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Lombardi A, de Lange P, Silvestri E, Busiello RA, Lanni A, Goglia F, Moreno M. 3,5-Diiodo-L-thyronine rapidly enhances mitochondrial fatty acid oxidation rate and thermogenesis in rat skeletal muscle: AMP-activated protein kinase involvement. *Am J Physiol Endocrinol Metab* 296: E497–E502, 2009. First published December 30, 2008; doi:10.1152/ajpendo.90642.2008.—Triiodothyronine regulates energy metabolism and thermogenesis. Among triiodothyronine derivatives, 3,5-diiodo-L-thyronine (T_2) has been shown to exert marked effects on energy metabolism by acting mainly at the mitochondrial level. Here we investigated the capacity of T_2 to affect both skeletal muscle mitochondrial substrate oxidation and thermogenesis within 1 h after its injection into hypothyroid rats. Administration of T_2 induced an increase in mitochondrial oxidation when palmitoyl-CoA (+104%), palmitoylcarnitine (+80%), or succinate (+30%) was used as substrate, but it had no effect when pyruvate was used. T_2 was able to *1*) activate the AMPK-ACC-malonyl-CoA metabolic signaling pathway known to direct lipid partitioning toward oxidation and *2*) increase the importing of fatty acids into the mitochondrion. These results suggest that T_2 stimulates mitochondrial fatty acid oxidation by activating several metabolic pathways, such as the fatty acid import/β-oxidation cycle/$FADH_2$-linked respiratory pathways, where fatty acids are imported. T_2 also enhanced skeletal muscle mitochondrial thermogenesis by activating pathways involved in the dissipation of the proton-motive force not associated with ATP synthesis ("proton leak"), the effect being dependent on the presence of free fatty acids inside mitochondria. We conclude that skeletal muscle is a target for T_2, and we propose that, by activating processes able to enhance mitochondrial fatty acid oxidation and thermogenesis, T_2 could play a role in protecting skeletal muscle against excessive intramyocellular lipid storage, possibly allowing it to avoid functional disorders.

adenosine 5′-monophosphate; thyroid hormone; mitochondria

AMONG THE ENDOCRINE FACTORS able to regulate substrate metabolism and thermogenesis, thyroid hormones (THs) play important roles. 3,5,3′-Triiodothyronine (T_3) exerts a plethora of effects, including upregulation of peripheral and hepatic glucose uptake, cholesterol reduction, loss of body weight and adiposity, cardiac acceleration, and increases in metabolic rate (28, 32). In adults, T_3 regulates energy metabolism by increasing respiration and energy expenditure and by lowering metabolic efficiency (for review, see Ref. 11). Because of this, T_3 was tested in the past as an antiobesity and hypolipidemic agent. However, due to its undesirable side effects, particularly within the cardiovascular system, its use was not continued (19). The development of TH derivatives that, while retaining lipid-lowering and antiobesity efficacy, are devoid of cardiovascular side effects would represent a potentially valuable therapeutic tool for the reduction of some important risk factors. Many laboratories have demonstrated metabolic effects of 3,5-diiodothyronine (T_2; a TH derivative) (2, 8–10, 12, 14, 22–27, 29, 30) that are independent of protein synthesis. Among the effects exerted by T_2, its ability to affect whole animal metabolic rate is of growing interest. In this context, we have shown that T_2 is able to *1*) rapidly increase the resting metabolic rate of hypothyroid rats (24, 29, 30), *2*) powerfully reduce adiposity in rats fed a high-fat diet by increasing the burning of fats (23), and *3*) improve the survival of hypothyroid rats in the cold (25). Importantly, T_2 administration does not induce hypertrophy or hyperplasia of metabolically very active tissues (29, 25) and does not induce thyrotoxicosis or cardiac acceleration in rodents or in humans (23). Moreover, within 1 h after its injection into hypothyroid rats, T_2 induces a rapid increase in mitochondrial oxidative capacity in skeletal muscle without involving transcription or translation mechanisms (27).

