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EFFECTS OF HYPERTHYROIDISM ON LIPID CONTENT AND COMPOSITION IN OXIDATIVE AND GLYCOLYTIC MUSCLES IN RATS

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Triiodothyronine (T_3) can influence lipid metabolism *via* multiple mechanisms, which generally result in an increase of fatty acids (FAs) oxidation. Consequently, we hypothesize that hyperthyroidism may influence intramuscular lipids accumulation. This increased intramuscular lipid turn-over is possibly accompanied by an increase in fatty acid transporters expression (FAT/CD36, FABPpm, FATP-1,4). In the present study we examined the lipid content and fatty acid saturation status of free fatty acids (FFA), triacylglycerols (TAG), diacylglycerols (DAG) and phospholipids (PL) in skeletal muscle of hyperthyroid rats (n=8). We measured also fatty acid transporters as well as AMP-activated protein kinase (pAMPK/AMPK), peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α), acetyl-CoA carboxylase (pACC/ACC), carnitine palmitoyltransferase I (CPT I) and citrate synthase (CS) protein expression in these muscles. *In vivo* T_3 administration, decreased the content of FFA, particularly in the red gastrocnemius and the TAG fraction, in both the red and white portions of the gastrocnemius muscle. Concomitantly, saturated/unsaturated fatty acids (SFA/UFA) ratio was also decreased, but only in the FFA fraction, irrespectively of muscle's fiber composition. In contrast, T_3 treatment had no effect on the lipid content and saturation status in PL fraction. Triiodothyronine induced also modest activation of AMPK/ACC axis with subsequent increased expression of mitochondrial proteins: CPT I and CS. This was accompanied by increased content of FAT/CD36, but only in the red part of gastrocnemius muscle. These findings support the conclusion that hyperthyroidism increases lipid metabolism, especially in skeletal muscles with high capacity for fatty acid oxidation.

Key words: *fatty acids, acetyl-CoA carboxylase, AMP-activated protein kinase, carnitine palmitoyltransferase I, citrate synthase, lipids, peroxisome proliferator-activated receptor-γ coactivator-1α, skeletal muscles, triiodothyronine, transporters*

INTRODUCTION

Triiodothyronine (T_3), regulates cellular energy metabolism, increasing substrate usage and energy expenditure (1). Furthermore, T_3 has been shown to influence whole body lipid metabolism, particularly adipose tissue lipolysis is increased in the fasting state, resulting in greater release of nonesterified fatty acids into blood stream. However, postprandially this effect is considerably reduced (2). Skeletal muscles, due to their metabolic plasticity and high oxidative capacity, constitute an important target for the action of T_3 (3). Especially oxidative fibers exhibit a high fractional extraction of plasma free fatty acids (FFA), which are then either oxidized or esterified into intramuscular lipid pools (*i.e.* triacylglycerols (TAG)) or membrane phospholipids (PL) (4, 5). Surprisingly, there are only very few data regarding the role of thyroid hormones on lipid metabolism in skeletal muscles. Previous studies demonstrated that hyperthyroidism induced accumulation of triacylglycerols in soleus muscle (5), but others showed no change in the total content of phospholipids after 6 days of thyroid hormone (T_3) treatment (6).

In recent years AMPK has emerged as a key kinase promoting fatty acids oxidation in skeletal muscle (7, 8). When activated, AMPK phosphorylates its downstream substrate acetyl-CoA-carboxylase (ACC). Phosphorylation of ACC inactivates this enzyme and prevents production of malonyl-CoA, a potent inhibitor of carnitine palmitoyltransferase I (CPT I). Enhanced activity of CPT-1, increases the transport of long chain fatty acyl-CoA into the mitochondria for β-oxidation. Therefore, the AMPK/ACC system is thought to play a central role in the regulation of cellular lipid homeostasis (9-12). Several studies indicate that thyroid hormones activate AMPK in a short-term, non-genomic mode (8, 13). Moreover, the peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) is a strong coactivator of thyroid hormone receptor-β (TRβ), and this is likely to be an important site in mediating some effects of thyroid hormone on mitochondrial biogenesis (14). Whether, there is T_3-induced PGC-1α and AMPK coactivation is less apparent.

Currently, there is limited data regarding the role of hyperthyroidism on the regulation of skeletal muscle fatty acid transport. Recent years prove that fatty acids movement across plasma membrane is facilitated by a number of specific membrane proteins, including fatty acid translocase (FAT/CD36), plasma membrane-associated fatty acid binding proteins (FABPpm), and a family of fatty acid transport proteins

(FATP1-6) (15). The expression of these transporters can be regulated chronically by altering their transcription or acutely by shifting their subcellular location from an intracellular pool to the plasma membrane (16). In fact, oxidative muscle fibers exhibit a higher amounts of these transporters than glycolytic fibers and are more sensitive for such regulation (15). Therefore, we examined different muscle types, namely red gastrocnemius, predominantly consisting of fast twitch oxidative-glycolytic fibers, and white part of gastrocnemius muscle, mainly composed of fast twitch glycolytic fibers (17).

