Tissue-specific regulation of medium-chain acyl-CoA dehydrogenase gene by thyroid hormones in the developing rat

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During development, gene expression of medium-chain acyl-CoA dehydrogenase (MCAD), a nuclear-encoded mitochondrial enzyme that catalyses the first step of medium-chain fatty acid β-oxidation, is highly regulated in tissues in accordance with fatty acid utilization, but the factors involved in this regulation are largely unknown. To investigate a possible role of thyroid hormones, rat pups were made hypothyroid by the administration of propylthiouracyl to the mother from day 12 of gestation, and their kidneys, heart and liver were removed on postnatal day 16 to determine MCAD mRNA abundance, protein level and enzyme activity. Similar experiments were run in 3,3′,5-tri-iodothyronine (T_3)-replaced hypothyroid (1 μg of T_3/100 g body weight from postnatal day 5 to 15) and euthyroid pups. Hypothyroidism led to an increase in MCAD mRNA abundance in kidney and a decrease in abundance in heart, but had no effect in liver. The protein levels and enzyme activity were lowered in hypothyroid heart and kidney, suggesting that hypothyroidism affects post-transcriptional steps of gene expression in the kidney. All the effects of hypothyroidism were completely reversed in both heart and kidney by T_3 replacement. Injection of a single T_3 dose into 16-day-old euthyroid rats also led to tissue-specific changes in mRNA abundance. Nuclear run-on assays performed from hypothyroid and hypothyroid plus T_3 rats showed that T_3 stimulates MCAD gene transcription in heart and represses it in the kidney. These results indicate that the postnatal rise in circulating T_3 is essential to the developmental regulation of the MCAD gene *in vivo*.

INTRODUCTION

Medium-chain acyl-CoA dehydrogenase (MCAD) is a nuclear-encoded mitochondrial flavoenzyme that catalyses the first step of the β-oxidation of medium-chain fatty acids. Numerous results point to the importance of medium-chain fatty acid oxidation in energy production of suckling newborn mammals, in both human and animal species. Medium-chain fatty acids are supplied in much higher amounts in maternal milk than in the adult diet [1]. Medium-chain triacylglycerols are used preferentially to other fat components of the milk in the newborn [2], because they are more efficiently solubilized, digested and absorbed by the immature gastrointestinal tract than long-chain fatty acids [3]. Because of their lower hydrophobicity, transport of medium-chain fatty acids into the cells, and into cell cytoplasm, is not strictly dependent on the expression of specific fatty acid-binding proteins, as happens for long-chain fatty acids [4]. Further, medium-chain fatty acids can freely cross the mitochondrial membranes [1], in contrast with long-chain fatty acids, and therefore represent a readily available substrate for ATP production by mitochondrial β-oxidation. The critical role of MCAD in energy metabolism is underscored by the severity of clinical features associated with MCAD deficiency [5]. Inborn deficit of MCAD, which was first described in the early 1980s, is not only a potentially fatal β-oxidation deficit, but also represents one of the most common inborn errors of metabolism [6].

The changes in MCAD gene expression during the fetal and postnatal developmental stages have been studied in a number of rat tissues. Steady-state levels of MCAD mRNA are generally low in all fetal tissues, and increase markedly after birth in organs that depend upon fatty acid oxidation for ATP production [7–9]. The developmental patterns of MCAD gene expression in rat liver, heart and kidney cortex [7,9,10] exhibit a marked tissue specificity. In the three organs, however, the highest steady-state levels of MCAD mRNA are found during the third postnatal week, i.e. at the end of the suckling period; MCAD mRNA decreases thereafter in kidney and liver, and remains high in the adult heart [7,9,10]. These results raise the question of the regulatory factors that might be involved in triggering MCAD gene expression in these tissues during the postnatal period. There are few studies dealing with control of the nuclear gene encoding MCAD and other mitochondrial β-oxidation enzymes by physiological factors such as the dietary or hormonal changes that occur during the postnatal period [9,10]. In the present study we have sought to determine whether thyroid hormone could be involved in the control of MCAD gene expression in rat kidney, heart and liver during the suckling period. Thyroid hormone is an excellent candidate for regulation of the MCAD gene *in vivo*, because a thyroid hormone response element (TRE) has been identified in the human MCAD gene promoter [11,12]. Moreover, thyroid hormone is a potent modulator of cellular energy metabolism [13] and is necessary for the activation of developmental gene regulation programmes [14,15]. We therefore studied the changes in MCAD mRNA, protein and enzyme activity in response to modifications in the thyroid status of developing rats, in three organs that use large amounts of fatty acids as energy substrates: liver, heart and kidney. Our results demonstrate that 3,3′,5-tri-iodothyronine (T_3) regulates MCAD gene expression at the transcriptional and translational or post-translational levels, in a tissue-specific manner during postnatal development.