Skeletal muscle, richly endowed with mitochondria, accounts for about 40% of body mass in mammals, and it is an important site for substrate oxidation and thermogenesis (6, 15, 35). An impairment of skeletal muscle metabolic energy transduction pathways can lead to functional disorders such as lipotoxicity and insulin resistance (3, 6–7, 15–18, 20). The mechanism by which mitochondrial dysfunction causes insulin resistance remains unclear, although one attractive hypothesis involves a high availability of fatty acids for lipid accumulation resulting from an impaired capacity for lipid oxidation (39). The identification of molecules able to direct lipid partitioning toward oxidation is a key focus in attempts to counteract obesity and metabolism-associated disorders. Pharmacological strategies designed to boost fatty acid oxidation, however, have to be focused on an enhancement of the complete oxidation of fatty acids; that is to say, the enhanced β-oxidation has to be coordinated with downstream pathways such as the TCA cycle and respiratory chain activity (20). Indeed, recent data indicate that, in skeletal muscle, insulin resistance may be related to mitochondrial fatty acid overload and incomplete fatty acid oxidation (20).

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In recent years, AMP-activated protein kinase (AMPK) has emerged as a key kinase driving lipid oxidation in skeletal muscle, and this function has important implications for metabolic disorders (for review, see Ref. 37). AMPK, when activated by an increase in the AMP/ATP ratio, switches on catabolic pathways (such as fatty acid oxidation) and switches off anabolic pathways (such as lipogenesis or gluconeogenesis). Once activated, AMPK inhibits acetyl-CoA carboxylase (ACC) through phosphorylation. A decrease in ACC activity reduces intracellular malonyl-CoA levels and stimulates carnitine palmitoyl transferase (CPT) I. This ultimately increases the influx of long-chain fatty acids into the mitochondria, where they are oxidized (37).

Here, to investigate the capacity of T_2 to affect mitochondrial fatty acid oxidation, we analyzed the pathways leading to fatty acid utilization, including the effect of T_2 on the efficiency of mitochondrial energy transduction. To this end, we evaluated in skeletal muscle and in the short term (after 1 h) the effects of T_2 on *1*) fatty acid uptake and oxidation, including the respiratory chain activity; *2*) the AMPK-ACC-malonyl-CoA signaling pathway; and *3*) mitochondrial proton leak kinetics.

As our animal model, we chose hypothyroid rats, in which thermogenesis and oxidative capacities are decreased (21).

EXPERIMENTAL PROCEDURES

Animal treatment. Male Wistar rats (250–300 g) (Charles River) were kept one per cage in a temperature-controlled room at 28°C under a 12:12-h light-dark cycle. A commercial mash and water were available ad libitum. Two groups of rats were used throughout, hypothyroid controls (referred to as hypo) and acutely T_2-injected hypothyroid rats (referred to as hypo + T_2). All of the experiments were performed in accordance with local and national guidelines covering animal experiments. Authorization to perform these experiments on rats was given by the Italian Ministero della Sanità (decree no. 176/2005-A). In both of the above groups (each consisting of 6 animals), hypothyroidism was induced by the intraperitoneal (ip) administration of propylthiouracil (1 mg/100 g body wt) for 4 wk together with a weekly ip injection of iopanoic acid (6 mg/100 g body wt) (24, 29). At the end of this treatment, hypo + T_2 rats received a single ip injection of T_2 (25 μg/100 g body wt), whereas hypo rats received a saline injection. The dose of 25 μg/100 g body wt T_2 was used because it produces a clear-cut, rapid effect on energy expenditure (29). One hour after the injection, rats were anesthetized by an ip injection of cloral hydrate (40 mg/100 g body wt) and killed by decapitation. Then, gastrocnemius and tibialis muscles were excised, weighed, and immediately processed for mitochondrial isolation.

Mitochondria isolation. Total skeletal muscle mitochondria were isolated at 8,000 *g* by differential centrifugation. Briefly, tissue fragments were gently homogenized in 5 vol of an isolation medium consisting of 220 mM mannitol, 70 mM sucrose, 20 mM Tris·HCl, 1 mM EDTA, and 5 mM EGTA (pH 7.4). Then, 1 mg protease/g tissue (nagarse 12 U/mg protein) was added to the homogenate and incubated for 4 min at 4°C, after which another 5 vol of isolation medium, supplemented with 0.5% BSA, was added. The homogenate was centrifuged at 8,000 *g* for 10 min at 4°C. The resulting pellet was resuspended in 10 vol of BSA-supplemented isolation medium, subjected to very gentle homogenization, and centrifuged 500 *g* for 10 min at 4°C. The resulting supernatant was centrifuged at 8,000 *g*. The mitochondrial pellet was then washed twice, resuspended in a minimal volume, and kept on ice until further determinations.

