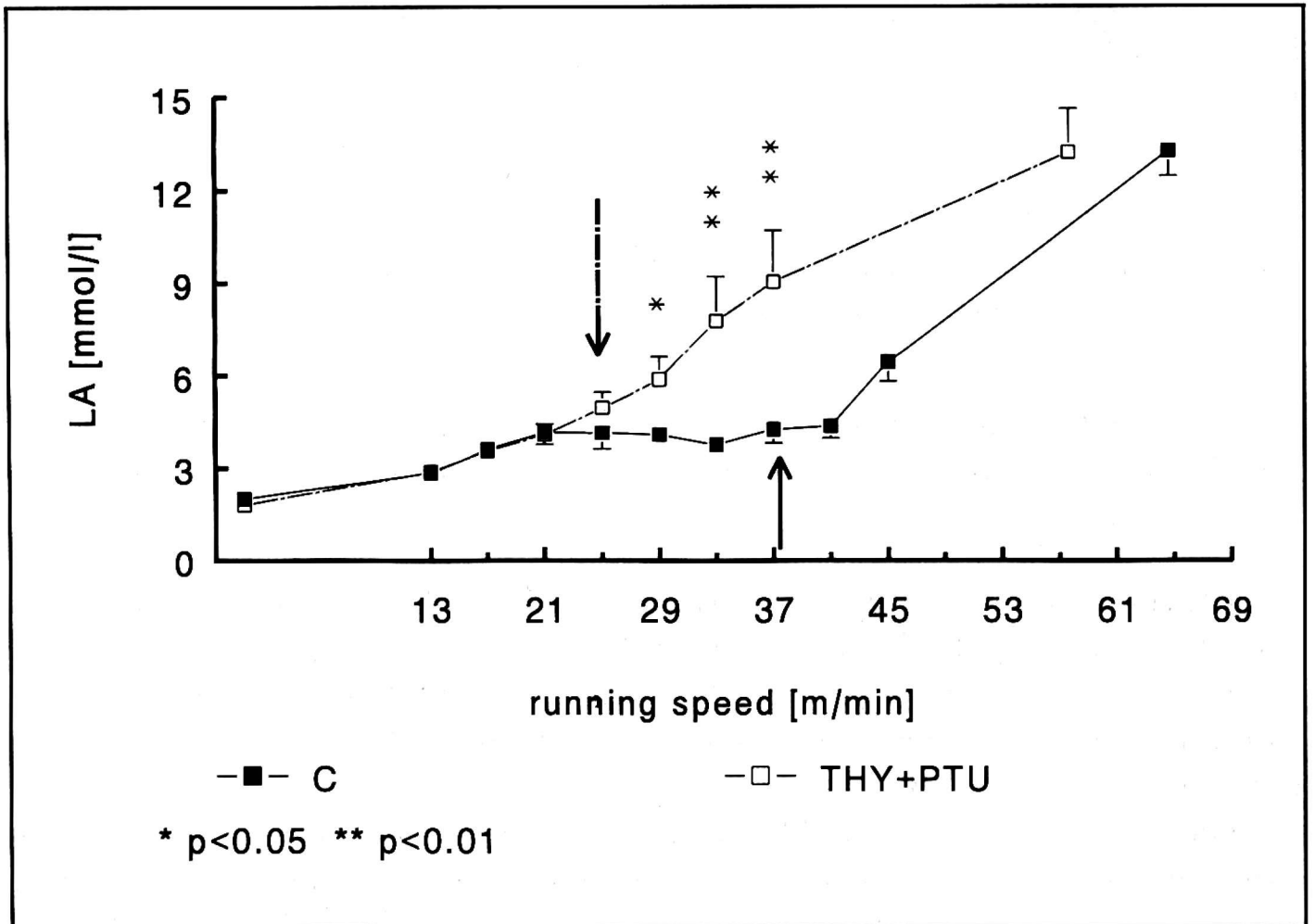


Fig. 5. Blood lactate (LA) concentrations in relation to running speed in trained control (C) and hypothyroid (THY+PTU) rats. Values are means  $\pm$  SE; asterisks denote significance of differences between C and THY+PTU groups; Arrows indicate the anaerobic threshold.