In view of the scarcely elucidated role of hyperthyroidism on the regulation of skeletal muscle lipid metabolism, we sought to investigate the effects of hyperthyroidism on the content and composition of intramuscular lipids namely free fatty acids, triacylglycerols, diacylglycerols and phospholipids. In addition we measured the expression of fatty acid transporters: FAT/CD36, FATP-1,4 and FABPpm, as well as the expression of proteins involved in regulation of lipid metabolism such as: AMPK, PGC-1α, ACC, CPT I and CS. For this purpose rats were made hyperthyroid for 10 days and subsequently white (fast-twitch glycolytic) and red (fast-twitch oxidative-glycolytic) sections of gastrocnemius muscle were taken for the analysis.

MATERIALS AND METHODS

Animals and study design

All experimental procedures were performed in accordance with the guidelines of the Ethical Committee for Animal Experiments at the Medical University of Bialystok. Adult male Wistar rats (250-280 g) were housed in controlled conditions (21°C±2, 12 h light/dark cycle) with unrestricted access to water and to a commercial chow. Rats were randomly divided into two groups: control (n=8) and treated with triiodothyronine (T_3) (n=8). Hyperthyroidism was induced by the subcutaneous administration of triiodothyronine (50 μg/100 g body weight, Sigma), daily for 10 days, as reported elsewhere (18, 19). Control animals were treated with 0.9% saline. After the last T_3 injection (24 h), the rats were anesthetized with intraperitoneal injection of pentobarbital in a dose of 80 mg/kg body weight. The animals were in a fasted state at the time of sacrifice and tissue collection. The white and red sections of gastrocnemius were taken and immediately frozen in liquid nitrogen. The blood was collected, centrifuged and subsequently plasma was saved frozen in -80°C until further analyses.

Plasma T_3 and glucose concentration

T_3 concentration was measured in plasma, with commercially available kit, according to manufacture instruction (rat tri-iodothyronine, T_3 ELISA kit, EIAab). Glucose was measured by the enzymatic method using a 2300 STAT Plus glucose analyzer (YSI, US).

Lipid analyses

Plasma levels of FFA and intramuscular lipids (FFA, TAG, DAG and PL) were analyzed by gas-liquid chromatography. Studied lipid fractions were extracted using the Folch method of extraction (20) and modified according to van der Vusse *et al.* (21). Briefly, muscle and serum samples were extracted in chloroform-methanol (2:1, vol/vol) containing the antioxidant butylated hydroxytoluene (0.01%), and an internal standard (heptadecanoic acid) was added. The lipid fractions were separated by thin-layer chromatography silica plates (Kieselgel 60, 0.22 mm, Merck, Darmstadt, Germany) with a heptane: isopropyl ether: acetic acid (60:40:3, vol/vol/vol) resolving solution was used. Lipid bands were visualized by spraying with a 0.2% solution of 3'7'-dichlorofluorescin in methanol and identified under ultraviolet light using standards on the plates. The gel bands were scraped off the plate, transferred into screw tubes and transmethylated with BF_3/methanol. The fatty acid methyl esters (FAMEs) were dissolved in hexane and analyzed by gas-liquid chromatography. A Hewlett-Packard 5890 Series II gas chromatograph with Varian CP-SIL capillary column (50 m 0.25 mm internal diameter) and flame-ionization detector (Agilent Technologies, Santa Clara, CA) were used. Injector and detector temperatures were set at 250°C. The oven temperature was increased linearly from 160°C to 225°C at a rate of 5°C/min. According to the retention times of standards, the individual long-chain fatty acids were quantified. Total content of intramuscular lipids (FFA, TAG, DAG and PL) were estimated as the sum of the particular fatty acid species of the assessed fraction and it was expressed in nanomoles per gram of the muscle weight. While, serum levels of FFA was expressed in nanomoles per milliliter of the plasma. We have also calculated the mole percentage of saturated fatty acids (SFA) and unsaturated fatty acids (UFA) and the ratio SFA/UFA in the plasma FFA and in each intramuscular lipids.

Western blot analyses

Routine Western blotting procedure was applied to detect proteins as described previously (22, 23). Protein content in muscle homogenates were determined using the bicinchonic acid (BCA) assay with bovine serum albumin as a standard. The homogenates (60 μg) and prestained molecular weight markers (Bio-Rad) were separated on 10% SDS-polyacrylamide gels (150 V for 1 h). The proteins were then transferred from the gel to a polyvinylidene fluoride membranes (0.75 A for 1 h) and blocked for 90 min at room temperature in 5% nonfat dry milk in TBST. Subsequently the membranes were immunoblotted with primary antibodies: anti-AMPKα, anti-pAMPKα (Thr172), anti-ACC, anti-pACC (Ser79), anti-PGC-1α (Cell Signaling Technology, CA), anti-CPT I, anti-CS, anti-FAT/CD36, anti-FATP-1,4 and β-actin (Santa Cruz Biotechnology, Santa Cruz, CA), anti-FABPpm (a generous gift from A. Bonen). The primary antibodies were detected with a horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Proteins were visualized using an enhanced chemiluminescence substrat (Thermo Scientific, USA) and quantified by densitometrically (Biorad, Poland). Equal protein concentrations were loaded in each lane as confirmed by Ponceau S staining on the blot membrane. The protein expression (Optical Density Arbitrary Units) was standardized to β-actin expression and finally the control was set to 100% and the experimental group were expressed relative to the control.

