Triiodothyronine-mediated up-regulation of UCP2 and UCP3 mRNA expression in human skeletal muscle without coordinated induction of mitochondrial respiratory chain genes[1]

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SPECIFIC AIMS

Triiodothyronine (T3) increases mitochondrial respiration and promotes, in rodents, the uncoupling between oxygen consumption and ATP synthesis. The T3 effect is mediated partly through transcriptional control of genes encoding mitochondrial proteins. Here, we determined the effect of T3 on mRNA levels of uncoupling proteins (UCP) and proteins involved in the biogenesis of the respiratory chain in human skeletal muscle and on UCP2 mRNA expression in adipose tissue.

PRINCIPAL FINDINGS

1. *In vivo* treatment with T3 increases UCP2 and UCP3 mRNA levels in human skeletal muscle without induction of respiratory chain gene expression

Ten young healthy men were treated for 14 days with 75 μg per day of T3. The treatment induced a 1.7-fold increase in free T3 levels ($P < 0.005$). Resting metabolic rate adjusted for lean body mass was increased by 15% ($P < 0.05$). The respiratory quotient was decreased ($P < 0.05$) and plasma NEFA levels were not significantly modified by the treatment.

T3 treatment induced a 1.7- and 2.4-fold increase in UCP2 and total UCP3 mRNA levels ($P < 0.01$), respectively (**Fig. 1**). The mRNA levels of proteins of the respiratory chain and factors controlling their expression were also determined. There was no change in nucleus-encoded cytochrome *c* oxidase subunit 4 (COX4), nuclear respiratory factor 1 (NRF1), and PPARγ coactivator 1 (PGC1) mRNA expression. Before and during the T3 treatment, NRF1 and PGC1 mRNA levels were positively correlated ($r=0.62$, $P<0.05$ and $r=0.76$, $P<0.02$, respectively). Mitochondrial transcription factor A (mtTFA) and mitochondrion-encoded COX2 mRNA expression were not modified by the treatment.

2. T3 induces UCP2 and UCP3 mRNA expression in primary culture of human skeletal muscle cell

The effect of T3 was studied in human muscle cells differentiated into myotubes. After 24 h of incubation, T3 induced a dose-dependent increase in UCP2 mRNA levels with a 4.5-fold induction at 100 nM. In untreated myotubes, UCP3 mRNA levels were much lower than in skeletal muscle biopsies, as previously reported in rodent skeletal muscle cell lines. However, T3 at 100 nM induced a 2.5-fold increase in UCP3 mRNA levels.

3. UCP2 mRNA expression was also increased by T3 *in vivo* and *in vitro* in human adipose tissue

In subcutaneous adipose tissue (**Fig. 2**), a threefold increase in UCP2 mRNA expression was observed *in vivo* ($P<0.05$). The effect of T3 was also studied in a culture system based on drug treatment of human subcutaneous adipose tissue explants. UCP2 mRNA levels were determined on mature adipocytes isolated at the end of the treatment period (Fig. 2). T3 induced a dose-dependent increase in UCP2 mRNA levels with a twofold increase at 100 nM.

CONCLUSIONS AND SIGNIFICANCE

This study shows, in humans, that a doubling of plasma T3 levels led to an up-regulation of UCP2 and UCP3

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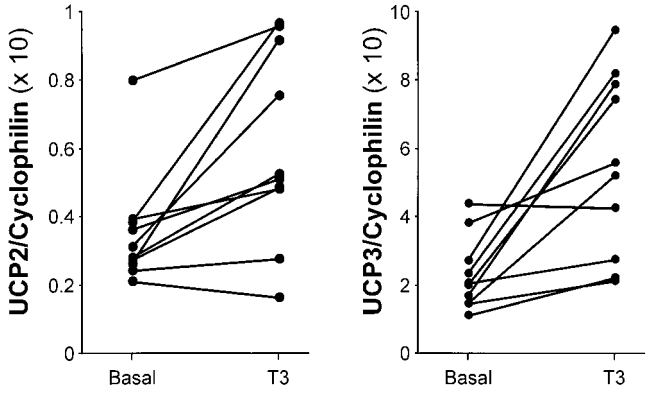

Figure 1. Individual variations in skeletal muscle UCP2 and UCP3 mRNA levels of 10 healthy men treated with T3 for 14 days.

mRNA levels in skeletal muscle and adipose tissue. In skeletal muscle, the increased expression of UCP2 and UCP3 mRNA was not associated with changes in mRNA levels of respiratory chain proteins and factors controlling their expression. Furthermore, the induction of UCP mRNA expression by T3 was demonstrated *in vitro* in primary cultures of human skeletal muscle cells and adipose tissue explants.