In some experiments (reported and indicated in Table 1 and Fig. 4), BSA was omitted from the isolation medium.

Table 1. *State 4, state 3, and RCR in skeletal muscle mitochondria isolated from Hypo and Hypo + T_2 rats*

	Hypo	Hypo + T_2
State 4 + BSA	85±12	100±16
State 3 + BSA	405±58	514±60*
RCR + BSA	4.8±0.4	5.1±0.4
State 4 − BSA	154±17	218±17*
State 3 − BSA	360±30	456±16*
RCR-BSA	2.3±0.2	2.1±0.1

Values are reported as the mean ± SE of 6 different mitochondrial preparations for each group. RCR, respitatory control ratio; Hypo, hypothyroid control; Hypo + T_2, 3,5-diiodothyronine-treated hypothyroid. *$P < 0.05$ vs. hypothyroid group. Bioenergetic parameters were measured in the presence of BSA (present in both the isolation and respiratory medium) or in the absence of BSA (omitted from both the isolation and the respiratory medium). State 4 and state 3 respiration rates, obtained by using succinate + rotenone as substrate, are expressed as nAtoms O/min mg prot.

Determination of respiratory parameters. A Clark-type oxygen electrode (Rank Brothers, Botttisham, Cambridge, UK) with an incubation chamber maintained at 30°C was used for oxygen consumption detection. Mitochondrial proteins were incubated in 1 ml of respiratory buffer (80 mM KCl, 50 mM HEPES, 1 mM EGTA, 5 mM KH_2PO_4, 2 mM $MgCl_2$, 1% BSA, pH 7).

Oxidation of succinate, in the presence of rotenone, was used to detect the capacity of T_2 to affect FADH-linked respiratory pathways. To this aim, incubation medium was supplemented with 4 μM rotenone, and mitochondria (0.2 mg/ml) were energized with 5 mM succinate in both the absence of ADP [to obtain indication of the uncoupled respiration in which there is no phosphorylation of ADP to ATP (state 4)] and presence of 200 μM ADP [to obtain indication on the coupled respiration in which phosphorylation of ADP is at the maximal rate (state 3)]. To test the involvement of free fatty acids (FFA) in the effect of T_2 on bioenergetics parameters, succinate-energized mitochondrial respiration has been detected in mitochondria isolated in the presence and in the absence of BSA. In the latter case (reported and indicated in Table 1), BSA was omitted from isolation and respiratory medium.

To detect to capacity of T_2 to affect NADH-linked respiratory pathways, incubation medium was supplemented with 2.5 mM malate and 200 μM ADP, and mitochondria (0.5 mg/ml) were energized with pyruvate (10 mM).

To detect whether T_2 is able to affect mitochondrial capacity to oxidize fatty acid, it was assessed either with palmitoyl-CoA (the oxidation of which requires its import into the mitochondrion, an influx mediated by CPT) or with palmitoylcarnitine (the oxidation of which does not require CPT). To detect mitochondrial palmitoyl-CoA oxidation, incubation medium (A) was supplemented with 2 mM carnitine, 2.5 mM malate, and 200 μM ADP, and the mitochondria (0.5 mg/ml) were energized with palmitoyl-CoA (40 μM). To detect mitochondrial palmitoylcarnitine oxidation, incubation medium was supplemented with 2.5 mM malonate, 2 mM carnitine, and 200 μM ADP, and the reaction was started by the addition of palmitoylcarnitine (40 μM).

Enzyme activity. Mitochondrial thioesterase-1 (MTE-1) and CPT system (CPT I + CPT II) activities were measured spectrophotometrically (1). For both enzymes, palmitoyl-CoA was used at a saturating concentration (50 μM), whereas total CPT activity was determined in the presence of both palmitoyl-CoA and saturating carnitine (5 mM).

