Involvement of the peroxisome proliferator-activated receptor α in regulating long-chain acyl-CoA thioesterases

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Abstract Long-chain acyl-CoA thioesterases catalyze the hydrolysis of acyl-CoAs to the corresponding free fatty acid and CoA. We recently cloned four members of a novel multigene family of peroxisome proliferator-induced genes encoding cytosolic (CTE-I), mitochondrial (MTE-I), and peroxisomal (PTE-Ia and PTE-Ib) acyl-CoA thioesterases (Hunt et al. 1999. *J. Biol. Chem.* 274: 34317–34326). As the peroxisome proliferator-activated receptor alpha (PPARα) plays a central role in regulating genes involved in lipid metabolism, we examined the involvement of this receptor in regulation of the thioesterases, particularly CTE-I and MTE-I. Northern blot analysis shows that the induction of these thioesterases by clofibrate is mediated through a strictly PPARα-dependent mechanism. All four acyl-CoA thioesterases are induced at mRNA level by fasting and using PPARα-null mice, it is evident that the increase in CTE-I due to fasting is mainly independent of the PPARα in liver and heart. The CTE-I gene responds rapidly to fasting, with induction of mRNA and protein evident after 6 h. This fasting effect is rapidly reversible, with CTE-I mRNA returning almost to control levels after 3 h refeeding, and being further repressed to 20% of control after 9 h refeeding. Although CTE-I mRNA shows a low basal expression in liver, it can be suppressed 90% by feeding a fat-free diet. These data demonstrate that the nutritional regulation of the thioesterases involves the PPARα and other signaling pathways responsible for activation and repression. Putative physiological functions for the acyl-CoA thioesterases are discussed.—Hunt, M. C., P. J. G. Lindquist, J. M. Peters, F. J. Gonzalez, U. Diczfalusy, and S. E. H. Alexson. **Involvement of the peroxisome proliferator-activated receptor α in regulating long-chain acyl-CoA thioesterases. *J. Lipid Res.* 2000.** 41: **814–823.**

Supplementary key words acyl-CoA thioesterases • acyl-CoA • fasting • peroxisome proliferator-activated receptor • lipid metabolism • fat free diet • clofibrate

Acyl-CoAs are important sources of metabolic energy and play roles in degradation and synthesis of fatty acids and their incorporation into complex lipids (for review, see ref. 1). It is proposed that acyl-CoA esters are key regulators of fatty acid synthesis, and long-chain acyl-CoA esters also affect numerous cellular systems and functions such as regulation of ion channels, ion pumps, and translocators, together with membrane fusion and protein acylation (for review, see ref. 2). Recently, acyl-CoAs and non-esterified fatty acids have also been implicated in other cellular processes, such as gene regulation, by acting as ligands for the hepatic nuclear factor 4 alpha (HNF-4α) (3) and the peroxisome proliferator-activated receptor alpha (PPARα) (4, 5), respectively. As long-chain acyl-CoA thioesterases (EC 3.1.2.2) belong to a family of enzymes that catalyze the hydrolysis of acyl-CoAs to free fatty acids and CoA, they can therefore be considered important mediators in many of the above cellular processes by regulating intracellular levels of non-esterified fatty acids and acyl-CoAs.

Long-chain acyl-CoA thioesterases are ubiquitously expressed in different organisms and cell types and show activity in several subcellular compartments, although their physiological functions remain unknown. The acyl-CoA thioesterase activity is strongly induced in rat and mouse liver by treatment with peroxisome proliferators. This induction occurs mainly in cytosol and mitochondria (6–8), and to a much lesser extent in peroxisomes (9, 10). The induced activity is due to two families of thioesterases of approximately 40 and 110–150 kDa, which are expressed in cytosol, mitochondria, and peroxisomes and which are only weakly expressed in liver under normal conditions (8). The low molecular mass enzymes are named CTE-I and MTE-I (cytosolic and mitochondrial acyl-CoA thioesterase, respectively) and the high molecular mass enzymes

Abbreviations: CTE-I, cytosolic acyl-CoA thioesterase I; MTE-I, mitochondrial acyl-CoA thioesterase I; PTE-Ia and Ib, peroxisomal acyl-CoA thioesterase Ia and b; PPAR, peroxisome proliferator-activated receptor; PPRE, peroxisome proliferator response element; CoA, coenzyme A; ACOX, acyl-CoA oxidase; PCR, polymerase chain reaction; bp, base pairs; kb, kilo base pairs; PIPES, 1,4-piperazinediethanesulfonic acid; BAT, brown adipose tissue; SDS, sodium dodecylsulfate; ARP-1, apolipoprotein A-I regulatory protein I.