In rodents, T3 has been shown to affect the leak of protons across the inner mitochondrial membrane. UCP2 and UCP3 are candidates to mediate the proton leak. Using heterologous yeast and mammalian cell expression systems, UCP2 and UCP3 have been shown to decrease mitochondrial membrane potential. State 4 respiration rate, i.e., respiration in the absence of ADP due primarily to proton leak, and basal oxygen consumption are increased in yeasts expressing UCP2 and UCP3. Accordingly, skeletal muscle mitochondria from mice lacking and overexpressing UCP3 have increased and decreased coupling, respectively. UCP3 gene knockout mice do not show gross phenotypic abnormalities whereas overexpression of human UCP3 in skeletal muscle results in increased energy expenditure and decreased adipose tissue mass.

Our data identify the two proteins as targets for T3-mediated gene regulation in human skeletal muscle and adipose tissue, and suggest that one of the mechanisms through which T3 increases uncoupling activity is the up-regulation of UCP2 and UCP3. Data in rodents support this hypothesis. Comparing rats with different thyroid status, a correlation was found between UCP3 mRNA level and state 4 respiration rate. Moreover, chronic administration of T3 induces a 60% decrease *in vivo* in rat skeletal muscle mitochondrial energy coupling that correlates with an increase in UCP3 mRNA and protein expression, suggesting that UCP3 up-regulation by T3 may contribute to the variations in skeletal muscle mitochondrial proton leak. Whereas these data argue for a link between T3 and UCP3-mediated uncoupling in skeletal muscle, the involvement of UCP3 in thyroid hormone-induced whole body thermogenesis is more elusive. Through the modulation of mitochondrial energy coupling, UCP2 and UCP3 could limit reactive oxygen species (ROS) production generated by the electron transport chain. Such a role was suggested for UCP2 in non-parenchymal liver cells, spleen, thymus, and hepatocytes. Moreover, skeletal muscle mitochondria lacking UCP3 overproduce ROS *in vivo*. Thyroid hormone increases oxidative stress in skeletal muscle. In that context, the up-regulation of UCP expression could be viewed as a protective mechanism counteracting T3-mediated increase in oxygen free radical production. T3 is also known to stimulate fatty acid oxidation. The decrease in respiratory quotient observed in our study suggests that T3 treatment induced an increase in fatty acid oxidation. In humans, we previously found a correlation between UCP3 mRNA levels and lipid oxidation rate. The up-regulation of skeletal muscle UCP3 mRNA expression by T3 is therefore consistent with a link between UCP3 and fatty acid oxidation.

The regulation of UCP2 and UCP3 gene expression in humans is not well documented. Previous studies showed that fatty acids are involved in the *in vivo* regulation of UCP3 mRNA expression in human skeletal muscle. Positive correlations have been reported between plasma NEFA and UCP3 mRNA levels. An increase in plasma NEFA levels resulting from a triglyceride infusion led to an up-regulation of UCP3 mRNA expression. In our experimental conditions, plasma NEFA levels were not modified during the T3 treatment, but it is known that T3 increases both adipose tissue lipolysis and fatty acid oxidation. These data thus raised the question as to whether the *in vivo* enhanced UCP expression by T3 was the result of an indirect effect through an increase in NEFA fluxes. Our *in vitro* data do not support this assumption, since we clearly showed that T3 acts directly on human adipocytes and myotubes to up-regulate UCP mRNA expression.

