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EFFECT OF TRIIODOTHYRONINE ON PHOSPHOLIPID METABOLISM IN SKELETAL MUSCLES OF THE RAT

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The aim of the present study was to examine the effect of treatment with triiodothyronine (T_3) on certain aspects of phospholipid metabolism in skeletal muscles. Rats were injected with triiodothyronine (T_3) daily ($10\ \mu g \times 100\ g^{-1}$ b.w., s.c.) for six days. Saline — treated rats served as controls. 24 h after the last dose of T_3, 14C palmitic acid suspended in the serum of a donor rat, was administered intravenously. Thirty min later, samples of the soleus, white and red section of the gastrocnemius and blood from the abdominal aorta were taken. The muscle phospholipids were extracted and separated into different fractions by means of thin layer chromatography. The following fractions were obtained: shingomeylin, phosphatidylcholine phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine and cardiolipin. The phospholipids were quantified and their radioactivity was measured. The plasma free fatty acid concentration and radioactivity was also determined. Treatment with T_3 reduced the content of phosphatidylinositol and phosphatidylserine in each muscle type, whereas the concentration of other phospholipids remained stable. T_3 increased markedly incorporation of the blood-borne fatty acids into each phospholipid fraction in the muscles. It is concluded that an excess of T_3 influences the metabolism of phospholipids in skeletal muscles.

Key words: *triiodothyronine, phospholipids, skeletal muscle, rat.*

INTRODUCTION

Thyroid hormones exert profound effects on intermediary metabolism (1). Much attention has been paid to the effect of these hormones on carbohydrate metabolism in skeletal muscles. The major effects of hyperthyroidism are: reduction in the glycogen, glucose — 6-phosphate and glucose 1-phosphate contents (2—6), elevation in free glucose and lactate contents (6) elevation in

the activity of hexokinase, glycogen phosphorylase and glycogen synthase (7, 8), increased insulin-stimulated glycolysis, glucose oxidation and glycogen synthesis (3, 9, 10).

Surprisingly, there are only few data on the role of thyroid hormones in muscle fat metabolism. Hyperthyroidism has been shown to reduce the content of triacylglycerols and activity of lipoprotein lipase in the red section of vastus lateralis in the rat. Hypothyroidism is accompamied by increased activity of the enzyme and decreased content of triacylglycerols in the muscle (11, 12). Deficit of thyroid hormones has also been shown to affect the content and composition of phospholipid fatty acids in the sarcoplasmic reticulum of the skeletal muscle of the rat (13).

The aim of the present study was to investigate the effect of treatment with triiodothyronine on the content of phospholipids and incorporation of the blood borne 14C-palmitic acid into the phospholipid moieties in different skeletal muscle types in the rat. The results obtained clearly showed that hyperthyroidism markedly affects the muscle phospolipid metabolism.

MATERIALS AND METHODS

The experiments were carried out on male Wistar rats fed ad libitum a commercial pellet diet for rodents. A 12 h light/dark cycle was maintained in the animal quarters. The animals were divided into two groups: control (C) and treated with triiodothyronine (T_3). Triiodothyronine (Sigma) was injected subcutaneously in a dose of $10 \mu g \cdot 100^{-1}$ g of body weight, daily for six days. Control rats were treated with saline according to the same protocol. The body mass of rats before treatment was: C-291 $\pm$ 12 g and T — 294 $\pm$ 13 g, and after the treatment: C — 295 $\pm$ 14 g, T — 274 $\pm$ 16 g (in case of T_3, $p < 0.01$ vs. the respective mass before treatment). 24 h after the last injection of saline or T_3 rats were anaesthetised with pentobarbital sodium (ip.) and 14C-palmitic acid (DuPont, S.A. 57 $mCi \cdot mmol^{-1}$) suspended in the serum of a donor rat ($0.1\ ml \cdot 100\ g^{-1}$) was administered to the femoral vein. 30 minutes later samples of the white and red section of the gastrocnemius, and the soleus muscles as well as blood from the abdominal aorta were taken. The muscle samples are composed mostly of the fast-twitch glycolytic, fast-twitch oxidative — glycolytic and slow-twitch oxidative fibers, respectively (14, 15). The muscle samples were homogenised in chloroform/methanol in a glass homogenizer and lipids were extracted according to Bligh and Dyer (16). The muscle phospholipids were fractionated by means of thin layer chromatography using silica plates (Kieselgel 60, 0.25 mm, Merck) and a developing solvent composed of chloroform, methanol, acetic acid ad water (50:37.5:3.5:2 v/v/v/v) (17). The phospholipid spots were visualised under an ultraviolet lamp after spraying the plates with 0.5% solution of 2'7'-dichlorofluorescine in absolute methanol and short exposure to ammonium vapours. The following phospholipids were obtained: sphingomyelin, phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine, phosphatidylserine and cardiolipin. The phospholipids obtained from muscles of one hind limb were quantified by measuring the content of phosphorus (18). The gel spots containing phospholipids obtained