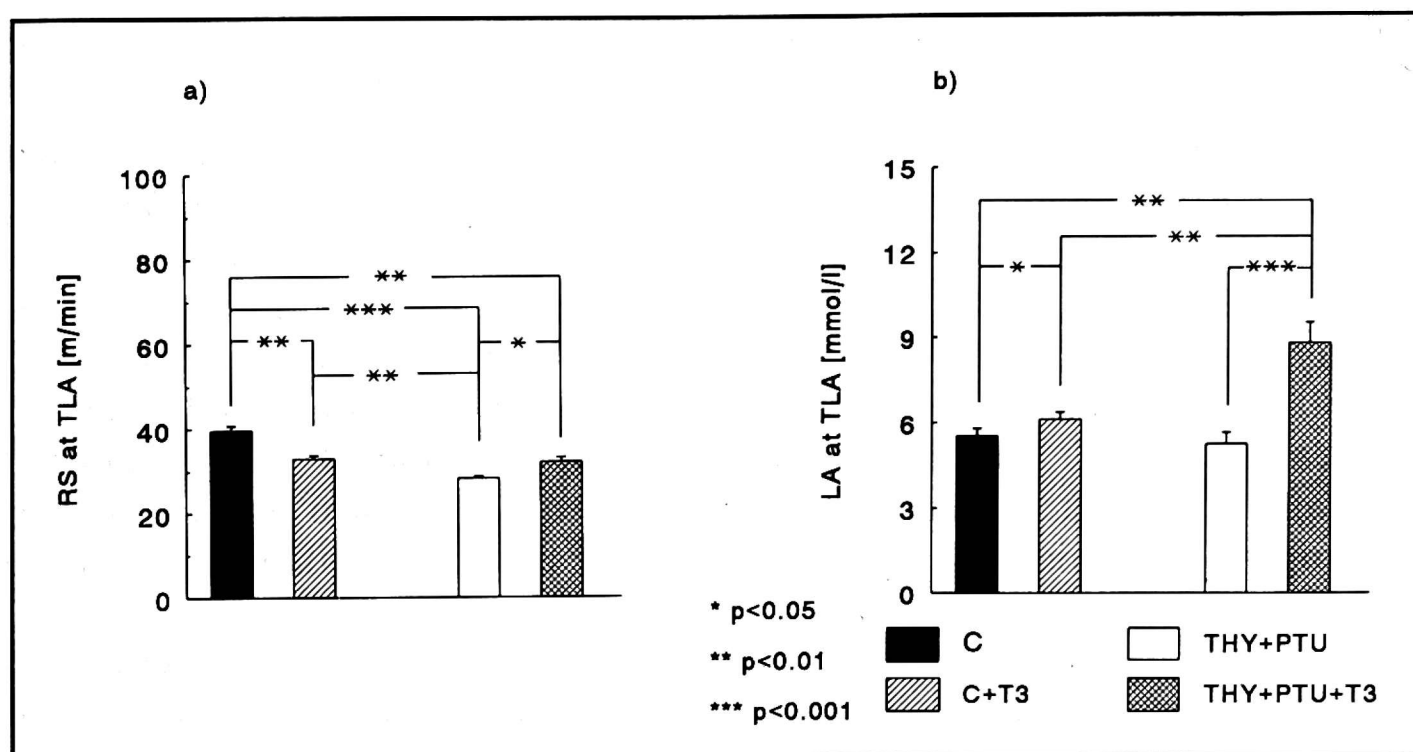


Fig. 6. The running speed at the lactate threshold —  $T_{LA}$  (a) and corresponding blood lactate (LA) concentrations (b) in trained rats. Other denotations as in Fig. 1.

After  $T_3$  injections  $RS_{max}$  achieved by trained C rats was diminished but in trained THY + PTU animals this value remained unchanged (Fig. 4 a). Blood LA concentrations at  $RS_{max}$  after  $T_3$  injections was significantly higher in trained THY + PTU than in trained C animals and in trained THY + PTU rats before  $T_3$  injections (Fig. 4 b). In trained C group injected with  $T_3$   $T_{LA}$  expressed as the running speed was diminished, whereas in the trained THY + PTU rats this value significantly increased. As a result no significant differences between trained C and THY + PTU rats were found after  $T_3$  injections (Fig. 6 a). Blood LA concentrations at  $T_{LA}$  were enhanced after  $T_3$  injections in both trained C and THY + PTU animals in comparison with the preinjection values (Fig. 6 b).  $T_3$ -treatment resulted in significantly higher ( $p < 0.01$ ) blood LA level at  $T_{LA}$  in trained THY + PTU than in C rats (Table 1).

Table 1. Influence of endurance training on maximal running speed ( $RS_{max}$ ), maximal blood LA concentrations ( $LA_{max}$ ), lactate threshold expressed as running speed ( $T_{LA}$ ) and lactate concentration at  $T_{LA}$  (LA at  $T_{LA}$ ) in control (C) and thyroid hormone deficient (THY + PTU) rats.