Statistical analysis

Values are means ±S.E.M. All data were analyzed by one-way ANOVA followed by the Newman-Keuls post-hoc test, with the significance set at $p<0.05$.

RESULTS

General characteristics and plasma FFA saturation profile

Prolonged T_3 administration caused only a small decrease in body weight compared to control rats (*Table 1*, –16%, $p>0.05$). However, hyperthyroidism induced significant increases in the plasma T_3 (5-fold, $p<0.05$) and glucose (+22%, $p<0.05$) as well

Table 1. General characteristics and plasma FFA saturation profile.

	Control	T_3
Body weight (g)	307.0±24.3	254.2±28.2
Plasma glucose (nM)	6.77±1.22	8.23±1,11*
Plasma T_3 concentration (pg/dl)	240.4±36.4	1200.4±54.9*
Plasma FFA concentration (nmol/ml)	101.9±28.8	489.2±75.7*
Plasma FFA saturation profile:		
mole % SFA	45.8±0.9	51.4±0.5
mole % UFA	54.2±2.0	48.6±0.8
SFA/UFA ratio	0.85±0.4	1.1±0.6

T_3 - triiodothyronine; FFA - free fatty acids; SFA - saturated fatty acids; UFA - unsaturated fatty acids. Data are expressed as means ±S.E.M. (n=8 in each group). *p<0.05, T_3 treatment *vs.* control.

Table 2 Effects of T_3 treatment on the FA saturation status in the intramuscular lipids.

	White gastrocnemius		Red gastrocnemius	
	Control	T_3	Control	T_3
mole % SFA				
FFA	992.6 ± 2.3	67.1 ± 1.9*	86.6 ± 1.4	58.8 ± 0.9*
TAG	38.5 ± 0.9	48.3 ± 0.5	44.2 ± 0.5	45.1 ± 0.5
DAG	81.6 ± 2.0	71.5 ± 1.2	86.7 ± 1.3	67.3 ± 0.8*
PL	43.7 ± 0.5	47.2 ± 0.5	40.1 ± 0.5	43.9 ± 0.6
mole % UFA				
FFA	7.4 ± 0.3	32.9 ± 1.3*	13.4 ± 0.4	41.2 ± 0.4*
TAG	61.5 ± 2.0	51.7 ± 0.5	55.8 ± 0.7	54.9 ± 0.6
DAG	16.5 ± 0.3	28.5 ± 0.6*	13.3 ± 0.3	32.7 ± 4.0*
PL	56.3 ± 1.0	52.7 ± 1.2	59.9 ± 0.9	56.1 ± 1.2
SFA/UFA ratio				
FFA	12.6 ± 6.3	2.0 ± 1.2*	6.5 ± 2.5	1.4 ± 0.9*
TAG	0.6 ± 0.4	0.9 ± 0.4	0.8 ± 0.4	0.8 ± 0.4
DAG	4.9 ± 2.5	2.5 ± 1.2*	6.5 ± 2.9	2.1 ± 1.2*
PL	0.8 ± 0.3	0.9 ± 0.3	0.7 ± 0.3	0.8 ± 0.3

SFA - saturated fatty acids; UFA - unsaturated fatty acids; FFA - free fatty acids; TAG - triacylglycerols; DAG - diacylglycerols; PL - phospholipids; WG - white section of the gastrocnemius; RG - red section of the gastrocnemius; T_3 - triiodothyronine. Data are expressed as means ± S.E.M. (n = 8 in each group). *p<0.05, T_3 treatment *vs.* control.

as FFA levels (4,8-fold, p<0.05), with no apparent change in FA saturation status (*Table 1*).

Effects of T_3 treatment on the intramuscular lipids content

1. Free fatty acids

T_3 administration significantly reduced the content of free fatty acids in the red but not in the white part of the gastrocnemius muscle (*Fig. 1a*, –15%, p<0.05).

2. Triacylglycerols

Similarly, T_3 administration induced significant reduction in the content of triacylglycerols in both red and white muscles (*Fig. 1b*, –68% and –36%, p<0.05, respectively).

3. Diacylglycerols

In contrast, the content of diacylglycerols was significantly higher in the red section of gastrocnemius of hyperthyroid rats (*Fig. 1c*, +48%, p<0.05), and a similar trend for an increase was observed in white part of gastrocnemius (*Fig. 1c*, +15%, p>0.05).

4. Phospholipids

No effects of T_3 treatment on the content of phospholipids, in each muscle, was noticed (*Fig. 1d*, p>0.05).

