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<th>Journal:</th>
<th>Biochemistry and Cell Biology</th>
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<td>Manuscript ID</td>
<td>bcb-2015-0051.R1</td>
</tr>
<tr>
<td>Manuscript Type:</td>
<td>Article</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>05-Oct-2015</td>
</tr>
<tr>
<td>Complete List of Authors:</td>
<td>Górecka, Monika; Mossakowski Medical Research Centre, Department of Applied Physiology Synak, Marcin; Mossakowski Medical Research Centre, Department of Applied Physiology Brzezińska, Zofia; Mossakowski Medical Research Centre, Department of Applied Physiology Dąbrowski, Jan; Mossakowski Medical Research Centre, Department of Applied Physiology Zernicka, Ewa; Mossakowski Medical Research Centre, Department of Applied Physiology</td>
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Effect of triiodothyronine (T₃) excess on fatty acid metabolism in the soleus muscle from endurance-trained rats.

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Abstract

We studied whether short-term T3 administration at the end of endurance training will influence the rate of palmitic acid (PA) uptake and metabolism in rat soleus muscle in vitro. Training per se did not affect the rate of PA uptake by the soleus, however, T3 excess increased the rate of this process at 1.5mM PA and the rate of PA incorporation into intramuscular triacylglycerols (TG). In trained euthyroid rats the rate of TG synthesis was reduced after exercise (1.5mM PA). In all trained rats immediately after exercise the rate of PA oxidation was enhanced compared to sedentary value. Hyperthyroidism additionally increased the rate of this process at 1.5mM PA. After recovery the rate of PA oxidation returned to control value in both groups. High-energy phosphates content demonstrated that elevated PA oxidation after exercise-training in euthyroid rats was associated with stable ATP concentration and increased ADP and AMP content thus reducing energy cellular potential (ECP). In hyperthyroid rats ADP and AMP were increased in sedentary and exercise-trained rats. After recovery in hyperthyroid rats ECP was high resulting from high ATP and decreased ADP and AMP. In conclusion, already short-term hyperthyroidism accelerates PA utilization in well-trained soleus muscle.

Keywords: Short-term hyperthyroidism, Fatty acid uptake, Triacylglycerols, Skeletal muscle
Introduction

Hyperthyroidism induces many biochemical, functional and structural changes in skeletal muscles leading to an increase in energy expenditure (Simonides and van Hardeveld 2008). To cover the increased energy requirements of skeletal muscles utilization of energetic fuel is modified and both anaerobic and aerobic metabolic pathways are stimulated.

In hyperthyroid state both glucose and fatty acids (FA) are intensively metabolized. The availability of FA in blood is increased due to catecholamines-induced lipolysis in white adipose tissue (Oppenheimer et al. 1991, Germack et al. 2000). This is accompanied by an increased uptake of FA from albumin-FA complex by skeletal muscles from hyperthyroid animals in vivo (Klieverik et al. 2009) and in vitro (Górecka et al. 2004). It was suggested that T₃ excess may also increase the muscle FA uptake from triacylglycerol-rich lipoproteins circulating in blood (Kaciuba-Uściłko et al. 1980, Klieverik et al. 2009) although the activity of lipoprotein lipase (LPL), the rate-limiting enzyme of plasma triacylglycerol (TG) hydrolysis, is not affected (Klieverik et al. 2009) or decreased (Kaciuba-Uściłko et al. 1980, Żernicka et al. 1994). Thus, both increased skeletal muscles oxidative capacity and elevated FA availability in experimental hyperthyroidism lead to an increased FA oxidation (Silvestri et al. 2005). T₃ excess also affects the incorporation of FA into intramuscular lipids (Kaciuba-Uściłko et al. 1980, Żendzian-Piotrowska et al. 2000). This effect of T₃ excess on intramuscular TG depends on skeletal muscle phenotype: in slow-twitch, oxidative muscle the rate of FA incorporation into cellular TG is elevated and a higher TG content is noted (Żernicka et al. 1994, Górecka et al. 2004), whereas in fast-twitch oxidative-glycolytic muscle the intramuscular TG content is decreased (Kaciuba-Uściłko et al. 1980, Miklosz et al. 2012).

In our previous study we confirmed the impact of T₃ excess on lipid metabolism in skeletal muscle of sedentary rats (Górecka et al. 2004) resulting in an increased rate of palmitic acid (PA) uptake by soleus m. in vitro as well as an increased rate of PA oxidation and incorporation into intramuscular lipids.

FAs are the main energetic fuel for skeletal muscle not only at rest but also during prolonged exercise of moderate intensity. Endurance training leads to an additional increase in lipids contribution to muscle oxidative metabolism (Dyck et al. 2000, Kiens 2006).

It is well known that despite the increased oxidative capacity of skeletal muscles in hyperthyroid state the body tolerance to exercise is greatly reduced, the reason for which is not fully understood. The influence of thyroid hormone excess on FA uptake and metabolism in endurance trained rats is not well studied either. Thus, the main aim of this study was to determine whether the administration of T₃ for the last three days of a five-week endurance training would affect the rate of FA uptake from FA-albumin complex and its metabolism in soleus m. in vitro immediately after the end of exercise-training and following 24h recovery.
Materials and methods

Experimental animals

This study protocol was approved by the 1st Ethics Committee at Polish Academy of Sciences in Warsaw in accordance with the Guide for the Care and Use of Laboratory Animals.