Proton leak kinetics. To evaluate proton leak kinetics, the respiration rate and membrane potential ($\Delta\Psi$) were measured simultaneously, the latter being detected using a triphenylmethylphosphonium ($TPMP^+$)-sensitive electrode. For these measurements, 0.5 mg of mitochondrial proteins was incubated in 1 ml of respiratory buffer supplemented with 1 μg/ml oligomycin and 80 ng/ml nigericin at

37°C. Mitochondria were incubated in the respiratory medium for 2 min to calibrate the $TPMP^+$-sensitive electrode, calibration being achieved by means of sequential additions of ≤2 μM $TPMP^+$. Then, mitochondria were energized using 6 mM succinate, and respiration was titrated with increasing amounts of malonate (≤2 mM). To test the involvement of FFA in the effect of T_2 on proton leak kinetics, it was detected on mitochondria isolated both in the presence and in the absence of BSA. In the last case, BSA was also omitted by incubation medium. A $TPMP^+$ binding correction factor of 0.4 was applied for mitochondria from both hypothyroid and hypothyroid + T_2-treated animal, as suggested by Rolfe et al. (36).

Preparation of total lysates. For Western blotting analysis, gastrocnemius muscle was homogenized in lysis buffer containing 20 mM Tris·HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 2.5 mM $Na_2H_2P_2O_7$, 1 mM b-$CH_3H_7O_6PNa_2$, 1 mM Na_3VO_4, 1 mM PMSF, 1 mg/ml leupeptin, and 1% Triton X-100 (all from Sigma-Aldrich, St. Louis, MO), using an ultraturrax, and centrifuged at 13,500 *g* for 10 min at 4°C (Beckman Optima TLX; Beckman Coulter, Milan, Italy). Supernatants were ultracentrifuged at 86,000 *g* for 10 min at 4°C (Beckman Optima TLX). The protein concentrations of the supernatants of the ultracentrifuged cleared lysates were determined using Bio-Rad's DC method (Bio-Rad Laboratories, Hercules, CA).

The total protein levels of AMPK and ACC in skeletal muscle were determined in the same lysates (using 10 and 5% SDS polyacrylamide gels, respectively) by employing specific antibodies against AMPK (Cell Signaling Technology) and ACC (Upstate Biotechnology, Lake Placid, NY). Phosphorylation of the α-subunit of AMPK was examined using an antibody against phosphopeptides on the basis of the amino acid sequence surrounding Thr^{172} of the α-subunit of human AMPK (Cell Signaling Technology). Phosphorylation of ACC was examined using an antibody against phosphopeptides on the basis of the amino acid sequence surrounding Ser^{79} of human ACC (Cell Signaling Technology). Proteins were detected by a chemiluminescence protein detection method based on the protocol supplied with a commercially available kit (NEN, Boston, MA) and using the indicated antibodies and secondary antibodies. The protein concentrations of the samples were determined using Bio-Rad's DC method (Bio-Rad Laboratories). In each case, membranes were stained with Ponceau S (Sigma) to confirm similar loading and transfers in each line. Western immunoblot bands were quantified by means of a Bio-Rad calibrated densitometer (GS-800), using the supplied software (Bio-Rad Laboratories).

Determination of FFA level in skeletal muscle. FFA content in skeletal muscle was determined on total lipid extracted from 500 mg wet wt of tissue. Briefly, lipids were extracted, as originally described by Folch et al. (5), and the chloroform phase was evaporated under nitrogen. FFA levels were then determined by using NEFA kit determination from Wako Diagnostic USA.

Statistical analysis. The statistical significance of differences between the two groups was determined by Student's *t*-test. Differences were considered significant when $P < 0.05$.

RESULTS

T_2 rapidly affects mitochondrial respiratory parameters. The administration of T_2 to hypothyroid rats rapidly increased their mitochondrial respiration rates (Table 1). When mitochondria used succinate as substrate and FFAs were chelated by the presence of BSA (in both isolation and respiratory medium), the values obtained for state 4 and state 3 appeared higher in hypothyroid-T_2-treated animals than in the hypothyroid controls (+18 and +27%, respectively), although only the change in state 3 was significant ($P < 0.05$) (Table 1). With both state 4 and state 3 being affected by T_2 treatment, respiratory control ratio (RCR) values did not change. To examine whether FFAs are involved in some of the effects of T_2 on succinate-supported respiration, we also measured respiratory parameters on mitochondria isolated in the absence of BSA. In addition, BSA was also omitted by respiration medium. In such conditions, both state 4 and state 3 were significantly stimulated, with state 4 showing the greatest increase (+42%). RCR value was not significantly affected by T_2 treatment when BSA was absent.