	C untrained	C trained	THY + PTU untrained	THY + PTU trained
$RS_{max}$ [m/min]	52.63	64.57***	29.40	57.60***
$LA_{max}$ [mmol/l]	7.88	13.27***	9.29	12.18
$T_{LA}$ [m/min]	26.26	39.50***	21.37	28.27***
LA at $T_{LA}$ [mmol/l]	3.08	5.52***	6.67	5.25
	0.24	0.28	0.69	0.41

\*\*\*  $p < 0.001$

## DISCUSSION

The previous study from this laboratory (25) showed that thyroidectomy followed by PTU treatment decreases plasma  $T_3$  concentration from  $1.48 \pm 0.15$  to  $0.55 \pm 0.04$  ng/ml ( $p < 0.001$ ) in rats, whilst daily administration of  $75 \mu\text{g}/100$  g b.w.  $T_3$  to euthyroid rats for 3 days significantly elevates ( $p < 0.001$ ) plasma  $T_3$  level ( $12.70 \pm 2.57$  ng/ml) in comparison with control values ( $1.48 \pm 0.15$  ng/ml). Similar concentrations of plasma  $T_3$  were found in trained and untrained animals. The data evidence that the procedures applied in the present investigation provide clearly differentiated models of thyroid hormone deficit and excess.

The data presented in this paper demonstrated that in experimental hypothyroidism produced in rats not only the maximal running speed achieved during graded exercise but also the lactate threshold were reduced. The latter has been widely accepted as the best predictor of endurance performance (for rev. see 20) although the physiological basis of rapid accumulation of lactate in blood during graded exercise is still not fully understood. It was proved, however, that the threshold is connected with accelerated LA production in working muscles (26). Deficit of thyroid hormones may influence the rate of lactate production at least by two mechanisms: 1) reducing the oxidative potential of muscle cells (2, 3, 7), and 2) diminishing free fatty acid (FFA) availability (2, 3, 5, 6). It should be mentioned that in hypothyroid rats  $T_{LA}$  occurred at a significantly higher blood LA and both submaximal and maximal LA concentrations were higher than in euthyroid controls. The 3-day administration of triiodothyronine to hypothyroid rats shifted the LA threshold to almost normal exercise intensities, but the corresponding blood LA concentrations were still markedly higher than in control animals. This indicates that the level of  $T_{LA}$  is not a direct function of the factors increasing the rate of LA production.

An excess of triiodothyronine, induced by 3-day injections of  $T_3$  to control rats, decreased their lactate threshold, and increased the threshold blood LA concentration. Thus, the effects of thyroid hormone excess and deficit appeared to be similar, although the mechanisms behind them are not identical. Prolonged hyperthyroidism is known to enhance FFA mobilization and muscle oxidative capacity (27) which obviously does not promote greater LA production. However, the short-time experimentally induced  $T_3$  excess may induce opposite changes in skeletal muscles. A decline of activities of both oxydative and glycolytic enzymes in skeletal muscles, a selective decrease in FTa muscle fibre cross-sectional area, and increased density of beta adrenergic receptors in all types of skeletal muscle fibers was reported in men after 14 days



of  $T_3$  administration (11—13). This was accompanied by an increase in blood LA concentration during submaximal exercise. In rats treated with  $T_3$ , transformation of ST to FT fibers was reported (14) but it is not certain whether this change may occur already during three days of the hormone administration.

Numerous studies have demonstrated that endurance training induces in men a shift of LA-threshold towards higher work loads (20, 28). The present investigation showed that this training effect occurs also in rats. This proved that the animal model can be of value for studies directed to explore the physiological mechanisms underlying the anaerobic threshold phenomenon, e.g. during experimental endocrine modifications.

Both in control and in THY + PTU animals the maximal work load as well as LA-threshold values were significantly higher in comparison with those obtained in untrained animals. The maximal running speed in hypothyroid rats increased more than in euthyroid controls, whereas the increment of  $T_{LA}$  was slightly smaller in the former. The changes in maximal and threshold speeds were accompanied by increments in corresponding blood LA levels in control but not in hypothyroid rats. The present data are, therefore, in agreement with the findings of other authors (4, 7) showing that hypothyroidism does not prevent development of training adaptation in skeletal muscles increasing their oxydative potential.

The pattern of changes produced by triiodothyronine administration to euthyroid trained animals was in general similar to that found in sedentary controls, although the changes were less pronounced. In fact, the speed at the LA threshold was not altered significantly. In trained hypothyroid rats  $T_3$  injections increased significantly only the threshold exercise load, without further improvements of maximal running performance. Both the maximal and threshold blood LA levels increased in this group of animals.

In summary, the present study demonstrated that among factors affecting the lactate threshold, thyroid hormones should be considered. Both their deficit and excess shift the threshold towards lower exercise rates and increase the overall blood lactate response to graded exercise in the rat. Both these thyroid states impair work tolerance expressed as the maximal running speed attained during incremental test. Endurance training and a short-term treatment with  $T_3$  reverse, to a great extent, the deleterious effects of hypothyroidism on work tolerance and LA threshold. However, only training reduces the increased blood lactate concentrations at submaximal loads whilst  $T_3$  administration causes further blood LA elevation.

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