MATERIALS AND METHODS

Animals

Pregnant Wistar rats were rendered hypothyroid by giving them propylthiouracyl (PTU; 0.01 %) in the drinking water from the

Abbreviations used: MCAD, medium-chain acyl-CoA dehydrogenase; PTU, propylthiouracyl; T_3, 3,3′,5-tri-iodothyronine.

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12th day of gestation through the entire postnatal period. They had free access to standard solid food (UAR 113; UAR Villemoisson sur Orge, France). Each litter was reduced to 10 pups at birth.

T_3 replacement (1 μg/100 g body weight) was given to hypothyroid rats by subcutaneous injection once daily from 5 to 15 days after birth. T_3 (Sigma, St Louis, MO, U.S.A.) was dissolved in 0.01 M NaOH and injected after neutralization of this solution. With this replacement dose, T_3 plasma levels measured in 16-day-old treated rats were no different from those found in age-matched euthyroid 16-day-old control rats (see the Results section). Hypothyroid controls from the same litter received only the vehicle for the same periods. For the nuclear run-on experiments, hypothyroid rats received T_3 subcutaneously at the dose of 10 μg/100 g body weight once daily from day 13 to day 15 after birth. This T_3 dosage was chosen in accordance with Schmitt and McDonough [16] in order to saturate T_3 nuclear receptors. On postnatal day 16, all the animals were anaesthetized with ketamine (50 mg/kg body weight) (Imalgène, Rhône-Mérieux Lyon, France), and liver, heart and kidneys were rapidly removed, immediately frozen in liquid nitrogen, and stored at -80 °C. Kidney cortex was dissected by hand at -20 °C from frozen organs.

For the studies of T_3 effects in euthyroid pups, control litters of 16-day-old rats were given a single subcutaneous injection of T_3 (10 μg/100 g body weight), or received vehicle only, and the organs were removed 6 h later.

Blood samples were collected from all the animals and the plasma T_3 levels were measured by radioimmunoassay (Amerlex T_3 RIA kit IM.3001; Amersham).

Northern blot analysis

Total RNA was extracted from liver, heart and kidney cortex with the RNAzol B technique [17]. The concentration of RNA was measured by A_{260}. Total RNA (15 μg) was fractionated through a formaldehyde/agarose gel and transferred to a nylon membrane. Membranes were incubated with [α-^{32}P]dCTP-labelled cDNA. The cDNA probe used in this study was a rat MCAD *Eco*RI fragment of 871 bp [18]. Prehybridization and hybridization were performed in 5 × SSPE [1 × SSPE = 0.15 M NaCl/10 mM sodium phosphate (pH 7.4)/1 mM EDTA]/50 % (v/v) deionized formamide/5 × Denhardt's solution/1 % SDS/100 μg/ml salmon sperm DNA. Prehybridization was performed in a 42 °C shaker bath for 4 h and hybridization for 18–24 h under the same conditions with labelled cDNA probe. The membranes were then washed twice with 2 × SSC (1 × SSC = 0.15 M NaCl/0.015 M sodium citrate) for 10 min at room temperature, once with 2 × SSC and 1 % (w/v) SDS for 10 min at room temperature and twice with 1 × SSC and 1 % SDS at 42 °C for 20 min. Autoradiographs were obtained by exposing the membranes to Kodak X-OMAT film with two intensifying screens at -80 °C. Multiple exposures were made to ensure that the signals were within the linear range of the film sensitivity. Quantification of signal intensity was performed by densitometric analysis of the autoradiograms with a computerized video densitometer (Biocom, Les Ulis, France). The blots were also hybridized with a 18 S rRNA cDNA probe to allow corrections for variations in the amount of RNA loaded.