Male Wistar rats weighing initially 70-80g were housed in groups of ten in temperature controlled room (22-24°C), fed a standard rat chow and provided with drinking water ad libitum.

The animals were randomly divided into two main groups: 1) hyperthyroid rats given i.p. triiodothyronine (T3, L-triiodothyronine sodium B.P.; Glaxo Laboratories, Greenford, Middlesex, UK) at a daily dose of 75µg/100 g body mass for 3 days prior to the experiments, leading to a ninefold increase in circulating T3, as described previously (Dubaniewicz et al. 1989, Żernicka et al. 1994); 2) euthyroid - control rats treated with saline (0.9% NaCl) for three days.

Each group was divided into three subgroups: a) sedentary, b) endurance-trained animals, running on a treadmill 5 days per week for 5 weeks for 40-60min daily from 16m/min during the first week up to 28m/min during the last two weeks, with the last run just prior to the experiment or c) endurance-trained animals, which recovered from the last bout of exercise for 24h prior to the experiment. Thus, there were six experimental groups of rats: hyperthyroid sedentary (HS), hyperthyroid trained-exercising (HTEx), hyperthyroid trained-recovering (HTR), control sedentary (CS), control trained-exercising (CTEx), control trained-recovering (CTR). All animals learned to run on a motor-driven rodent treadmill for a short period of time before training.

Experimental procedure

Rats, deprived of food for 14-16h, were anaesthetized with sodium pentobarbital (60 mg/kg b.w. i.p.) and killed by crushing the spinal cord in the neck. Then, soleus muscles were extracted from both hindlimbs. Soleus from left legs deprived of visible connective and adipose tissue were frozen in liquid nitrogen and then stored at -70°C for further analyses. The soleus m. from right legs were used for in vitro incubation. Blood samples were collected, centrifuged and serum was frozen in liquid nitrogen and stored at -70°C until analyses.

An in vitro procedure was applied to measure FA incorporation and utilization (Górecka et al. 2001). Briefly, the soleus m. was dissected longitudinally into two halves of similar weight to optimize diffusion. The muscle strips were tied to stainless steel clips, and immediately transferred to Erlenmeyer flasks containing pre-incubation medium. They were pre-incubated for 15 min at 37°C in 5 ml of modified Krebs-Ringer bicarbonate buffer (pH 7.4) containing 2% bovine serum albumin, 5.5mM glucose and 0.5mM or 1.5mM palmitic acid (PA). After pre-incubation, the muscles were transferred to flasks with the same but fresh incubation medium additionally containing traces of labelled [1-14C]palmitic acid (0.6 µCi/ml; 1 Ci = 37 GBq) (NEN, Boston, USA).
in which they were incubated for 20 min. The palmitic acid used in all experiments was solubilized in 5% de-fatted serum bovine albumin in 0.9% saline solution at pH 7.4 (Borgstrom and Olivecrona 1961). The stock solution was then diluted with the incubation buffer to achieve the desired final concentrations. The medium was gassed continuously with O₂/CO₂ (95:5 v/v) during the pre-incubation and incubation periods.

At the end of the incubation muscle strips were removed from the flasks and freeze-clamped in liquid nitrogen. Paper filters saturated with 10% NaOH were then suspended in the flask (Christiansen et al. 1976), the flasks were rapidly resealed and the medium was acidified with 50% HClO₄ (Leighton et al. 1985). The released CO₂ was absorbed onto a blotting filters for 90 min in room temperature. The filter papers were then transferred into scintillation vials and the ¹⁴CO₂ counted using a liquid scintillation counter (Tri-Carb 2100TR, Packard, USA).

Biochemical assays

Lipids from muscle strips were extracted overnight in the chloroform-methanol (2:1) mixture according to Folch et al. (1957). Thin layer chromatography (TLC) was used to separate and quantify the lipid classes (Mangold 1969). The lipid extracts containing fatty acids (FA), triacylglycerols (TG), mono- (MG) and diacylglycerols (DG) and phospholipids (PL) were dried under a stream of nitrogen and re-dissolved in a known, small volume of Folch mixture. Then, they were quantitatively applied on TLC plates (Silica Gel 60, Merck, Germany, art. 5554) and developed in heptane : diethyl ether : acetic acid (160:80:3). The lipid spots were visualized in iodine vapour using standards as references. Then the lipid spots were scrapped off the plates and transferred to scintillation vials for counting (Tri-Carb 2100TR, Packard, USA).

TG concentration in plasma and soleus m. was measured using the enzymatic method (Eggstein and Kuhlmann 1973). Nonesterified free fatty acid (FFA) levels in plasma were measured using standard enzymatic colorimetric assays (NEFA C test, Wako Chemicals GmbH, Neuss, Germany). Glycogen content in freeze-dried soleus m. was determined after hydrolyze in 1M hydrochloric acid at 100°C (Harris et al. 1974) with the use of the Glucose HK Assay Kit (Sigma-Aldrich, USA). Freeze-dried muscle samples were analyzed for ATP, ADP and AMP content using the enzymatic micro-methods described by Harris et al. (1974). The energy cellular potential (ECP) was calculated from Atkinson’s equation (1968).