Effects of triiodothyronine treatment on the fatty acids saturation status in the intramuscular lipids (Table 2)

1. Saturated fatty acids

In the FFA fraction, prolonged exposure to T_3 resulted in significant reduction of the content of SFA in both the white and red portions of the gastrocnemius muscle (*Table 2*, –28% and –32%, p<0.05, respectively). Similarly, in the DAG fraction the amount of saturated fatty acids (SFA) was significantly lower in the red section of gastrocnemius (–22%, p<0.05), but not in the white part of the gastrocnemius of hyperthyroid rats. In TAG and PL fraction induced hyperthyreosis had no effects on the SFA content in each muscles.

2. Unsaturated fatty acids

In the FFA fraction, T_3 induced significant increases in the content of unsaturated fatty acids (UFA) in examined muscles (*Table 2*, 4,5-fold and 3-fold, p<0.05, respectively). Correspondingly in the DAG fraction T_3 administration resulted in significant increases in UFA levels in both the white and red part of the gastrocnemius muscles (+72% and 2,5-fold, p<0.05, respectively). In contrast, in the TAG and PL fractions no significant changes were noticed.

3. Saturated fatty acid/ unsaturated fatty acids

Accordingly, T_3 treatment resulted in a reduction ratio SAT/UFA in the FFA fraction in both the white and red section of the gastrocnemius muscle (*Table 2*, 6-fold and 4,5-fold, p<0.05, respectively), and in the DAG fraction (2-fold and 3-fold, p<0.05, respectively). In the fractions of (TAG, PL) no significant differences were observed.

Effects of thyroid hormone on expression of proteins involved in lipid metabolism

1. Phospho-AMP-activated protein kinase ratio

T_3 administration induced significant increase in the AMPK phosphorylation status, with only minor change in the total amount of AMPK protein in both white and red part of the gastrocnemius, which resulted in overall increase in pAMPK/ AMPK ratio (*Fig. 2a*, +27% and + 37%, p<0.05, respectively).

2. phospho-ACC/ACC ratio

Similarly, but to a greater extend, T_3 treatment induced increases in pACC /ACC ratio in both white and red muscles (*Fig. 2b*, +75% and +90%, p<0.05).

3. Peroxisome proliferator-activated receptor-γ coactivator-1α

After T_3 treatment the expression of PGC-1α was upregulated in the both red and white sections of the gastrocnemius (*Fig. 2c*, +14%, p=0.09).

4. Carnitine palmitoyltransferase I

Modest increases were also observed for CPT I expression followed by T_3 administration (*Fig. 2d*, +11% and +22%, p<0.05).