Measurement of MCAD activity

The activity of MCAD was determined spectrophotometrically by following the decrease in ferricenium ion absorbance at 300 nm, as previously described [10]. Briefly, tissue samples (20–50 mg) were weighed frozen, and homogenates immediately prepared as a 20 % (w/v) suspension in ice-cold 100 mM Hepes (pH 7.6)/0.1 mM EDTA with a motor-driven Teflon/glass homogenizer. The homogenates were then centrifuged at 7000 ***g*** for 2 min and the determination of MCAD activity was immediately performed on the supernatant. This supernatant contained all detectable MCAD enzyme activity, because further treatment of the pellet by homogenization and freeze–thaw cycles yielded a second supernatant with no detectable enzyme activity. MCAD activity was measured at 37 °C by adding 5 μl of supernatant to 500 μl of reaction mixture containing 100 mM Hepes (pH 7.6)/0.1 mM EDTA/200 μM ferricenium hexafluorophosphate/0.5 mM sodium tetrathionate/50 μM octanoyl-CoA. The decrease in absorbance of ferricenium hexafluorophosphate in the presence of homogenate was found to be stable for at least 3 min; the results were calculated from the decrease observed over the initial 60 s period. The results were corrected for a tissue blank measured in the absence of octanoyl-CoA from the reaction mixture.

Western blot analysis

Small pieces of frozen tissue were homogenized in ice-cold 10 mM Tris/HCl, pH 7.4, containing 320 mM sucrose, 1 mM EDTA and the protease inhibitors 2 μg/ml leupeptin, 10 μg/ml aprotinin and 1 mM PMSF, in a small Potter homogenizer at approx. 50 mg/ml. The homogenates were centrifuged at 750 ***g*** for 10 min and the supernatant was stored at -80 °C until use. Protein concentrations were determined by the method of Bradford [19].

Protein fractionation of rat tissue homogenates was performed by SDS/PAGE [12 % (w/v) gel] by the method of Laemmli [20] with Mini-Protean II apparatus from Bio-Rad. The gel was electrically blotted on a nitrocellulose membrane. After blotting, the gel was stained with Coomassie Blue to examine the blotting efficiency. Before the Western-blot procedure and to ensure that the variations observed in the amount of MCAD protein between the various groups were not due to variations in protein loading, the blots were coloured with Ponceau S. This procedure revealed no obvious differences in the amount of total proteins loaded in the gels. Blots were blocked for 1 h in 5 % (w/v) non-fat milk in Tris-buffered saline containing 0.05 % Tween-20 (TBS-T). The blots were then incubated overnight at 4 °C with rabbit antibodies raised against MCAD [18]. After being washed in TBS-T, the membranes were incubated for 1 h at room temperature with a donkey anti-[rabbit IgG (H + L)] used as the secondary antibody. Finally, after washing, the secondary antibody was coupled to rabbit peroxidase anti-peroxidase and detected with the enhanced chemoluminescence system (Amersham) by exposure to X-ray film. The approx. 45 kDa immunoreactive bands corresponding to the subunit of MCAD tetrameric flavoprotein [21] were scanned by densitometry with a computerized video densitometer.

Nuclear run-on assay *in vitro*

Nuclei from 16-day-old hypothyroid and T_3-treated 16-day-old hypothyroid rat heart or kidney cortex were isolated by the method of Sierra et al. [22]. Briefly, 1 g of tissue was homogenized in approx. 6 ml of ice-cold homogenization buffer containing 10 mM Hepes, pH 7.6, 15 mM KCl, 0.15 mM spermine, 0.5 mM spermidine, 1 mM EDTA, 2.4 M sucrose (RNase-free), 0.5 mM dithiothreitol and 0.5 mM PMSF. The homogenate was filtered through nylon cloth, layered carefully on top of a 10 ml sucrose cushion and centrifuged at 75000 ***g*** for 60 min at 4 °C. In this procedure, the homogenization buffer is used both for homogenization and for preparation of the cushions. The clean