Calculation

As we found previously (Górecka et al. 2001, 2004) the FA fraction extracted from soleus muscle originated from the extracellular space so the rate of total palmitate uptake was calculated according to the equation:

\[ \frac{([R_m + R_{CO_2}] - R_{FA})/A_1)}{\text{muscle weight x incubation time}}, \]

where \( R_m \) is the total radioactivity extracted from the muscle, \( R_{CO_2} \) designates the radioactivity of released CO₂, \( R_{FA} \) is the radioactivity of FA fraction and \( A_1 \) stands for the specific radioactivity of the incubation medium (in counts per minute/nmol PA).
Statistical analysis

Data are presented as means ± SEM. The significance of differences between groups was calculated using Student’s t-test for unpaired data. Within-group analyses were performed using one-way ANOVA: parametric ANOVA followed by Tukey test or a non-parametric Mann-Whitney test depending on whether the data were normally distributed and passed Levene’s test for homogeneity of variance. Statistical threshold was set at p<0.05.

Results

There are no statistically significant differences in body weight between the experimental groups. However, the rats treated with T3 for three days were found to have significantly higher (by 32-35%, p<0.001) ratio of heart mass to body mass than euthyroid controls, independently of their physical activity (Table 1). This is an indirect indication that the hyperthyroid state was achieved.

Lipids serum concentration is shown in Table 2. FFA concentration was elevated in HS group (by 56%, p<0.001) as compared to CS one and remained elevated in all hyperthyroid trained rats (N.S.). Endurance training had no effect on FFA concentration in control and hyperthyroid rats. TG concentration was markedly increased HS and HTR groups (p<0.001 and p<0.01, respectively) when compared with controls. In both CTEx and HTEx groups a significant decrease (p<0.001) in TG level was observed when compared to sedentary values. After 24h recovery TG concentration still remained significantly reduced in CTR rats, while in HTR rats the TG level increased close to sedentary value.

Intramuscular TG content in the soleus m. from control euthyroid rats amounted to 4.97 ± 0.57 µmol/g w.w. and was slightly increased (N.S.) after training (Table 2). T3 excess caused a marked increase (p<0.001) in intramuscular TG content only in sedentary rats.

It was found that T3 excess had no effect on glycogen content in the soleus m. (Table 2). As predicted, immediately after exercise-training glycogen level was markedly decreased as compared to sedentary value in both CTEx and HTEx rats, however, in HTEx group the decrease was less pronounced (CTEx: p<0.001; HTEx: p<0.01). After 24h recovery glycogen content rose over sedentary level by 30% and 48% in CTR and HTR, respectively.

Soleus m. concentrations of adenine nucleotides as well as energy cellular potential (ECP) are shown in Table 3. In sedentary rats short-term hyperthyroidism caused a significant reduction in ATP content and a rise in concentrations of ADP (p<0.001) and AMP (p<0.001), which led to a marked reduction (p<0.001) in ECP value as compared to euthyroid control. In CTEx and CTR groups ATP concentration did not change from sedentary value, but an increase in concentrations of ADP (CTEx: p<0.01; CTR: p<0.05) and AMP (CTEx: p<0.01; CTR: p<0.001) was found. These changes led to reduction in ECP values (CTEx: p<0.05; CTR: p<0.01) as compared
with sedentary values. In HTEx group a further increase in ADP (p<0.01) and AMP (p<0.05) concentrations, but without changes in ATP concentration and ECP value compared to CTEx rats were observed. However, after 24h recovery from the last bout of exercise in HTR group a significantly higher ATP concentration and reduced content of AMP compared to CTR group (p<0.05) as well as to HTEx one (ATP: p<0.001; AMP: p<0.01) were found. Thus, in HTR group the ECP of soleus m. had markedly higher values than the ones obtained in CTR or HS and HTEx rats.

In all experimental groups, as in our previous studies (Górecka et al. 2001, 2004), the rates of PA uptake and metabolism in soleus m. *in vitro* rose significantly with an increasing concentration from 0.5mM to 1.5mM in the incubation medium (Table 4-6).

The rate of PA oxidation to CO$_2$ in the soleus m. of hyperthyroid rats at 0.5mM PA was slightly elevated when compared to control. At 1.5mM PA the rate of this process was also increased by T$_3$ excess in all groups, however only in HS and HTEx rats was the rise significant (Table 4). In both CTEx and HTEx rats the rate of PA oxidation to CO$_2$ was significantly (p<0.01) higher than in sedentary ones at both PA concentrations, whereas after 24h of recovery the process rate returned to sedentary value.

A marked increase (by 21-46%) in the rate of PA incorporation into intramuscular TG in soleus m. in HS and HTEx rats was found, independently of PA concentration in the medium, while in HTR group the rate of this process was significantly increased compared to controls only at 1.5mM PA (Table 5). It was noted that in CTEx rats at 1.5mM PA the rate of TG synthesis was reduced by 15% (p<0.01), while in CTR rats the process rate returned to sedentary level. In HTEx animals the rate of PA incorporation into intramuscular TG tended to decrease as compared with sedentary value, T$_3$ excess had no effect on the rate of PA incorporation into MG/DG in the soleus m. except for a significant increase in the process rate which was found in HS rats at 0.5mM PA (Table 5). In both CTEx and HTEx rats the rate of PA incorporation into MG/DG had a tendency to decrease (by 21-39%) at 1.5mM PA, while in CTR and HTR groups it did not differ from sedentary levels. The rate of PA incorporation into phospholipids in soleus m. from HS rats was slightly but significantly decreased at 0.5mM PA, while at 1.5mM PA it did not differ from control value (Table 5). Neither in euthyroid nor in hyperthyroid animals did the endurance training affect the rate of this process.