T_2 rapidly enhances the mitochondrial capacity to import and oxidize fatty acids. We next investigated the effect of T_2 on mitochondrial lipid uptake and handling by assessing the capacity of T_2 to affect *1*) the activity of the CPT system (the rate-limiting step for mitochondrial fatty acid uptake), *2*) the activity of MTE (considered to be a key enzyme in the modulation of mitochondrial fatty acid utilization), and *3*) the mitochondrial ability to oxidize various substrates.

Mitochondrial fatty acid uptake is stimulated by T_2. Indeed, mitochondria from hypothyroid + T_2-treated rats displayed a significant, enhanced CPT activity (+32% vs. those from the hypothyroid controls; Fig. 1*A*).

The cleavage of activated fatty acid (acyl-CoA) into fatty acid anions and CoA was also significantly increased by T_2, with MTE-1 activity being enhanced by 57% in hypothyroid + T_2-treated rats vs. hypothyroid control ones (Fig. 1*B*).

T_2 treatment also increased the mitochondrial fatty acid oxidation rate. When using palmitoyl-CoA (+carnitine + malate) as substrate, mitochondria from hypothyroid + T_2-treated rats showed an almost doubled oxygen consumption compared with the hypothyroid control ones (+104%; Fig. 1*C*). When palmitoylcarnitine (+malate; a lipid substrate able to bypass CPT I) was used as substrate, T_2 still induced an increase in the fatty acid oxidation rate (+80% in hypothyroid + T_2-treated rats vs. the hypothyroid controls; Fig. 1*D*).

No significant difference in mitochondrial oxygen consumption rate was detected between hypothyroid and hypothyroid + T_2-treated rats, when pyruvate plus malate was used as substrate (Fig. 1*F*).

The maximal rate of succinate (+rotenone) oxidation was significantly greater in hypothyroid + T_2-treated rats than in the hypothyroid control (Fig. 1*E* and Table 1).

T_2 rapidly stimulates phosphorylation of AMPK and ACC. We next investigated whether the AMPK-ACC-malonyl-CoA pathway might be involved in the effects of T_2 on fatty acid oxidation. Western immunoblotting (Fig. 2) revealed that AMPK phosphorylation was increased following T_2 administration, although the total amount of AMPK protein was not. Phosphorylation of ACC was also significantly increased following T_2 injection (Fig. 3), but the total protein level was unaffected.

FFAs are involved in the effect of T_2 on mitochondrial thermogenesis. Administration of T_2 to hypothyroid rats induces mitochondrial uncoupling. Indeed, when the phosphorylation of ADP to ATP is inhibited, hypothyroid + T_2-treated mitochondria exhibit an enhanced flux of protons across the inner membrane towards the matrix. Proton leak kinetics revealed that hypothyroid + T_2-treated mitochondria respire at a higher level than hypothyroid control ones to maintain the same membrane potential (Fig. 4).

Free fatty acids are involved in the effect of T_2 on mitochondrial uncoupling, since with FFA chelated by BSA, T_2 lacked its uncoupling effect (Fig. 4). Actually, BSA signifi-