nuclei were resuspended in nuclei storage buffer [50 mM Tris/HCl (pH 8.3)/40 % (v/v) glycerol/5 mM $MgCl_2$/0.1 mM EDTA] and stored at −80 °C. The transcription rate was determined by the method described by Greenberg [23]. Aliquots of 10^7 nuclei were incubated for 30 min at 30 °C in 200 μl of reaction mixture containing 10 mM Tris/HCl, pH 8.0, 5 mM $MgCl_2$, 0.3 mM KCl, 1 mM of each of ATP, CTP and GTP, 5 mM dithiothreitol, 500 units/ml RNase inhibitor (RNasin) and 200 μCi of [α-^{32}P]UTP (3000 Ci/mmol). The reaction mixture was then treated with RNase-free DNase I (20 μg/ml) for 5 min at 30 °C, and deproteinized by digestion with 100 μg/ml proteinase K at 42 °C for 30 min. After extraction with phenol/chloroform (1:1, v/v), the aqueous phase was precipitated for 30 min on ice with cold 10 % (w/v) trichloroacetic acid in the presence of 60 mM $Na_4P_2O_7$ and 10 μg/ml *Escherichia coli* tRNA carrier to remove unincorporated ^{32}P-labelled nucleotides. The precipitate was filtered on a Whatman GF/A glass-fibre filter; the filters were washed three times with 5 % (w/v) trichloroacetic acid/30 mM $Na_4P_2O_7$. The filters were then incubated for 30 min at 37 °C in a mixture containing 20 mM Hepes, pH 7.5, 5 mM $MgCl_2$, 1 mM $CaCl_2$ and 25 μg/ml RNase-free DNase I. The reaction was quenched by 15 mM EDTA and 1 % (w/v) SDS. The samples were heated for 10 min at 65 °C to elute the RNA, after which the supernatants were removed and saved. This procedure removes more than 95 % of the radioactivity from the filters. The supernatants containing ^{32}P-labelled RNA were again digested with proteinase K (30 μg/ml) for 30 min at 37 °C and extracted with phenol/chloroform. The aqueous phase was precipitated overnight at −20 °C with 0.1 vol. of 3 M sodium acetate and 2.5 vol. of ethanol. After washing the precipitate with 75 % (v/v) ethanol, the radiolabelled RNA were resuspended in 200 μl of hybridization mixture. For specific detection of radioactive nascent RNA transcripts, 6×10^6 c.p.m. of isolated RNA was hybridized for 72 h at 42 °C with cDNA plasmids previously immobilized on nylon membranes. Portions (5 μg) of each linearized MCAD plasmid, β-actin plasmid and PGEM3Z and PBR322 control plasmids were denatured and blotted under vacuum on a nylon membrane. Washing, autoradiography and image analysis were performed as described for Northern blot analysis.

Expression of results and statistical analysis

The mRNA and protein abundance were expressed on a relative percentage basis; the results were obtained from at least two different Northern or Western blots. MCAD enzyme activity was expressed as μmol of octanoyl-CoA oxidized/min per g wet weight. The data are expressed as means ± S.E.M. The means for five to ten individual rats in each experimental group were subjected to one-way analysis of variance (ANOVA) and the Fisher test; $P < 0.05$ was considered significant.

RESULTS

Animals

Table 1 shows the plasma level of T_3, and body and organ weights in the various groups of animals. As expected, treatment by PTU decreased the T_3 in circulation to undetectable levels. In the animals supplemented during days 5–15 after birth, plasma T_3 levels were restored to near-physiological values (Table 1). PTU-induced hypothyroidism resulted in postnatal growth retardation, since body weights on day 16 were decreased by 24 % ($P < 0.001$) compared with 16-day-old euthyroid rats. Similarly, heart and kidney weights were lowered by an average of 34 % in hypothyroid rats. Administration of T_3 during days 5–15 to pups born to PTU-treated dams stimulated growth, but did not fully restore body weights to the control levels. Treatment with T_3 had contrasting effects on heart and kidney growth. In fact, heart growth was strongly stimulated in response to T_3 administration, since heart weights of hypothyroid pups receiving T_3 were similar to those in control age-matched pups. By comparison, replacement by T_3 led to a modest (+15 %) increase in kidney weight and therefore did not restore the values found in euthyroid rats.