The rate of total PA uptake by the soleus m. in sedentary rats was significantly increased by T$_3$ excess (by 16-31%, p<0.01), independently of PA concentration in the medium (Table 6). In trained groups the rate of this process was markedly elevated by hyperthyroidism at 1.5mM PA in the medium (by 16% and 30% in HTEx and HTR, respectively). In both CTEx and HTEx groups the rate of PA uptake by soleus m. was at sedentary levels. In HTR animals after 24h of recovery the rate of this process was higher than in HTEx (p<0.05) and HS (N.S.) groups.
Discussion

In our previous paper (Górecka et al. 2004) we demonstrated that three-day T₃ excess in vivo changed lipid metabolism in sedentary rats by augmenting the rate of PA uptake by soleus m. in vitro and its oxidation and incorporation into intramuscular lipids. These findings led us to the question whether in exercise trained rats the injection of T₃ would influence the PA uptake and its further utilization by soleus m. as both T₃ and endurance training are known to increase the oxidative capacity of skeletal muscles.

Lipid profile of rats

In this study we were particularly interested in the effects of experimental short-term hyperthyreosis in trained rats. It is well known that endurance training results in an increased adipose TG lipolysis followed by higher FFA contribution to energy metabolism (Dyck et al. 2000). We found that short-term hyperthyroidism caused a marked increase in plasma FFA concentration in sedentary rats, while in hyperthyroid trained rats plasma FFA concentration was only slightly higher than in controls. Elevated FFA concentration was observed in HS rats by others (Żernicka et al. 1994, Górecka et al. 2004, Klieverik et al. 2009, Miklosz et al. 2012), however, no effect of short-term hyperthyroidism on FFA concentration in trained rats after 24h recovery was observed in our previous study (Żernicka et al. 1994). Now we found a more pronounced effect of T₃ on blood TG concentration which was markedly increased in HS and HTR rats when compared to controls, probably as a result of decreased LPL activity in skeletal muscles of these rats (Zernicka PhD Thesis, unpublished). However, the impact of free glycerol which is insignificantly elevated by T₃ excess cannot be ruled out (Bernardes et al. 2014). The decrease in blood TG concentration in CTR animals as compared to CS ones was previously described by others (Mondon et al. 1984, Lira et al. 2008) and may result from decreased hepatic VLDL-TG secretion and increased LPL activity in oxidative skeletal muscle and the heart (Żernicka et al. 1994). Moreover, a significant reduction in blood TG concentration noted in both CTEx and HTEx groups was more pronounced in hyperthyroid rats. Interestingly, there are no data on the effect of training on LPL activity in rats with short-term T₃ excess directly after exercise but, contrary to euthyroid trained rats, we may expect no increase in this enzyme activity. As found by Kaciuba-Uściłko et al. (1980), the uptake of TG by skeletal muscle is markedly increased in rats with T₃ excess. It seems possible that during exercise this process is further accelerated.

The influence of endurance training on intramuscular TG content in soleus m. is not clear. Lee et al. (2002) found no changes in TG content in soleus m., other authors noted a decrease (Nikolaidis et al. 2004) or an increase (Nadeau et al. 2006, Chabowski et al. 2012). In all our trained controls and hyperthyroid rats we found no changes in TG content in soleus m. pointing to the maintenance of a dynamic balance between the processes of TG synthesis and lipolysis. Short-term T₃ excess augmented intramuscular TG content in soleus m.
only in sedentary group despite the fact that the rate of PA incorporation into TG pool \textit{in vitro} was increased independently of the animal physical activity.

\textit{Cellular energy state of soleus muscles}

Hyperthyroidism is known to increase basal metabolic rate by increasing ATP turnover and expenditure but it also leads to a decrease in the efficiency of ATP synthesis (Silva 1995, Arruda et al. 2003, Bahi et al. 2005). Additionally, T\textsubscript{3} excess affects physical activity and its energy cost. We found it interesting to compare the high-energy phosphates content in soleus m. from eu- and hyperthyroid rats, especially after prolonged endurance training. Our present results show that short-term hyperthyroidism in sedentary rats reduces ATP content in soleus m. which is accompanied by a marked increase in ADP and AMP concentration probably resulting from decreased efficiency of ATP synthesis (Silva 1995). It should be kept in mind that in sedentary rats hyperthyroidism strongly activates ATP-consuming processes in skeletal muscles (Arruda et al. 2003, Yamada et al. 2004) and increases the rate of ATP synthesis (Short et al. 2001, Silvestri 2005). Kuwahara et al. (2010) reported that reduced ATP concentration in rodent skeletal muscles may result from an increased production of reactive oxygen species and oxidative damage in mitochondria. Additionally, Venditti et al. (2007) observed that these processes are increased by short-term T\textsubscript{3} excess. However, in our HTEx rats ATP concentration as well as ECP value in the soleus m. were similar to those noted in CTEx group. This finding may support the hypothesis that endurance training has protective effects on hyperthyroid skeletal muscles (Venditti et al. 2009).