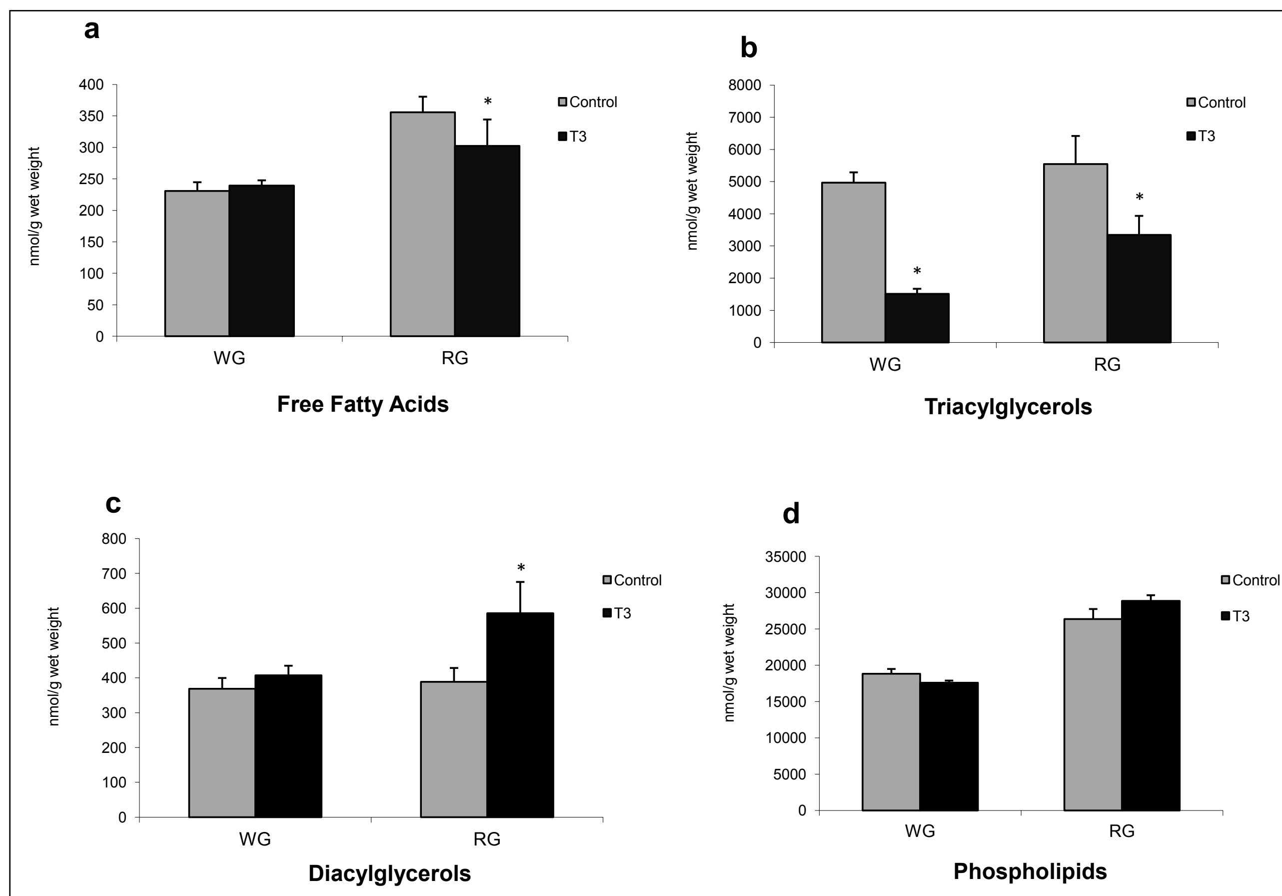

Fig. 1. Effect of T_3 administration on the content of: free fatty acids (a), triacylglycerols (b), diacylglycerols (c), phospholipids (d) in rat skeletal muscles. WG - white section of the gastrocnemius; RG - red section of the gastrocnemius; T_3 - triiodothyronine. Data are expressed as means ±S.E.M. (n=8 in each group). *p<0.05, T_3 treatment *vs*. control.

5. Citrate synthase

Similarly, T_3 induced only modest changes in the expression of CS, reaching the significance level only in the red gastrocnemius muscle (*Fig. 2e*, +12%, p<0.05).

Effects of triiodothyronine treatment on the expression of fatty acid transporters

1. FAT/CD36

In the red part of gastrocnemius, FAT/CD36 protein expression was increased after T_3 treatment (*Fig. 3a*, +20%, p<0.05), but in the white part it remained unaltered (*Fig. 3a*, p>0.05).

2. FATP-4

Similarly, a trend towards an increase in the expressions of FATP-4 was observed in both sections of the gastrocnemius from the hyperthyroid rats, but the differences were not statistically significant (*Fig. 3b*).

3. FABPpm

There was no difference in the protein expression of FABPpm in the white and red section of the gastrocnemius after T_3 treatment (*Fig. 3c*).

FATP-1

FATP-1 expression was decreased (*Fig. 3d*, -31% and -25%, p=0.07, respectively) in the white and red of hyperthyroid muscles compared with control muscles.

DISCUSSION

The aim of the present study was to determine whether prolong (10 days), *in vivo* treatment with triiodothyronine would influence lipid metabolism in rat skeletal muscles. We have demonstrated that T_3 administration decreased the content of FFA, particularly in the red gastrocnemius, and TAG fraction in the both parts of the gastrocnemius muscles. Interestingly, T_3 treatment had ambiguous effects on fatty acid transport proteins, favoring an increase in FAT/CD36 content with concomitant decrease in FATP-1 expression. Most likely, T_3-induced effects resulted from the AMPK activation, followed by modest enhancements in the expression of CPT-1 and CS mitochondrial proteins.

Fatty acids metabolism in the hyperthyroid muscles

Myocytes have virtually no ability to synthesize *de novo* fatty acids and therefore, they must obtain long-chain fatty acids (LCFAs) from the plasma (24). Following entry, LCFAs are channeled into different metabolic routes and a limited part remains as free fatty acids fraction (25). The increased entry of