Table 1 T_3 plasma levels, body weight, and heart and kidney weights in euthyroid, hypothyroid and T_3-replaced hypothyroid 16-day-old rats

Rat pups were made hypothyroid by the administration of PTU to the mother from day 12 of gestation. T_3-replaced hypothyroid pups (PTU + T_3) received one daily injection of 1 μg of T_3/100 g body weight from postnatal day 5 to day 15. The plasma and organs were sampled on day 16, and T_3 levels were measured by radioimmunoassay as described in the Materials and methods section. Results are means ± S.E.M. for five to nine rats. Abbreviation: n.d., not detectable. *$P < 0.05$, **$P < 0.01$ compared with euthyroid values. ††$P < 0.01$ compared with hypothyroid values.

	T_3 (nM)	Body weight (g)	Heart weight (mg)	Kidney weight (mg)
Euthyroid	0.8 ± 0.2	30.0 ± 0.6	188 ± 8	217 ± 5
PTU	n.d.	22.8 ± 1.1**	122 ± 6**	146 ± 9**
PTU + T_3	0.59 ± 0.15††	26.7 ± 0.4**††	179 ± 8††	168 ± 4*††

Effect of hypothyroidism and T_3 replacement on MCAD gene expression in liver, kidney and heart

The effects of hypothyroidism on MCAD gene expression were markedly different from one organ to another (Figure 1). In the liver, no differences in MCAD mRNA abundance, protein level and enzyme activity were found between 16-day-old euthyroid and 16-day-old PTU-treated rats (Figure 1, Table 2). In the kidney cortex, hypothyroidism resulted in a large (+80 %) increase in mRNA steady-state level together with decreases in MCAD protein level (−24 %) and enzyme activity (−10 %) (Table 2). In the heart, hypothyroidism led to a significant ($P < 0.001$) decrease in the levels of MCAD mRNA, protein and enzyme activity (Table 2).

In the rat, T_3 plasma levels are exceedingly low at birth, start to increase 1 week after birth and reach high levels during the second postnatal week [24,25]. The T_3 replacement to hypothyroid rats during postnatal days 5–15 therefore covered the whole period during which T_3 level normally increases in euthyroid rat pups. This T_3 replacement to hypothyroid rats resulted in a decrease in MCAD mRNA steady-state levels in the kidney cortex (Figure 1) and restored the values found in euthyroid rats (Table 2). Kidney cortex MCAD protein and enzyme activity were also restored to control values after T_3 administration (Table 2). In the heart, the levels of MCAD mRNA, protein and enzyme activity were significantly increased in response to T_3 replacement to hypothyroid rats (Figure 1), reaching levels similar to those found in euthyroid rat heart (Table 2).

Effect of T_3 administration to 16-day-old euthyroid rats on MCAD mRNA

To ascertain whether the up-regulation of MCAD mRNA by T_3 in the heart, and its down-regulation in the kidney, involve a direct and rapid hormonal effect, 16-day-old euthyroid animals were injected with a single high dose of T_3 (10 μg/100 g body weight) to ensure that the maximum effects were elicited. This led

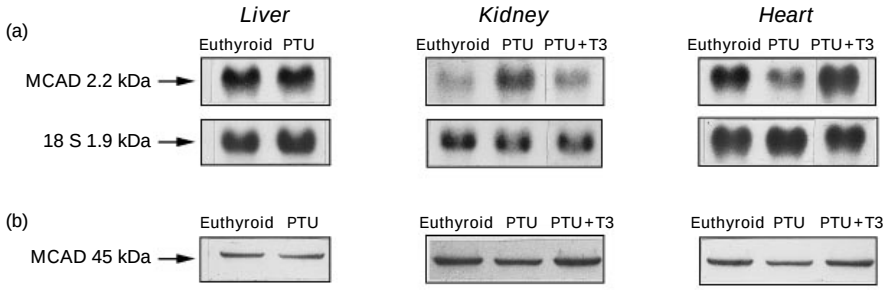

Figure 1 Tissue-specific expression of MCAD in euthyroid, hypothyroid (PTU) and T_3-replaced hypothyroid (PTU + T_3) 16-day-old rats

(**a**) Representative Northern blot analysis of 15 μg of total RNA extracted from the various tissues, probed with the MCAD and 18 S cDNAs, as described in the Materials and methods section. (**b**) Representative Western blot analysis from tissue proteins size-fractionated and blotted with an anti-MCAD antibody, as described in the Materials and methods section.