In euthyroid rats slow-twitch oxidative muscles such as soleus m. can maintain almost constant level of ATP during contraction (Whitlock and Terjung 1987). This finding is in line with our results showing no change in ATP content in the soleus m. of sedentary euthyroid rats and those after exercise-training followed by 24h of recovery. However, a significant decrease in ECP values in both euthyroid trained groups resulting from the increase in ADP and AMP content was noted.

As mentioned before, both CTEx and HTEx groups had showed a similar cellular energy state at the end of exercise, however, marked discrepancies were found after 24h recovery. In HTR rats the ATP content reached higher values than in other groups with a simultaneous reduction in ADP and AMP content leading to an important increase in ECP value even over sedentary level. These results may indicate that short-term hyperthyroidism in trained soleus m. causes a mismatch between ATP synthesis and degradation. Three days’ T\textsubscript{3} excess is probably too short to additionally increase mitochondrial biogenesis in endurance training skeletal muscles (Venditti et al. 2009).

AMP in muscles is formed in a reaction catalyzed by adenylate kinase (AK). According to Hancock et
al. (2006), AMP formation in skeletal muscles is mainly determined by ADP concentration as AK activity is already very high. This finding corresponds well with our results showing that AMP concentration in soleus m. reflects changes in ADP content. The maintenance of low AMP content in myocytes during exercise depends on AMP deaminase. However, as noted by Norman et al. (1998) endurance training decreases AMP deaminase activity and this may be the reason for the increased AMP content in CTR rats.

Effects of short-term hyperthyroidism and endurance training on PA metabolism in soleus muscle in vitro

In this study we confirmed our previous results (Górecka et al. 2004) that in sedentary state the rate of PA uptake, oxidation to CO$_2$ and incorporation into intramuscular acylglycerols (TG, MGDG) is elevated by T$_3$ excess. This may be related to the recent finding that short-term hyperthyroidism can affect the expression of proteins facilitating FA uptake in skeletal muscles. Miklosz et al. (2012) showed that T$_3$ excess causes an increase in fatty acid translocase (FAT) content, but does not affect the plasma membrane-associated fatty acid binding proteins (FABPpm) and fatty acid transport protein 4 (FATP4), while leading to a decrease fatty acid transport protein 1 (FATP1) content in red gastrocnemius m. Moreover, it was shown that a week’s T$_3$ treatment to hypothyroid rats increased redistribution of FAT to sarcolemma and mitochondria in gastrocnemius m. (Lombardi et al. 2012). There is no data available regarding T$_3$ influence on the content of these proteins in soleus m.

In skeletal muscles of the rats treated with T$_3$ an increased phosphorylation and/or content of AMP-activated protein kinase (AMPK) were noted (Ircher et al. 2003, Branvold et al. 2008, Miklosz et al. 2012). As recently found by Gowans et al. (2013) AMPK is also sensitive to changes in cellular energy status and allosteric regulation of AMPK by AMP. Now we found that the elevated rate of PA oxidation was associated with an increase in AMP content in HS rats. In our previous work we postulated that the increased rate of PA oxidation (Górecka et al. 2004) may result from the enhanced uncoupling of oxidative phosphorylation from ATP synthesis. In this study we noted a reduced ATP content in the soleus m. (analyzed before incubation) in HS rats and an increased rate of PA oxidation to CO$_2$ in incubated ones when compared to controls, which also suggests the uncoupling of substrate oxidation from ATP synthesis in HS rats as previously noted by Jucker et al. (2000).

There is lot of available data on the effects of endurance training on FA metabolism in skeletal muscle of rats. Surprisingly, the data on the influence of T$_3$ on FA uptake and metabolism in skeletal muscles of trained rats are limited. It is well established that soleus m. despite being a postural muscle activated for 5-8 h per day in freely moving rats (Hennig and Lømo 1985) can further adapt to endurance training by enhanced mitochondrial biogenesis and increased enzymes activity (Durante et al. 2002). Hence, it is not surprising that directly after exercise-training in euthyroid as well as in hyperthyroid rats the rate of PA oxidation to CO$_2$ was increased by 68-75% compared with sedentary values independently of PA concentration. Moreover, short-term
T₃ excess caused a further increase in the rate of this process in HTEx rats at 1.5mM PA. 24 h recovery decreased the rate of CO₂ production by soleus m. to the sedentary level, independently of thyroid state. Our results are in line with those of McFarlan et al. (2012) and Dyck et al. (2000) who did not observe any changes in the rate of FA oxidation in trained soleus m. after 48-60h of recovery. Training-induced increase in the rate of FA oxidation was observed by these authors only when muscle samples for in vitro analyses were taken immediately after electrical or pharmacological stimulation. The increased FA oxidation found immediately after the last bout of exercise training may result from an increased level of FAT and FABPpm in sarcolemmal membrane of rodent hindlimb muscles (Bradley et al. 2012, McFarlan et al. 2012). In hyperthyroid trained rats an increase in the FAT content in sarcolemma directly after exercise can also be expected (Miklosz et al. 2012).