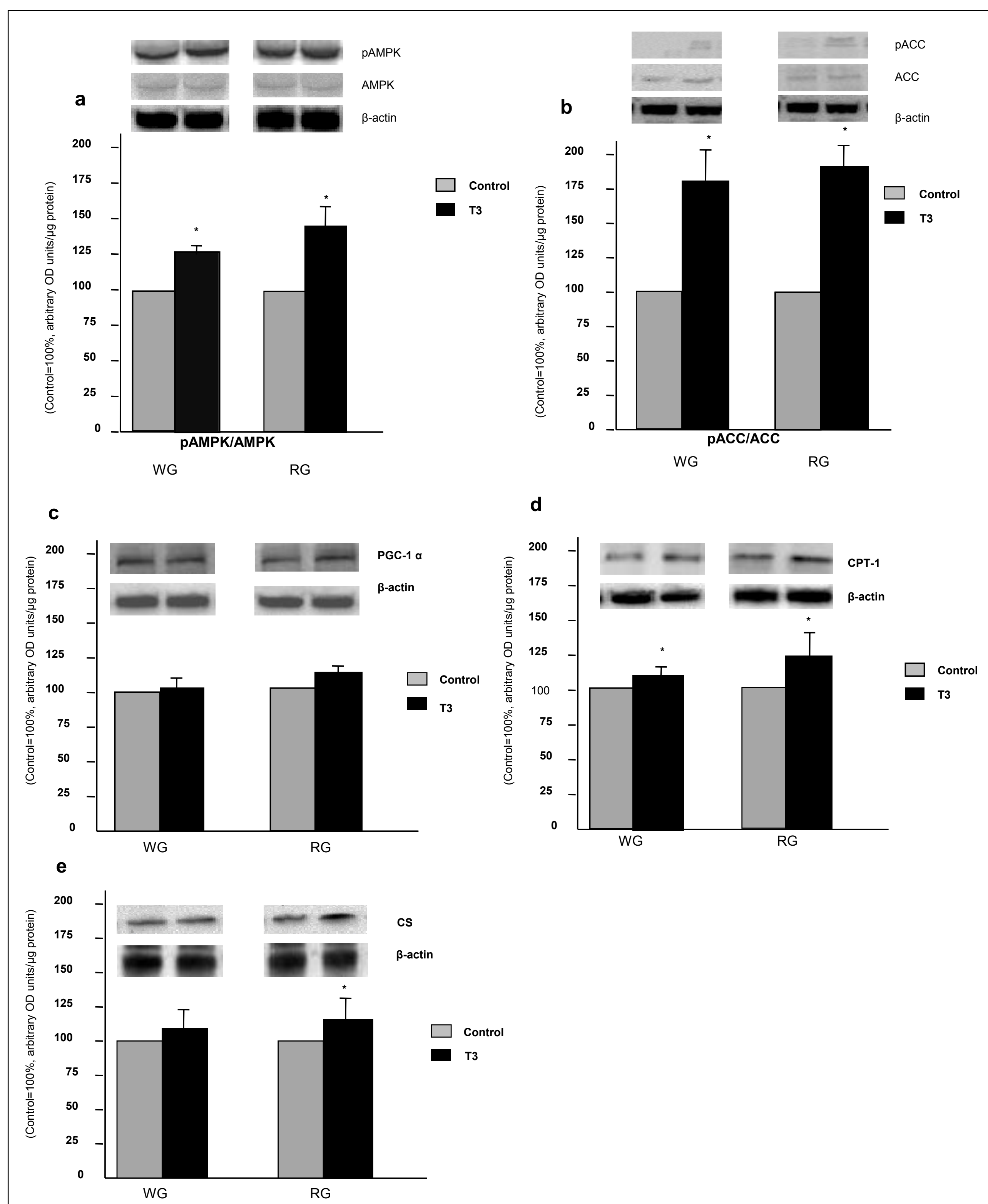

Fig. 2. Effect of T_3 administration on the expression of : phospho-AMPK /AMPK ratio (a), phospho-ACC/ACC ratio (b), PGC-1α (c), CPT I (d), CS (e) in rat skeletal muscles. Representative Western blots are shown. AMPK - AMP-activated protein kinase; pAMPK - phosphorylation of AMP-activated protein kinase; ACC - acetyl-CoA carboxylase; pACC - phosphorylation of acetyl-CoA carboxylase; PGC-1α - peroxisome proliferator-activated receptor-γ coactivator-1α; CPT I - carnitine palmitoyltransferase I; CS - citrate synthase; WG - white section of the gastrocnemius; RG - red section of the gastrocnemius; T_3 - triiodothyronine. Data are expressed as means ±S.E.M. (n=8 in each group). *p<0.05, T_3 treatment *vs.* control.

FFAs into the myocyte without a corresponding increase in FA oxidation contributes to the accumulation of intramuscular lipids.

In the present study, as well as, in previous reports (5, 6, 18, 19, 26) higher concentration of plasma FFAs were noticed in