from the muscles of the contralateral hind limb were scraped off the plate into the scintillation vials, scintillation cocktail (Ultima Gold, Packard) was added and radioactivity was measured (Tri-carb 1900 Packard). In a separate experiment 14C-palmitic acid was administered as above. Blood samples were taken from the carotid artery in 0.5, 1, 3, and 5 min after administration of the label. The plasma concentration of free fatty acids was determined according to the method of Duncombe (19). Radioactivity of the plasma free fatty acids was also measured. In order to do it, the plasma lipids were extracted according to Bligh and Dyer (16), and separated into different fractions on the silica plates using a developing solvent composed of heptane, isopropyl ether, acetic acid (60:40:3 v/v/v (20). The band containing free fatty acids was scraped off the plate and its radioactivity was counted as above. The results obtained were evaluated statistically using the Student t-test for unpaired data.

RESULTS

Plasma. The plasma free fatty acid concentration was: control -234.9 ± 29.1, T_3-treated — 717.4 ± 56.5 $\mu mol \cdot l^{-1}$. The rate of clearance of 14C-palmitic acid from plasma was similar in both groups. Most of the radioactivity disappeared within the first five minutes after administration of the label (*Fig. 1*). The specific activity of the plasma free fatty acids in T_3 — treated rats was much lower at each time point than the corresponding values in the control group (*Fig. 2*).

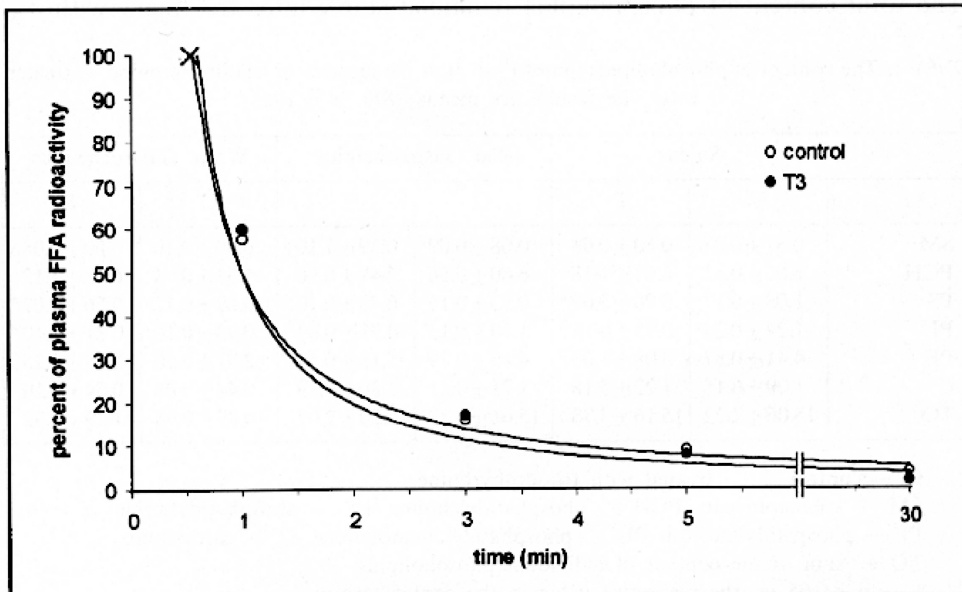

Fig. 1. 14C-palmitic acid clearance rate from plasma. Values represent percent of radioactivity in the 30s after administration of the label.