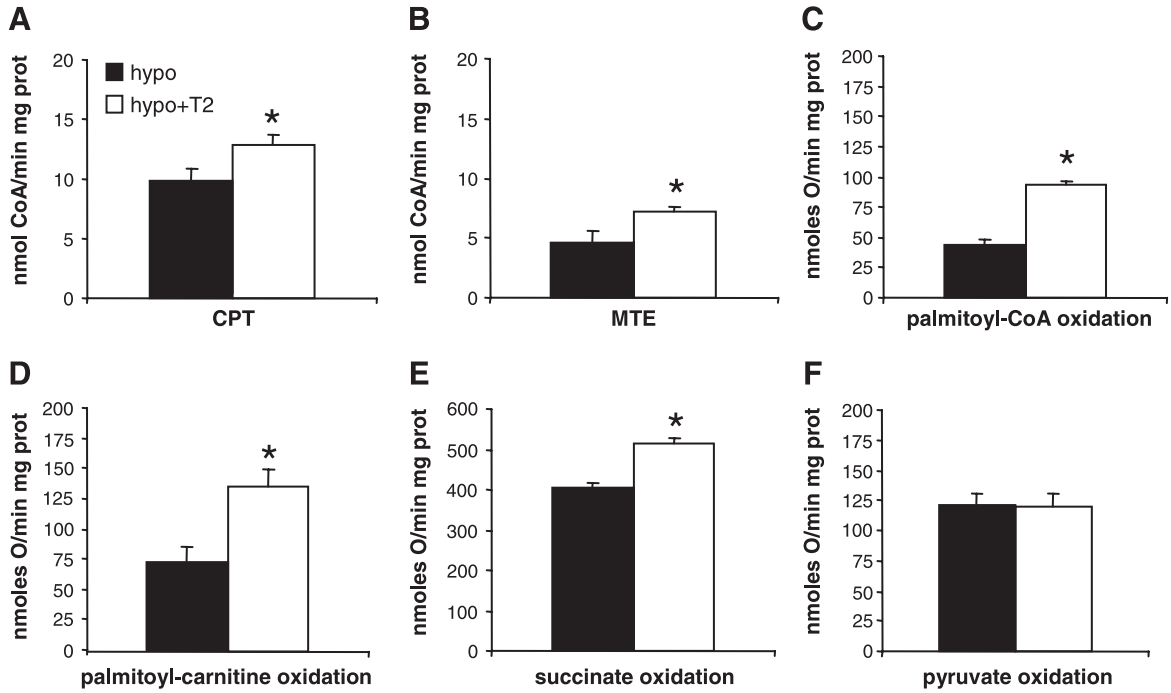

Fig. 1. Effect of 3,5-diiodothyronine (T_2) administration to hypothyroid rats on carnitine palmitoyl transferase (CPT) activity (*A*) and mitochondrial thioesterase (MTE) activity (*B*), palmitoyl-CoA oxidation (*C*), palmitoylcarnitine oxidation (*D*), succinate oxidation (*E*), and pyruvate oxidation (*F*). Results represented are the mean ± SE of 6 independent experiments, each one performed in duplicate. *Significant differences ($P < 0.05$) vs. hypothyroid control rat.

cantly inhibited proton leak kinetics in both hypothyroid control and hypothyroid + T_2-treated mitochondria and in so doing abolished the difference between the two groups. However, to exclude the possibility that in T_2-treated rats an increase in the muscle FFA concentration could lead to an increased FFA contamination of isolated mitochondria (thus affecting mitochondrial uncoupling), the actual FFA levels were measured in both groups. FFA levels were significantly reduced in skeletal muscle from T_2-treated hypothyroid animals compared with the hypothyroid control ones ($P < 0.05$), the nominal values being 24 ± 2 and 17 ± 1 nmol/100 mg tissue in skeletal muscle from hypothyroid and T_2-treated

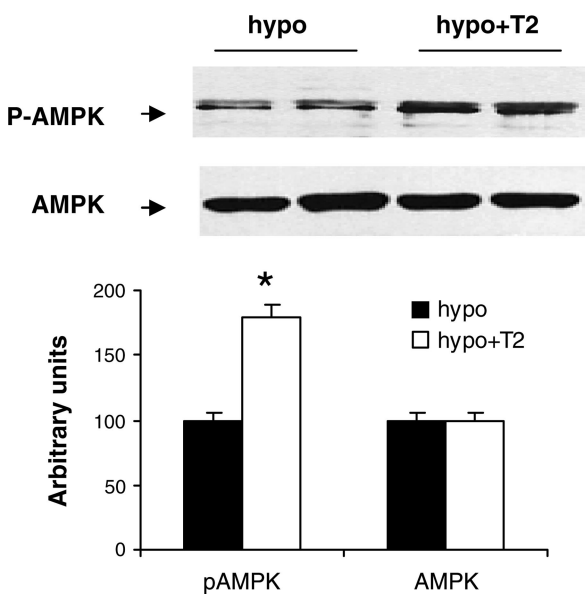

Fig. 2. Effect of T_2 administration to hypothyroid rats on AMP-activated protein kinase (AMPK) phosphorylation. Levels of phosphorylated AMPK (p-AMPK) (Thr^{172}) and total protein level. Each lane contains 30 μg of protein from a single rat. The histograms show quantification of the signals (expressed relative to the value obtained for 5 rats) Each point represents the mean ± SE of samples. *Significant differences ($P < 0.05$) vs. hypothyroid control rats.