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are named CTE-II and MTE-II (cytosolic and mitochondrial, respectively). Antibodies raised against MTE-I have been shown to cross-react with CTE-I and with peroxisomal acyl-CoA thioesterases (8, 11). This cross-reactivity suggested the existence of a structurally related family of peroxisome proliferator-inducible acyl-CoA thioesterases with multiple subcellular localizations. The cDNAs corresponding to both CTE-I and MTE-I from rat and CTE-I from mouse were recently cloned (12–14). The genes encoding mouse CTE-I, MTE-I, and two peroxisomal thioesterases, PTE-Ia and PTE-Ib, have also recently been cloned (15) and show a high degree of sequence similarity.

Previous studies showed that both CTE-I and MTE-I were inducible at mRNA level by treatment with the peroxisome proliferator clofibrate or by fasting (13). Peroxisome proliferators are a structurally diverse group of compounds that mediate their effects via the nuclear hormone receptor peroxisome proliferator-activated receptor alpha (PPARα). The PPARα regulates the expression of several genes involved in fatty acid oxidation by binding as a heterodimeric complex with the retinoid X receptor (RXR) to a peroxisome proliferator response element (PPRE) located in the promoter region of the target gene, such as acyl-CoA oxidase (ACOX) (16), the enoyl-CoA hydratase/3-hydroxyacyl-CoA bifunctional enzyme (BIEN) (17), and P450 4A1 (CYP4A1 (18)). These PPREs are an almost perfect direct repeat of the sequence AGGTCA, spaced by one nucleotide (DR1 element). The important role of the PPARα in transcriptional regulation of genes involved in fatty acid oxidation was demonstrated by targeted disruption of the gene (19, 20). The PPARα-null mice showed lack of peroxisome proliferation, no hepatomegaly and lack of induction of peroxisome proliferator-regulated genes in response to treatment with peroxisome proliferators. The PPARα evidently plays a critical role in the adaptive response to fasting in mice (21–23), as the induction of several genes involved in lipid catabolism is abolished in the PPARα-null mice. Fatty acids can activate the PPARα and may be the natural ligands for PPARs in vivo, such as oleic, linoleic, linolenic, and arachidonic acid (4, 5, 24). Therefore, under conditions of increased levels of free fatty acids such as those associated with fasting, increased expression of PPARα-regulated genes is expected.

Although the long-chain acyl-CoA thioesterases are a family of highly regulated genes, the physiological roles of these enzymes have not yet been elucidated. It has been speculated that they may be involved in lipid metabolism by modulation of cellular concentrations of acyl-CoAs and fatty acids. The current study was undertaken to examine the involvement of the PPARα in the physiological regulation of these enzymes with the idea of elucidating their roles in lipid metabolism.

MATERIALS AND METHODS

Animals and treatments

Adult male C57 BL/6 mice, obtained from B & K, Sollentuna, Sweden, were used as indicated throughout this study. Ten- to 12-week-old wild-type or PPARα-null male mice on a pure Sv/129 genetic background (derived from the original colony of mixed background mice) (19) were housed in a temperature- and light-controlled environment. In fasting experiments, mice were maintained on a normal chow diet (Lactamin R36, Vadstena, Sweden) prior to the start of the experiment and then transferred to new cages and fasted for the time-points indicated in the figure legends. In some cases, after 24 h of fasting, the animals were refed a normal chow diet for various lengths of time before killing. Treatment with a fat-free diet (containing 64% carbohydrate, 17.6% protein, 4.2% ash, 4% fibre, and 19% water, AnalyCen, Linköping, Sweden) or a 0.5% clofibrate-containing diet (Atromidin, Zeneca Ltd, Cheshire, United Kingdom) was for up to 1 week. Diurnal variation was investigated with mice maintained on a normal chow diet and killed at the time-points as indicated. The dark periods were between 18.00 and 06.00 h. All mice had access to water ad libitum and experiments were commenced at 9.00 am unless otherwise stated. Animals were killed by CO_2 asphyxiation followed by cervical dislocation, and weighed immediately. Tissues were then excised, weighed, and frozen in liquid nitrogen. Tissue samples were stored at −70°C for preparation of total RNA.