It is not clear how FA oxidation is regulated by AMPK-ACC axis in soleus m. during exercise in trained rats. McConell et al. (2008) observed that endurance training fully abolished phosphorylation of AMPK in soleus m. during exercise, while phosphorylation of ACC was attenuated. By contrast, Durante et al. (2002) demonstrated that in trained soleus m. directly after exercise the total AMPK activity was augmented, however, 24 hrs after last training session activities of AMPK as well as ACC did not differ from sedentary values. These results are in line with the changes in the rate of PA oxidation in euthyroid rats. Moreover, directly after exercise-training in CTEX rats AMP content in soleus m. (before incubation) was ca. 3.0 fold higher than in CS group pointing to activation of AMPK during exercise. Surprisingly, AMP level in soleus m. from CTR rats was still increased compared to the sedentary value.

It is interesting to note that in hyperthyroid rats the increased rate of PA oxidation in HTEx group compared to HS subjects was not associated with the change in AMP content resulting probably from already high AMP in HS rats. However, when the rate of PA oxidation in HTR rats returned to the sedentary value after recovery, a significant decrease in this parameter was found. These results suggest that short-term hyperthyroidism may alter the response of trained soleus m. to exercise.

There are limited data concerning the effect of hyperthyroidism on FA oxidation in contracting skeletal muscles. Branvold et al. (2008) demonstrated that long-term hyperthyroidism (4 weeks) in rats enhances the response of soleus m. to electrical stimulation causing an increase in AMPK activity and a stronger increase in ACC phosphorylation compared to that observed in euthyroid animals. Yamauchi et al. (2008) found that T₃ can activate AMPK also by intracellular Ca²⁺ and calcium/calmodulin-dependent protein kinase β thus leading to stimulation of FA oxidation. These findings may explain why T₃ excess increased the rate of PA oxidation in trained rats directly after exercise.

In our CTEX rats the rate of PA incorporation into intramuscular TG was significantly decreased at high
PA concentration. Moreover, a tendency to decrease was observed also in the rate of PA incorporation into MG/DG pool. This reduction in the rate of acylglycerols synthesis may results from reduced glycogen concentration in soleus m. in CTEx group (2.0 fold compared to sedentary level). According to Jensen et al. (2011), at the end of exercise the rate of glycogen synthesis in skeletal muscles is increased while the rate of glycolysis, a major metabolic pathway providing glycerol-3-phosphate to TG synthesis, is decreased. Contrastingly, in hyperthyroid animals the drop in glycogen content directly after exercise was slight (by 12.5%) as was the decrease in the rate of PA incorporation into TG at high PA concentration. After 24h of recovery the glycogen pool in soleus muscle was rebuilt in CTR and HTR rats and the rates of TG synthesis return to sedentary levels.

It should be noted that three-day T3 injection into HTEx rats caused an increase in the rate of PA incorporation into TG by 21-33% when compared to control values and it was independent of the PA concentration. However, after 24h recovery the incorporation into TG was elevated by T3 only at 1.5mM PA. It is known that T3 excess increases the rate of glycolysis in exercised soleus m. (Dubaniewicz et al. 1989), thus the higher rate of PA incorporation into TG in hyperthyroid trained rats may also be the result of an increased availability of glycerol-3-phosphate.

Summarizing, our results prove that short-term hyperthyroidism evoked in well-trained animals may accelerate the rate of PA uptake by soleus m. as well as PA oxidation and incorporation into intramuscular TG especially at high FA concentration.
References


Table 1. Body mass and ratio of heart mass to body mass (H/B) in control sedentary (CS), control trained-exercising (CTEx), control trained-recovering (CTR), hyperthyroid sedentary (HS), hyperthyroid trained-exercising (HTEx) and hyperthyroid trained-recovering (HTR) rats in the experimental day.

<table>
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<tr>
<th>Groups</th>
<th>CS</th>
<th>CTEx</th>
<th>CTR</th>
<th>HS</th>
<th>HTEx</th>
<th>HTR</th>
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<tr>
<td>Body mass (g)</td>
<td>254 ± 8 (28)</td>
<td>240 ± 4 (9)</td>
<td>236 ± 9 (8)</td>
<td>237 ± 9 (24)</td>
<td>234 ± 10 (5)</td>
<td>243 ± 5 (9)</td>
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<td>H/B (mg/g)</td>
<td>3.200 ± 0.036 (28)</td>
<td>3.333 ± 0.056 (9)</td>
<td>3.360 ± 0.088 (8)</td>
<td>4.334 ± 0.061 (24) *</td>
<td>4.397 ± 0.103 (5) *</td>
<td>4.509 ± 0.193 (9) *</td>
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</table>