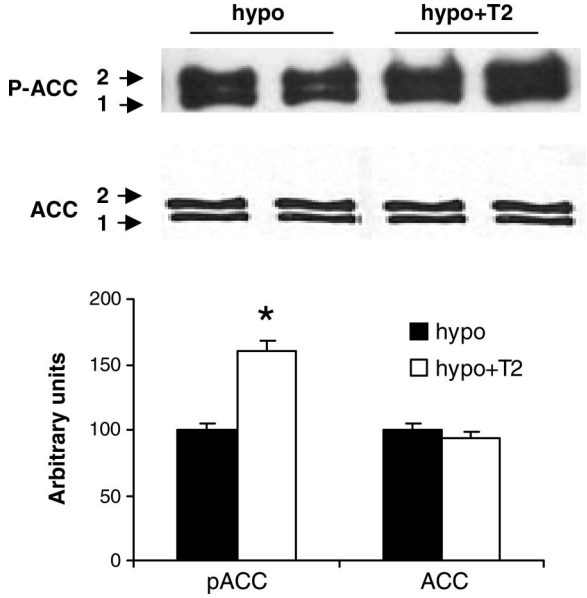

Fig. 3. Effect of T_2 administration to hypothyroid rats on acetyl-CoA carboxylase (ACC) phosphorylation. levels of phosphorylated ACC (Ser^{79}) and total protein level. Each lane contains 30 μg of protein from a single rat. The histograms show quantification of the signals (expressed relative to the value obtained for 5 rats). Each point represents the mean ± SE. *Significant differences ($P < 0.05$) vs. hypothyroid control rats.

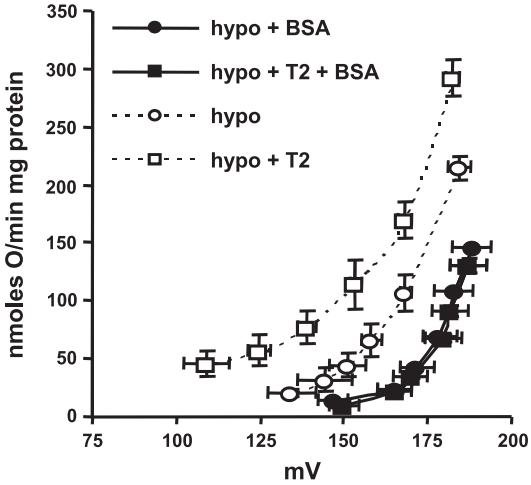

Fig. 4. Proton leak kinetics in hypothyroid and T_2-treated hypothyroid mitochondria measured in both the presence and absence of free fatty acids (FFA). To detect mitochondrial proton leak in the presence of FFA, BSA was omitted from both mitochondria isolation and respiration medium (hypo and hypo + T_2). To detect mitochondrial proton leak in the absence of FFA, BSA was present in both the isolation and respiratory medium (hypo + BSA and hypo + T_2 + BSA). Mitochondria were incubated in respiration buffer for 3 min before addition of 4 μM triphenylmethylphosphonium (to calibrate the electrode). Respiration was then initiated by addition of 5 mM succinate. To evaluate the kinetic response shown by mitochondrial proton leak to a change in $\Delta\Psi$, respiration was inhibited by sequential addition of malonate ($\leq$1 mM). Data points are means ± SE for 5 independent experiments, each one performed in duplicate.

hypothyroid rats, respectively. These data permit us to exclude the possibility that a higher concentration of FFA in skeletal muscle from T_2-treated hypothyroid rats could have affected data on mitochondrial uncoupling. These data as a whole indicate a crucial role for FFA in the effect exerted by T_2 on mitochondrial uncoupling.

DISCUSSION

In previous studies, we identified T_2 as a molecule able to increase the metabolic rate of hypothyroid rats (24, 30–31). Later, we showed that it has the ability to reduce adiposity, serum triglyceride and cholesterol levels (23), the fat content of the liver (steatosis) (12, 23), and body weight gain without a reduction in calorie/fat intake when administered to rats for 4 wk at the same time as a high-fat diet (23).