Table 2 Effects of thyroid status on MCAD mRNA, protein and enzyme activity levels in liver, heart and kidney cortex of 16-day-old rats

Abbreviations used are as used in Figure 1. Results are means ± S.E.M. for (a) densitometric scanning of MCAD Northern blot analysis, normalized against 18 S cDNA, (b) densitometric scanning of MCAD Western blot analysis, and (c) determination of enzyme activity in tissue homogenates expressed in μmol of octanoyl-CoA oxidized/min per g of wet weight, as described in the Materials and methods section. For MCAD mRNA and protein analysis, an arbitrary 100% value was given to the 16-day-old euthyroid values. The numbers of individual animals are shown in parentheses. $^{*}P < 0.05$, $^{***}P < 0.001$ compared with euthyroid values; †$P < 0.05$, †††$P < 0.001$ compared with hypothyroid values.

	Liver		Kidney			Heart		
	Euthyroid	PTU	Euthyroid	PTU	PTU + T_3	Euthyroid	PTU	PTU + T_3
(a) mRNA (%)	100 ± 6 (5)	103 ± 9 (6)	100 ± 3 (10)	181 ± 8 (10)***	120 ± 8 (5)†††	100 ± 6 (9)	44 ± 4 (9)***	107 ± 6 (5)†††
(b) Protein level (%)	100 ± 6 (5)	94 ± 10 (5)	100 ± 5 (5)	76 ± 9 (6)*	103 ± 8 (4)†	100 ± 5 (4)	39 ± 4 (4)***	103 ± 5 (4)†††
(c) Enzyme activity (μmol/min per g wet wt.)	9.2 ± 0.3 (10)	8.5 ± 0.3 (10)	5.1 ± 0.1 (10)	4.5 ± 0.1 (10)*	5.0 ± 0.3 (5)	8.6 ± 0.4 (10)	4.5 ± 0.3 (10)***	8.4 ± 0.4 (4)†††

Table 3 Effects of T_3 on kidney and heart MCAD mRNA levels in euthyroid pups

Control rats 16 days old received a subcutaneous injection of T_3 (10 μg/100 g body weight) (euthyroid + T_3) or were injected with T_3 vehicle only (euthyroid) in the morning of postnatal day 16; the organs were removed 6 h later. MCAD Northern blot analysis and mRNA quantifications were performed as described in the Materials and methods section. The results are means ± S. E. M. The numbers of subjects are indicated in parentheses. Euthyroid values were taken as 100% reference. $^{**}P < 0.01$ compared with euthyroid values.

	MCAD mRNA (%)	
	Euthyroid	Euthyroid + T_3
Kidney	100 ± 7 (10)	74 ± 6 (10)**
Heart	100 ± 5 (4)	148 ± 12 (12)**

within 6 h to a 50% increase in MCAD mRNA in the heart and to a 25% decrease in the kidney (Table 3), whereas no changes in enzyme activity were noted in any of the organs (results not shown).

Effect of T_3 on transcription of MCAD gene *in vitro*

To analyse further the mechanism by which thyroid hormones regulate MCAD gene expression, nuclear 'run-on assays' were performed with heart or kidney cortex nuclei prepared either from 16-day-old hypothyroid pups or from 16-day-old hypothyroid pups that had received a high daily dose of T_3 (10 μg/100g body weight) on days 13, 14 and 15. In the heart, the relative transcription rate of MCAD (normalized to the β-actin signal) was increased 1.8-fold in T_3-treated rats (Figure 2). In contrast, MCAD gene expression was lowered in the kidney in response to T_3 replacement (Figure 2).

Northern blotting experiments conducted in the same groups of animals showed that, in response to T_3 administration, the MCAD mRNA abundance was doubled in the heart (196 ± 21%, $n = 3$, in T_3-replaced hypothyroid; $P < 0.001$ compared with the 100% hypothyroid value), and was significantly lowered in the kidney (55 ± 8%, $n = 3$, in T_3-replaced hypothyroid; $P < 0.001$ compared with the 100% hypothyroid value). In heart and also in kidney, the T_3-induced changes in MCAD mRNA abundance and in relative transcription rates range over the same order of magnitude.