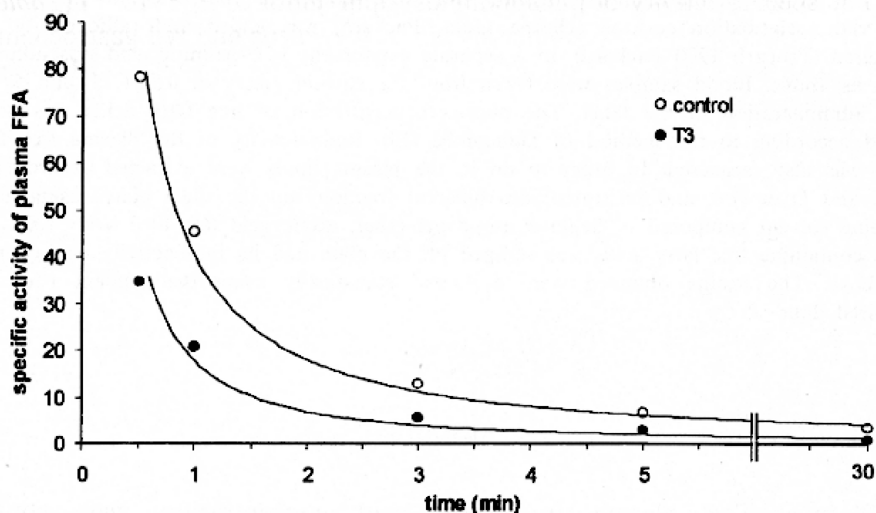

Fig. 2. Specific activity of the plasma free fatty acids (dpm · μmol^{-1} FFA · 10^{-4}).

The muscle phospholipid content (μmol of P · g^{-1}) (*Table 1*).

Treatment with T_3 had no effect on the content of sphingomyelin, phosphatidylcholine, phosphatidylethanolamine and cardiolipin, and it reduced the content of phosphatidylserine and phosphatidylinositol in each muscle. The total content of phospholipids remained stable after treatment with T_3.

Table 1. The content of phospholipids (μmol P · g^{-1}) in the muscles of triiodothyronine — treated rats. The results are means ± SD. N = 10.

	Soleus		Red Gastrocnemius		White Gastrocnemius	
	C	T	C	T	C	T
SM	0.67±0.07	0.60±0.08	0.68±0.09	0.59±1.10	0.60±0.10	0.50±0.08
PCH	6.66±0.81	6.67±1.18	6.60±0.56	7.49±0.96	5.31±0.74	6.18±1.12
PS	1.08±0.17	0.76±0.09 [c]	0.93±0.15	0.78±0.10 [a]	0.69±0.12	0.56±0.07 [a]
PI	1.24±0.24	0.95±0.16 [b]	1.10±0.13	0.91±0.09 [b]	0.93±0.10	0.81±0.10 [a]
PE	4.41±0.87	5.08±1.03	4.49±0.79	5.18±0.54	2.97±0.50	2.78±0.25
C	1.00±0.15	1.22±0.18	1.21±0.21	1.30±0.29	0.44±0.08	0.54±0.10
TO	15.06±2.22	15.16±3.73	15.01±1.27	16.20±2.07	10.89±0.98	11.29±0.32

C — control, T — treated with triiodothyronine.
SM — sphingomyelin, PCH — phosphatidylcholine, PS — phosphatidylserine.
PI — phospatidylinositol, PE — phosphatidylethanolamine, C — cardiolipin.
TO — sum of the content of individual phospholipids.
[a] — $p < 0.05$ vs. the respective value in the control group.
[b] — $p < 0.01$
[c] — $p < 0.001$

The specific activity of phospholipids (dpm · µmol of $P^{-1} \cdot 10^{-2}$) (*Table 2*).

The specific activity of sphingomyelin in the soleus and red gastroenemius, phosphatidylserine and phosphatidylinositol in the soleus and cardiolipin in both sections of the gastrocnemius was elevated in T_3 — treated rats. T_3 reduced the specific activity of phosphatidylcholine in each muscle and phosphatidylethanolamine in the soleus and red gastrocnemius.