In the present study, we focused on skeletal muscle since it accounts for about 40% of body mass in mammals and is an important site for lipid oxidation and thermogenesis (6, 15). Our results highlight the ability of T_2, when acutely injected into hypothyroid rats, to induce a rapid stimulation of fatty acid oxidation and thermogenesis in skeletal muscle.

Our results show that T_2 induced an increase in oxygen consumption rate when the mitochondria were provided with succinate as substrate in the presence of rotenone. This suggests that T_2 enhances $FADH_2$-linked respiratory pathways and confirms our previous findings of the ability of T_2 to affect the overall reactions involved in the oxidation of succinate and in the production of the membrane potential (26, 28). On the other hand, the inability of T_2 to significantly affect pyruvate plus malate-supported respiration seems to exclude the NADH-linked respiratory pathways as possible targets of T_2.

Our results also show that T_2 induced mitochondrial fatty acid oxidation, whatever the lipidic substrate used, the highest stimulatory effect of T_2 being observed when palmitoyl-CoA (plus malate) was used as substrate (+104% compared with +80% for palmitoylcarnintine + malate). This led us to hypothesize that CPT I, considered the rate-limiting step for fatty acid uptake into the mitochondrion, could also be a target of T_2. This idea was supported by our findings that, within 1 h after T_2 injection, total CPT activity was significantly enhanced (Fig. 1*A*).

The stimulation of fatty acid oxidation by T_2 appears to be mediated by activation of AMPK. Indeed, T_2 induced activation of AMPK (i.e., AMPK phosphorylation) and inactivation of its protein target ACC (i.e., phosphorylation) within 1 h after its injection to hypothyroid rats. Stimulation of the AMPK-ACC-malonyl-CoA pathways leads to a rapid increase in the CPT system with a consequent increase in long-chain fatty acyl-CoA uptake and oxidation by mitochondria. This strongly supports AMPK as the kinase that rapidly directs lipid partitioning toward oxidation in T_2-treated rats.

It has been reported that, inside mitochondrion, a decrease in the CoA-SH/acetyl-CoA ratio and an accumulation of β-oxidation intermediate metabolites can cause feedback inhibition of the β-oxidation pathway at several steps (4). Moreover, the presence of excess of acyl-CoA within the matrix has been reported to inhibit ADP/ATP transport (38) and to promote myocyte apoptosis (30, 38). Enhanced fatty acid oxidation may drastically decrease the level of matrix CoA-SH required to support the enhanced β-oxidation by converting most of it to acyl-CoA (4). However, the present data show that the T_2-induced increase in the fatty acid oxidation rate was accompanied by an enhanced activity of MTE-1 (Fig. 1*B*), an enzyme able to catalyze the cleavage of acyl-CoA to the relevant fatty acid and CoA. Taken as a whole, these data suggest that T_2 promotes reactions that support fatty acid utilization by preventing depletion of the free CoA pool, thus avoiding *1*) the accumulation of long-chain fatty acid metabolites and *2*) feedback inhibition of β-oxidation by its own products.

Alongside the enhanced fatty acid oxidation induced by T_2 administration to hypothyroid rats, there was a rapid decrease in oxidative phosphorylation efficiency as T_2 significantly increased proton leak kinetic, the effect being dependent on the presence of FFA (Fig. 4). In addition, the ability of T_2 to stimulate state 4 respiration (i.e., respiration in which phosphorylation of ADP to ATP is absent and mitochondria consume oxygen to balance the flux of proton back to the matrix) was almost doubled when assessed in the presence of FFA compared with the condition in which they were chelated by BSA addition to the incubation medium (Table 1).

Taken as a whole, the present data demonstrate that T_2 has the capacity to rapidly stimulate FFA handling in hypothyroid rats, leading to *1*) complete fatty acid utilization in skeletal muscle mitochondria, *2*) the direction of lipid partition to oxidation by activating AMPK, and *3*) the enhancement of proton leak kinetics.

Activation of process that lead to fatty acid oxidation and mitochondrial uncoupling underscores potentially important roles for T_2 affecting thermogenesis in skeletal muscle in its protection against excessive intramyocellular lipid storage.

GRANTS

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