DISCUSSION

During development, MCAD gene expression is highly regulated in tissues in accordance with cellular fatty acid oxidation rates [7]. In the present study we tested the hypothesis that thyroid hormones might represent a critical trigger for the development of MCAD gene expression in thyroid-responsive organs with high fatty acid utilization. The results obtained clearly indicate that each of the organs considered in this study exhibits a specific

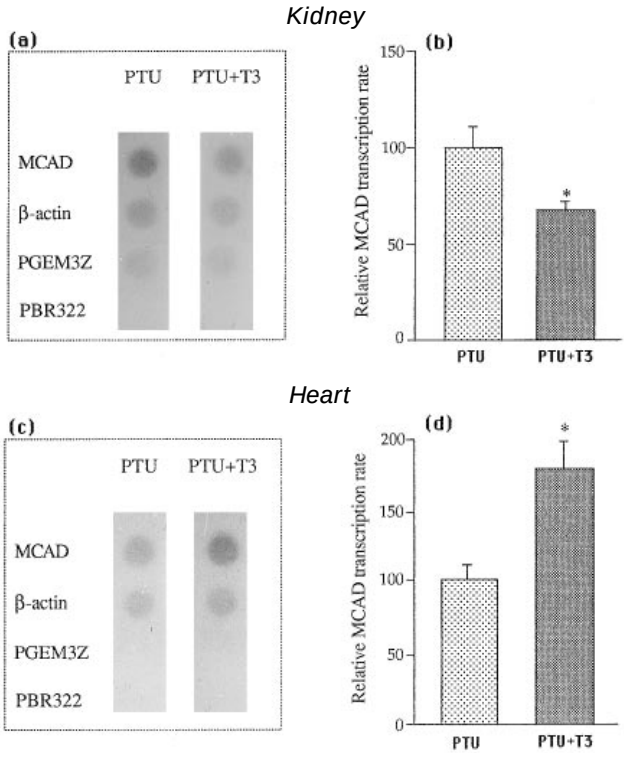

Figure 2 MCAD gene transcription rates in kidney and heart of hypothyroid and T_3-replaced hypothyroid 16-day-old rats

(**a**, **c**) Representative run-on assays performed from isolated nuclei of kidney (**a**) and heart (**c**) from 16-day-old hypothyroid (PTU) or from 16-day-old hypothyroid rats receiving T_3 replacement (10 μg/100 g body weight daily) from day 13 to day 15 (PTU + T3). Transcription rates of the MCAD gene were determined *in vitro* as described in the Materials and methods section. Vector DNAs (PGEM3Z and PBR322) were used as negative control for non-specific hybridization; the results were corrected for the slight background obtained in these experiments. (**b**, **d**) Quantifications of the run-on autoradiogram signals obtained from kidney (**b**) and heart (**d**) nuclei in the PTU and PTU + T_3 groups. Results are means ± S.E.M. for three experiments (in each experiment the kidneys of ten rats were pooled). An arbitrary 100% value was given to the hypothyroid values. $^*P < 0.05$ compared with the PTU values.

response with regard to the effects on MCAD gene expression of changes in the thyroid status of the rat pups. The liver is the only tissue in which the gene expression of MCAD remained unaffected by changes in the thyroid status of the rat pups. In contrast, thyroid hormones seem to control MCAD mRNA abundance in the developing heart and kidney, and exhibit opposite effects in both organs. Thus hypothyroidism resulted in a decrease in MCAD mRNA pool in the heart, and an increase in the kidney. In the hypothyroid kidney, the observed increase in MCAD mRNA levels was not accompanied by increases in protein and enzyme activity; on the contrary, the latter was decreased. This is not related to a generalized impairment of translational capacities, because MCAD activity was unaffected by hypothyroidism in the liver. Therefore the discrepancy between changes in levels of mRNA and enzyme activity in hypothyroid kidney could reflect a tissue-specific impairment of MCAD mRNA translation and/or changes in protein stability. Nevertheless, T_3 replacement during postnatal days 5–15 restored the levels of MCAD mRNA, protein and enzyme activities to normal in both heart and kidney, and therefore completely reversed all the effects of hypothyroidism.