Means ± SE; The number of animals indicated in parentheses *: P<0.05 between values obtained in hyperthyroid and euthyroid control animals.
Table 2. Blood concentration of free fatty acids (FFA) and triacylglycerols (TG) and the soleus muscle TG (TGm) and glycogen contents in control sedentary (CS), control trained-exercising (CTEx), control trained-recovering (CTR), hyperthyroid sedentary (HS), hyperthyroid trained-exercising (HTEx) and hyperthyroid trained-recovering (HTR) rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>CS (Mean ± SE)</th>
<th>CTEx (Mean ± SE)</th>
<th>CTR (Mean ± SE)</th>
<th>HS (Mean ± SE)</th>
<th>HTEx (Mean ± SE)</th>
<th>HTR (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFA</td>
<td>0.391 ± 0.018 (25)</td>
<td>0.435 ± 0.055 (6)</td>
<td>0.416 ± 0.064 (8)</td>
<td>0.612 ± 0.028 (22)*</td>
<td>0.504 ± 0.060 (5)</td>
<td>0.569 ± 0.063 (8)</td>
</tr>
<tr>
<td>TG</td>
<td>0.771 ± 0.042 (25)</td>
<td>0.570 ± 0.028 (5) †</td>
<td>0.521 ± 0.044 (8) †</td>
<td>1.129 ± 0.076 (22) *</td>
<td>0.620 ± 0.016 (4) †</td>
<td>0.960 ± 0.133 (7)*</td>
</tr>
<tr>
<td>TGm</td>
<td>4.97 ± 0.57 (16)</td>
<td>5.46 ± 1.05 (4)</td>
<td>5.99 ± 0.70 (6)</td>
<td>7.12 ± 0.50 (19) *</td>
<td>6.70 ± 0.76 (5)</td>
<td>5.95 ± 0.92 (8)</td>
</tr>
<tr>
<td>Glycogen</td>
<td>78.4 ± 3.5 (10)</td>
<td>40.2 ± 4.0 (6) †</td>
<td>101.6 ± 5.1 (3) † ‡</td>
<td>73.6 ± 2.6 (6)</td>
<td>64.4 ± 8.0 (4)</td>
<td>109.2 ± 4.5 (3) † ‡</td>
</tr>
</tbody>
</table>

Means ± SE; The number of animals indicated in parentheses. FFA and TG concentrations are expressed as mmol/l, whereas TGm as µmol/g wet weight and glycogen as µmol/g dry weight. *: P<0.05 between values obtained in hyperthyroid and euthyroid control animals; †: P<0.05 between values obtained in trained and sedentary groups; ‡: P<0.05 between values obtained in CTR and CTEx or HTR and HTEx groups.
Table 3. Concentration of high-energy phosphates (ATP, ADP, AMP) and the energy cellular potential (ECP) in the soleus muscle from control sedentary (CS), control trained-exercising (CTEx), control trained-recovering (CTR), hyperthyroid sedentary (HS), hyperthyroid trained-exercising (HTEx) and hyperthyroid trained-recovering (HTR) rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>CS</th>
<th>CTEx</th>
<th>CTR</th>
<th>HS</th>
<th>HTEx</th>
<th>HTR</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>22.5 ±0.5 (14)</td>
<td>22.1 ±0.6 (5)</td>
<td>22.9 ±1.1 (8)</td>
<td>20.9 ±0.5 (17)*</td>
<td>23.2 ±0.6 (5)</td>
<td>26.4 ±1.0 (7)* † ‡</td>
</tr>
<tr>
<td>ADP</td>
<td>2.32 ±0.24 (14)</td>
<td>4.17 ±0.09 (5)†</td>
<td>3.41 ±0.28 (8)†</td>
<td>3.91 ±0.27 (17) *</td>
<td>4.65 ±0.11 (5) *</td>
<td>2.25 ±0.62 (7)† ‡</td>
</tr>
<tr>
<td>AMP</td>
<td>0.230 ±0.053 (14)</td>
<td>0.701 ±0.028 (5)†</td>
<td>0.682 ±0.068 (8)†</td>
<td>0.775 ±0.076 (17) *</td>
<td>0.801 ±0.028 (5) *</td>
<td>0.331 ±0.122 (7)† ‡</td>
</tr>
<tr>
<td>ECP</td>
<td>0.94 ±0.007 (14)</td>
<td>0.90 ±0.003 (5)†</td>
<td>0.91 ±0.008 (8)†</td>
<td>0.89 ±0.007 (17) *</td>
<td>0.89 ±0.003 (5)</td>
<td>0.95 ±0.015 (7) * † ‡</td>
</tr>
</tbody>
</table>

Means ± SE; The number of animals indicated in parentheses; Concentration of ATP, ADP and AMP expressed as µmol/g dry weight.

*: P<0.05 between values obtained in hyperthyroid and euthyroid control animals; †: P<0.05 between values obtained in trained and sedentary groups; ‡: P<0.05 between values obtained in CTR and CTEx or HTR and HTEx groups.
Table 4. The rate of palmitic acid (PA) oxidation to CO$_2$ in stripped soleus muscles isolated from control sedentary (CS), control trained-exercising (CTEx), control trained-recovering (CTR), hyperthyroid sedentary (HS), hyperthyroid trained-exercising (HTEx) and hyperthyroid trained-recovering (HTR) rats.

<table>
<thead>
<tr>
<th>[PA]</th>
<th>Groups</th>
<th>CS</th>
<th>CTEx</th>
<th>CTR</th>
<th>HS</th>
<th>HTEx</th>
<th>HTR</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5mM</td>
<td>0.68 ±0.06 (22)</td>
<td>1.19 ±0.06 (6) †</td>
<td>0.72 ±0.05 (5)</td>
<td>0.78 ±0.06 (22)</td>
<td>1.37 ±0.15 (5) †</td>
<td>0.82 ±0.11 (8) ‡</td>
<td></td>
</tr>
<tr>
<td>1.5mM</td>
<td>1.78 ±0.15 (21)</td>
<td>3.02 ±0.15 (6) †</td>
<td>1.98 ±0.33 (7) ‡</td>
<td>2.34 ±0.19 (23) *</td>
<td>3.94 ±0.31 (5) * †</td>
<td>2.81 ±0.39 (9)</td>
<td></td>
</tr>
</tbody>
</table>

Means ± SE, expressed as nmol PA/min/g wet weight. The number of animals indicated in parentheses.