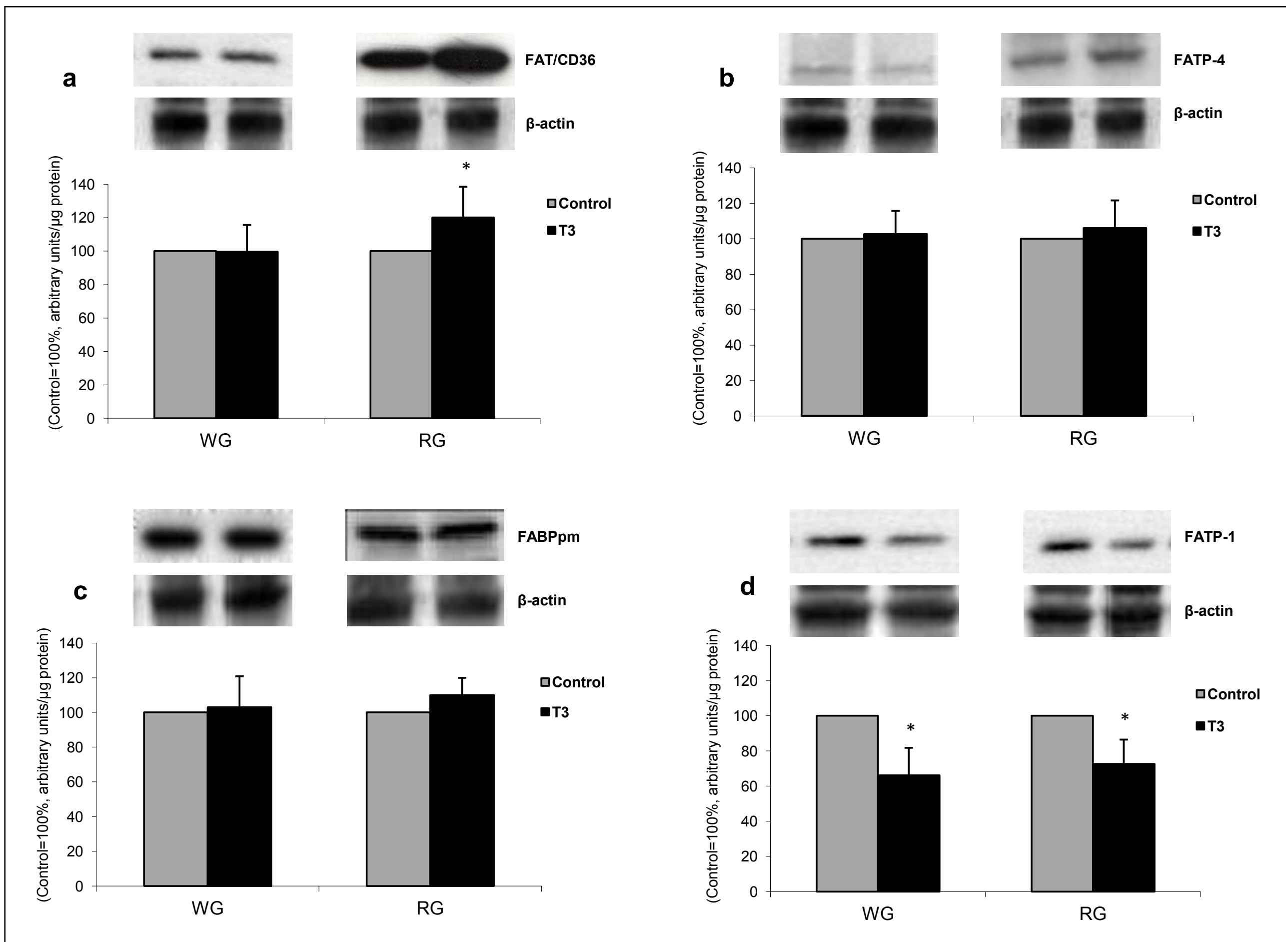

Fig. 3. Effect of T_3 administration on the expression of: FAT/CD36 (a), FATP-4 (b), FABPpm (c), FATP-1 (d) in rat skeletal muscles. Representative Western blots are shown. FAT/CD36 - fatty acid translocase; FATP-1,4 - fatty acid transport protein; FABPpm - plasma membrane associated fatty acid binding protein; WG - white section of the gastrocnemius; RG - red section of the gastrocnemius; T_3 - triiodothyronine. Data are expressed as means ±S.E.M. (n=8 in each group). *$p<0.05$, T_3 treatment *vs*. control.

hyperthyroid rats. Likely, greater plasma FFA availability resulted from T_3-induced lypolysis of adipose tissue (27, 28). Thus, hyperthyroidism, by increasing plasma FFA level leads to a considerably greater LCFA concentration gradient across the plasma membrane, which favors the entry of fatty acids into the myocytes *via* passive diffusion. As a result an increase in myocyte FFA fraction and/or other intramuscular lipid accumulation should appear. However, hyperthyroidism concomitantly increases FA oxidation in skeletal muscles (5, 7, 18, 26, 29). Accordingly, we observed an increase in AMPK activation, with subsequent increased expression of mitochondrial CPT-1 and CS proteins, which likely resulted in an increase of FAs oxidation. This may explain a decrease in the content of intramuscular FFA and TAG fractions, which are the major sources of energy in oxidative fibers of skeletal muscle. Recently, Chabowski *et al.* showed that contractile activity (which also triggers AMPK activation) can reduce intracellular FFA pool in soleus muscle (30). Summing up our results suggest that T_3 enhances intramuscular lipids metabolism (likely both TAG hydrolysis and FA uptake) by stimulation of FAs oxidation. Unexpectedly, we found that T_3 induced an increase in DAG content, in red part of gastrocnemius muscle. Most likely, higher DAG content serves as FA temporary reservoir, subsequently used for FA oxidation and/or synthesis of other lipid fractions (*i.e*. TAG and PL) (24, 31). On the other hand, both processes: FA oxidation and esterification seem to be highly dependent on the saturation profile FAs (31, 32). Unsaturated fatty acids are more readily oxidized than saturated, which are less efficiently incorporated into triacylglycerols, and thus it may lead to the increased accumulation of DAG (31). Further, our results show a general lack of significant changes in the levels of phospholipids in muscles of hyperthyroid rats. This is in agreement with Zendzian-Piotrowska *et al*. who found no change in the content of intramuscular PL`s fraction from hyperthyroid rats (6). This is not surprising, given that phospholipid fraction is a very stable pool of the membrane lipids (24).