Tissue-specific changes in MCAD mRNA abundance could also be induced by injecting euthyroid 16-day-old pups with a single dose of T_3. This suggests a direct control by thyroid hormone of the MCAD gene, at a transcriptional and/or post-transcriptional level. The nuclear run-on assays performed from hypothyroid and T_3-replaced hypothyroid rat pups showed that MCAD gene transcription rates were increased in heart and decreased in the kidney in response to T_3 replacement, and therefore demonstrated a transcriptional control by T_3 of MCAD gene expression in these tissues. Furthermore, the magnitude of changes in gene transcription induced by T_3 were similar to the corresponding changes in mRNA abundance in both organs. This is consistent with a T_3 effect mainly on gene transcription rather than on MCAD mRNA stability.

Thyroid hormone receptors belong to the nuclear receptor superfamily of ligand-regulated transcription factors [26]. In the developing heart, the T_3-induced stimulation of MCAD gene transcription could result from a direct interaction of the thyroid hormone receptor complex with *cis*-acting DNA elements present on the MCAD gene 5′ flanking region. Indeed, previous studies in NIH 3T3 cells have demonstrated that the MCAD gene 5′ flanking region contains a functional thyroid hormone response element [11]. In contrast with those obtained from heart, all the data obtained from kidney cortex indicate that T_3 represses MCAD gene expression in this tissue, during the postnatal period. However, it is known that MCAD mRNA and enzyme activity are up-regulated in the kidney cortex during the postnatal period [10]. It is therefore very likely that the physiological regulation of renal MCAD gene expression involves co-regulatory factors capable of counteracting the negative control exerted by T_3. Circulating glucocorticoids, which greatly increase together with thyroid hormones after birth [24], could effectively compete with T_3 because they have recently been shown to up-regulate MCAD gene transcription and mRNA abundance in the developing kidney [10]. The various effects of T_3 on MCAD gene expression in immature liver, heart and kidney might also be due to the presence of different thyroid hormone receptor isoforms [27,28], and/or to the expression of tissue-specific transcription factors that could modulate the effect of thyroid hormones. In support of the latter hypothesis, thyroid hormone receptors are known to bind as heterodimers with retinoid-X receptor [29], and the MCAD gene regulatory regions contain a pleiotropic DNA sequence element that can bind retinoid-X receptor and a variety of other nuclear transcription factors (RAR, HNF-4 and COUP-TF) [11,30,31]. Finally it should be mentioned that a new family of T_3 receptor-associating cofactors, named TRACs, has been cloned recently; these proteins are proposed to associate with a variety of nuclear hormone receptors to modulate their transcriptional properties [32–34].

The postnatal development of mitochondrial fatty acid oxidation is essential to produce ATP from milk lipids; it therefore has a crucial role in meeting the increasing energy demand of the developing organs. Much remains to be known about the regulatory factors involved in the timely and full expression of mitochondrial oxidative enzymes during the postnatal period. The results of this study are consistent with recent results supporting a role for thyroid hormone in the postnatal regulation of oxidative enzyme expression in various developing tissues. In particular, it was shown that thyroid hormone controls gene transcription of the β-F1 subunit of the respiratory-chain ATP synthetase complex in immature rat liver mitochondria [14,35,36]. In the developing rat brain, hypothyroidism leads to a decrease in the expression of cytochrome *c* oxidase subunit III, encoded by the mitochondrial genome, and of nuclear-encoded genes coding for other respiratory-chain components (cytochrome *c* oxidase subunits IV and VIc) [15]. In addition, we recently demonstrated that T_3 is essential to the postnatal development of

mitochondrial enzymes of the tricarboxylic acid cycle and ketone-body oxidation pathways in kidney cells with high ATP turnover rates [25].

In conclusion, the results of this study strongly suggest that the postnatal increase in circulating T_3 [25] could have an essential role in the developmental regulation of MCAD gene expression *in vivo*. It is also very likely that transcription factors other than T_3, acting as co-activators or co-repressors, might determine the tissue-specific effects of T_3 on MCAD gene expression. Further studies will help to delineate the nature of these co-regulatory factors, and to improve our understanding of the physiological and molecular basis of oxidative-enzyme gene expression during development.

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