*: P<0.05 between values obtained in hyperthyroid and euthyroid control animals; †: P<0.05 between values obtained in trained and sedentary groups; ‡: P<0.05 between values obtained in CTR and CTEx or HTR and HTEx groups.
Table 5. The rate of PA incorporation into intramuscular lipids: triacylglycerols (TG), mono-, diacylglycerols (MG/DG) and phospholipids (PL) in stripped soleus muscles isolated from control sedentary (CS), control trained-exercising (CTEx), control trained-recovering (CTR), hyperthyroid sedentary (HS), hyperthyroid trained-exercising (HTEx) and hyperthyroid trained-recovering (HTR) rats.

<table>
<thead>
<tr>
<th>[PA]</th>
<th>Groups</th>
<th>CGS</th>
<th>CTEx</th>
<th>CTR</th>
<th>HS</th>
<th>HTEx</th>
<th>HTR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5mM</td>
<td>2.59 ±0.10 (24)</td>
<td>2.50 ±0.04 (6)</td>
<td>3.17 ±0.34 (7)</td>
<td>3.78 ±0.17 (24) *</td>
<td>3.05 ±0.19 (5) *</td>
<td>3.39 ±0.29 (9)</td>
<td></td>
</tr>
<tr>
<td>1.5mM</td>
<td>11.54 ±0.39 (23)</td>
<td>9.79 ±0.33 (6) †</td>
<td>11.30 ±0.84 (6)</td>
<td>14.47 ±0.60 (22) *</td>
<td>11.82 ±0.37 (5) *</td>
<td>15.04 ±0.67 (8) * ‡</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MG/DG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5mM</td>
<td>0.84 ±0.05 (22)</td>
<td>0.90 ±0.09 (6)</td>
<td>1.02 ±0.10 (8)</td>
<td>1.00 ±0.04 (22) *</td>
<td>0.93 ±0.05 (5)</td>
<td>1.02 ±0.06 (9)</td>
<td></td>
</tr>
<tr>
<td>1.5mM</td>
<td>3.28 ±0.37 (21)</td>
<td>2.60 ±0.21 (6)</td>
<td>3.85 ±0.67 (7)</td>
<td>4.14 ±0.42 (22)</td>
<td>2.51 ±0.12 (5)</td>
<td>4.54 ±0.71 (9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5mM</td>
<td>0.80 ±0.05 (21)</td>
<td>0.70 ±0.06 (6)</td>
<td>0.68 ±0.12 (7)</td>
<td>0.65 ±0.03 (22) *</td>
<td>0.71 ±0.07 (5)</td>
<td>0.76 ±0.06 (8)</td>
<td></td>
</tr>
<tr>
<td>1.5mM</td>
<td>1.55 ±0.09 (23)</td>
<td>1.42 ±0.12 (6)</td>
<td>1.38 ±0.11 (8)</td>
<td>1.43 ±0.06 (23)</td>
<td>1.60 ±0.11 (4)</td>
<td>1.63 ±0.16 (9)</td>
<td></td>
</tr>
</tbody>
</table>

Means ± SE, expressed as nmol PA/min/g wet weight. The number of animals indicated in parentheses.

*: P<0.05 between values obtained in hyperthyroid and euthyroid control animals; †: P<0.05 between values obtained in trained and sedentary groups; ‡: P<0.05 between values obtained in CTR and CTEx or HTR and HTEx groups.
Table 6. The rate of PA uptake in stripped soleus muscles isolated from control sedentary (CS), control trained-exercising (CTEx), control trained-recovering (CTR), hyperthyroid sedentary (HS), hyperthyroid trained-exercising (HTEx) and hyperthyroid trained-recovering (HTR) rats.

<table>
<thead>
<tr>
<th>[PA]</th>
<th>Groups</th>
<th>CS</th>
<th>CTEx</th>
<th>CTR</th>
<th>HS</th>
<th>HTEx</th>
<th>HTR</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5mM</td>
<td>5.06 ±0.18 (22)</td>
<td>5.28 ±0.18 (6)</td>
<td>5.49 ±0.68 (8)</td>
<td>6.07 ±0.18 (23) *</td>
<td>6.07 ±0.31 (5)</td>
<td>5.86 ±0.29 (9)</td>
<td></td>
</tr>
<tr>
<td>1.5mM</td>
<td>18.43 ±0.70 (22)</td>
<td>16.99 ±0.57 (6)</td>
<td>18.59 ±1.24 (8)</td>
<td>21.48 ±0.71 (21) *</td>
<td>19.67 ±0.91 (5) *</td>
<td>24.14 ±1.24 (8) * ‡</td>
<td></td>
</tr>
</tbody>
</table>

Means ± SE, expressed as nmol PA/min/g wet weight. The number of animals indicated in parentheses.

*: P<0.05 between values obtained in hyperthyroid and euthyroid control animals; ‡: P<0.05 between values obtained in CTR and CTEx or HTR and HTEx groups.