Several mechanisms could account for the effect of

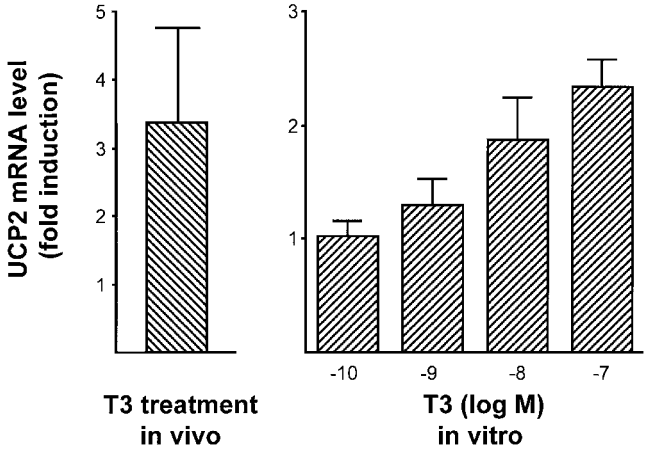

Figure 2. *In vivo* (left panel) and *in vitro* (right panel) effects of T3 on UCP2 mRNA expression in human adipose tissue. Values (means±SE of five and three experiments for *in vivo* and *in vitro* experiments, respectively) are expressed as fold induction of UCP2 mRNA level from untreated adipose tissues.

T3 on UCP mRNA expression. T3 stimulates transcription through thyroid hormone receptors, which bind as heterodimers with the retinoic acid receptor RXR to thyroid hormone response elements (TRE). The promoter of the human UCP3 gene contains a sequence that varies by a single base from the canonical motif, but it is not known whether this putative TRE is functional. Another potential mechanism is suggested by the effect of T3 on nuclear respiratory genes. For most of them, there is no evidence to date that T3 activates transcription through TRE located in the cognate promoters. In rodents, NRF1 *trans*-activates T3-inducible mitochondrial genes encoded in the nucleus. Stimulation of NRF1 and COX4 mRNA expression by thyroid hormone has been reported in rodent skeletal muscle. PGC1 greatly increases the transcriptional activity of thyroid hormone receptors. Ectopic expression of the coactivator in muscle cells induces NRF1 and UCP2 gene expression. The correlation between PGC1 and NRF1 mRNA levels suggests that the relationship between the two nuclear factors may also exist in human skeletal muscle. In our study, the doubling of plasma T3 levels for 14 days did not modify NRF1, PGC1, and COX4 mRNA levels. Moreover, the lack of induction of COX2 and mtTFA mRNA levels indicates that mitochondrial DNA transcription was not modified during the treatment. The data do not preclude an effect of higher plasma free T3 levels and prolonged thyroid status alteration on mitochondrial biogenesis in humans. However, they show that T3 regulation of UCP2 and UCP3 mRNA levels is not mediated through the respiratory chain transcription program (**Fig. 3**). The findings also suggest that an increase in uncoupling capacity due to UCP up-regulation may occur without concomitant changes in respiratory capacity, thereby contributing to decreased mitochondrial energy coupling. FJ

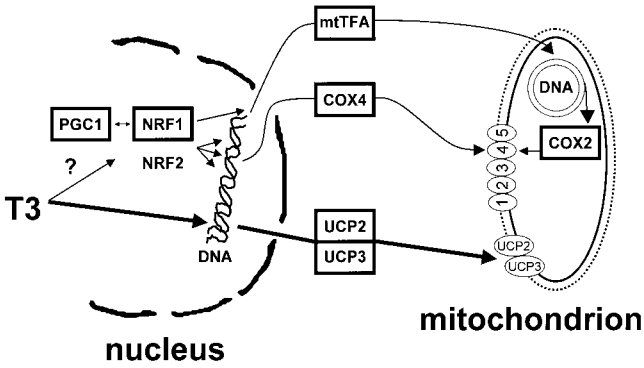

Figure 3. Schematic diagram of thyroid hormone effect on UCP and respiratory chain gene expression in human skeletal muscle. T3 up-regulates UCP2 and UCP3 mRNA expression without induction of the respiratory chain transcription program that controls expression of nucleus- and mitochondrion-encoded proteins. COX, cytochrome *c* oxidase subunit; mtTFA, mitochondrial transcription factor A; NRF, nuclear respiratory factor; PGC1, PPARγ coactivator 1. Respiratory chain complexes are numbered from 1 to 5. Boxes represent proteins which mRNA levels were determined in the study.