In the present study we also examined the saturation status of FAs present in each intramuscular lipid fractions (FFA, TAG, DAG and PL) and plasma FFA. Our results have shown T_3 induced significant decrease in FA saturation status, paralleled by a proportionate enhancement in unsaturated fatty acids content, but only in the FFA and DAG fractions. Likely, UFA taken up from plasma, are more readily oxidized, as it has been shown in cell cultures in which unsaturated long chain fatty acids present in incubation medium, promoted FAs oxidation, while introduction of saturated FA to this incubation increased lipid accumulation (31, 33).

Expression of proteins involved in lipid metabolism in the hyperthyroid muscles

The stimulation of fatty acid oxidation by T_3 appears to be mediated by activation of AMPK–ACC axis. Park *et al.*

demonstrated that AMPK expression was increased after T_3 administration, especially in the soleus (34, 35), which is further confirmed in our study. The role of AMPK in the orexigenic actions of thyroid hormones is still not fully explored (36), however, it is unlikely that thyroid hormones would directly activate AMPK, since most of thyroid hormone effects are thought to be mediated by regulation of gene transcription. It is more likely that AMPK is activated secondarily to the increased metabolic rate (depleted cellular ATP and elevated AMP levels) (34, 35). Presumably, in skeletal muscle, T_3 treatment activates also AMPK *via* CaMKKβ activation and intracellular Ca^{2+} mobilization (8). Increased AMPK activity, in turn, decreases the level of malonyl-CoA and stimulates mitochondrial fatty acid oxidation. In our study, T_3 administration resulted in activation of AMPK with subsequent phosphorylation of ACC and increased expression of CPT I, a rate limiting mitochondrial enzyme involved in FA oxidation. Recent study also supports our observation that muscle CPT I activity is increased after thyroid hormone treatment as the result of lowered concentration of malonyl-CoA (a potent inhibitor of CPT I) (7). It also appears that T_3-induced AMPK activation in skeletal muscles is directly associated with increased expression of PGC-1α (8, 14). This mechanism might be important in mediating hormone-induced increase in mitochondrial biogenesis as it was shown that PGC-1α transcription factor can influence fatty acid oxidation *via* its overall regulation of mitochondrial biogenesis (37). Irrcher *et al*. found that PGC-1α expression is increased in skeletal muscle after 5 days of triiodothyronine treatment, with the most pronounced changes occurring in oxidative muscles (14). We confirm the increased PGC-1α expression after T_3 administration in the red muscle, with concomitant increase in mitochondrial biogenesis as suggested by enhanced citrate synthase expression. Finally, the study by Benton *et al*. indicated that when PGC-1α expression was increased triacylglycerol synthesis was concomitantly reduced (38). Taken altogether these results support the observations that hyperthyroidism increased fatty acid oxidation in rat skeletal muscle.

Fatty acid transporters expression in the hyperthyroid muscles

Factors, such as serum free fatty acids availability and the content of FA transport proteins, may have an effect on FA metabolism in muscle (2). It has been suggested that upregulation of fatty acid transport into muscle could contribute to skeletal muscle lipid oversupply and subsequent accumulation of intramuscular lipids. Specifically, it has been shown that an increased abundance of the FA transporters (FAT/CD36, FATP-1,4, FABPpm) in the sarcolemma of skeletal muscle leads to increased FA transport into the cell, which is associated with subsequent muscle lipids accumulation (15, 16, 39). As far, most studies suggest that among putative FA transporters, FAT/CD36 is the major player as its expression is highly correlated with the FA transport rates into myocytes and muscles FA oxidative potential (15, 16, 40). Importantly, skeletal muscles containing more oxidative fibers have higher rates of FA transport and oxidation, correlated with higher content of fatty acid transport proteins (16, 40). Thus, in present study, greater protein expression of FAT/CD36 in red gastrocnemius of hyperthyroid rats most likely increased skeletal muscle fatty acid metabolism. Interestingly, the expression of other FA transporters (FABPpm and FATP-4) remained quite stable and the content of FATP-1 decreased along with T_3 administration. This further indicates that, the precise molecular mechanism of FA transporters actions remains ambiguous. Recently, Nickerson *et al*. showed that, FA transporters exhibit different capacities for fatty acid transport and metabolism in rat skeletal muscles, showing that, muscle FATP1 overexpression, unlike overexpression of other fatty acid transporters, is rather ineffectually correlated with either FA transport or oxydation (16).

In conclusion, the results from present study demonstrate that hyperthyroidism increased lipid metabolism in skeletal muscle, particularly in highly oxidative red muscles that are more reliant on fatty acid oxidation. Specifically, despite marked increases in circulating fatty acids in T_3 treated animals, there were reductions in intramuscular fatty acids and TAG fractions. These changes resulted T_3 induced activation of AMPK axis, with subsequent increased expression of mitochondrial proteins, that are associated with the upregulation of fatty acid oxidation.

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Conflict of interests: None declared.

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