Table 2. Specific activity of skeletal muscle phospholipids (dpm · µmol $P^{-1} \cdot 10^{-2}$) of rats treated with triiodothyronine. The muscle samples were taken in 30 min after administration of the label. N = 10.

	Soleus		Red Gastrocnemius		White Gastrocnemius	
	C	T	C	T	C	T
SM	21.8±4.6	35.1±7.4 [b]	20.2±3.2	30.2±4.9 [c]	22.8±2.4	25.7±3.8
PCH	25.5±4.8	17.9±3.7 [b]	39.6±7.6	16.9±3.2 [c]	27.3±6.1	16.6±1.9 [a]
PS	12.1±2.4	18.5±5.2 [b]	14.2±2.8	17.7±3.5	18.3±1.2	22.6±5.2
PI	11.7±1.3	17.4±3.8 [b]	13.0±2.3	15.0±2.3	13.9±1.3	16.6±3.6
PE	9.8±1.6	5.7±1.2 [c]	9.6±2.1	5.9±0.9 [c]	8.0±1.0	6.9±1.0
C	10.2±2.6	9.6±2.1	6.7±1.6	11.7±2.8 [b]	10.3±2.4	24.5±3.0 [c]

Legends as in Table 1.

DISCUSSION

The results obtained clearly show that treatment with triiodothyronine (T_3) affects metabolism of the skeletal muscle phospholipids.The content of phosphatidylinositol and phosphatidylserine was reduced, whereas the content of other phospholipids remained unchanged in each muscle. Since phosphatidylinositol and phosphatidylserine comprise only a small portion of muscle phospholipids the total content of phospholipids remained unchanged. Cardiolipin is synthesised and located in mitochondria (21). It is known that long term treatment with thyroid hormones increases the oxidative capacity of the muscle mitochondria. The elevation in mitochondrial enzyme activities is more pronounced in a muscle composed of slow-twitch fibers than in a muscle composed of fast — twitch fibers (22). Treatment with thyroid hormones for the shorter period of time had no effect on the activity of the mitochondrial enzymes in the muscles, though their activity in the liver increased markedly over the same period (23). Our rats were treated with T_3 for only 6 days. This might account for the lack of a significant elevation in the content of cardiolipin in the muscles. Studies performed on other tissues also indicate that the content of particular phospholipid fractions may be regulated individually. Treatment with isoprenaline increases incorporation of (^{32}P) Pi into the heart sphingomyelin to a greater extent than into the other phospholipids examined (24). Insulin stimulates turnover of phosphatidylcholine but not other choline

phospholipids in rat adipocytes (25). The effect of diabetes on the phospholipid composition in human blood cells depends both on the phospholipid fraction and the cell type (26). Triiodothyronine treatment increases the content of cardiolipin, phosphatidylinositol and phosphatidylserine but reduces the content of phosphatidylethanolamine and phosphatidylcholine in rat heart mitochondria (27). In the present study we have provided evidence for a selective effect of triiodothyronine excess on the phospholipid content in skeletal muscles. As it can be seen from *Fig. 1* T_3 excees did not affect the rate of 14C-palmitic acid clearance from the plasma and most radioactivity was eliminated within the first five minutes after administration of the label in both groups. However, at the same time, specific activity of the plasma free fatty acids in T_3 — treated rats was much lower than in the controls (by 54—59%). In the other words, the label was more "diluted" in the plasma free fatty acids of T_3 rats than in the controls ($\sim$2.3 times in the first five minutes). This indicates that the labeled palmitic acid represents a smaller percent of total fatty acids taken by the myocytes and incorporated into the phospholipid moieties in T_3 — treated than in the control rats. In consequence, the real amount of fatty acids incorporated into the muscle phospholipids of T_3 — treated rats was over twice as high as that shown by the specific activity of the phospholipids. Obviously, it was much higher than in the control group. Since the content of phospholipids remained either stable or was reduced (in the case of phosphatidylinositol and phosphatidylserine) in the experimentally hyperthyroid rats, the above data strongly suggest that T_3 increases not only acylation but also deacylation of the phospholipid moieties in the muscles. Several features of the cell membranes depend on the fatty acid composition of their phospholipids (28—30). Our results suggest that an excess of T_3 may facilitate changes in the composition of the myocyte phospholipid fatty acids, and thus may modify certain properties of the membranes.

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