Northern blot analysis

Total RNA was isolated from mouse tissue samples using either QuickPrep^R Total RNA Extraction Kit (Pharmacia Biotech, Uppsala, Sweden) or Ultraspec RNA Kit (Biotecx Laboratories Inc., Houston, TX). Total RNA (either 10 or 20 μg as outlined in figure legends) was denatured in formaldehyde/formamide and electrophoresed on 1% formaldehyde-containing agarose gels with ethidium bromide. The RNA was transferred to Hybond-N nylon membranes (Amersham Pharmacia Biotech) by capillary action. A cDNA probe was prepared corresponding to the full length cDNA for CTE-I as described (13). An MTE-I specific probe of 120 bp was prepared by PCR amplification of the mitochondrial leader peptide of MTE-I. Probes for mouse PTE-Ia and PTE-Ib were prepared by PCR in an area of lowest homology to both CTE-I and MTE-I. Oligonucleotides were designed for PCR amplification of the mouse PPARα cDNA, based on the published sequence (25) (5′-**TCTAGA**GGGGACCTGAG AAACGCTG-3′ and 5′-**TCTAGA**ATTAGCCTCCGATCACCCC-3′), with the addition of *Xba*I restriction sites (indicated in bold). Reverse transcription for RT-PCR with mouse liver total RNA as a template was carried out using RT-PCR Kit (Amersham Pharmacia Biotech). PCR was performed in an Eppendorf Thermal cycler and the product obtained was cloned into the pcDNA3.1(+) expression vector (Invitrogen). Sequences were verified using the ABI Prism Dye Terminator Ready-Reaction Kit (Perkin-Elmer) and analysed at Cybergene AB (Huddinge, Sweden). Probes corresponding to ACOX and β-actin were also used. All probes were labeled with α-^{32}P dCTP (NEN Life Science Products, Belgium) by random priming (Oligolabeling Kit, Amersham Pharmacia Biotech). RNA filters were pre-hybridized for 1 h in a solution containing 50 mm PIPES, 100 mm NaCl, 50 mm sodium dihydrogen phosphate, 1 mm EDTA, and 5% SDS at pH 6.8, together with 100 μg salmon sperm DNA (Sigma) at 65°C. After prehybridization, the filters were further hybridized overnight at 65°C with the relevant α-^{32}P-labeled probe. After hybridization, the filters were initially washed at 65°C in 1× SSC−5% SDS and further washed in decreasing concentrations of SSC-SDS solutions. Bands were visualized by exposure to X-ray film (CEA RP medical X-ray screen film) or phosphor imaging plates (Fujifilm). Signals were analyzed in a Fujifilm BAS-1800 using Image Gauge Software Version 3.0 or quantitated in an Image Master VDS (Amersham Pharmacia Biotech) using Image Master Program Version 3.0. Filters were stripped in boiling 0.5% SDS solution and re-probed as above.

Gel electrophoresis and Western blotting

Liver pieces from C57 BL/6 mice fasted for various time-points up to 24 h or treated with a 0.5% clofibrate-containing diet for 1 week were homogenized in 50 mm potassium phosphate buffer, pH 7.0 (0.1 g tissue in 200 μl buffer). Cytosolic fractions were prepared by centrifugation in an Eppendorf centrifuge at 15,000 *g* for 30 min at 4°C. Twenty-five micrograms total cytosolic protein was separated on a 10% sodium dodecylsulfate polyacrylamide gel (SDS-PAGE). Western blotting was performed by electrophoretic transfer of the separated proteins onto a nitrocellulose filter (Nitropure, Micron Separations Inc., Westborough, MA) using a Protean II xi cell (BioRad Laboratories, Hercules, CA). The blots were probed with an anti-MTE-I antibody previously described (11) and subsequently with horseradish peroxidase-conjugated secondary antibodies and visualized by enhanced chemiluminescence (ECL™, Amersham Pharmacia Biotech) using X-ray film. Signals were analyzed using an Image Master VDS (Amersham Pharmacia Biotech) and Image Master Program Version 3.0.

Other methods

Acyl-CoA thioesterase activity and protein were measured essentially as described previously (26, 27).

RESULTS

CTE-I shows a diurnal regulation

The diurnal regulation of the cytosolic acyl-CoA thioesterase was investigated in C57 BL/6 mice over a 24-h period (**Fig. 1**). Mice were fed a normal chow diet and were killed every 4th h starting at 09.00 h. Quantitation of the mRNA signal for CTE-I, which shows a weak basal expression in liver, showed that during the light period (between 06.00 h and 18.00), when the animals were less active, levels of mRNA were increased, indicating an induction by fasting. During the dark period, when feeding takes place (between 18.00 h and 06.00), mRNA levels declined steadily, indicating a rapid nutritional regulation in response to refeeding.

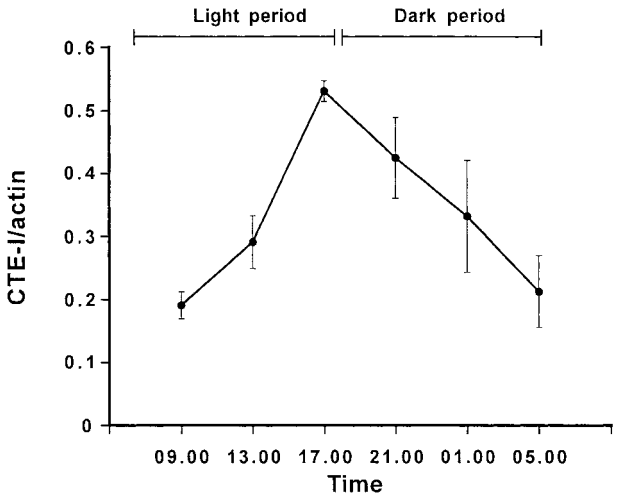

Fig. 1. Diurnal variation of CTE-I mRNA expression in liver. Mice (C57 BL/6) were maintained on a normal chow diet ad libitum and were killed at the time-points indicated. The light period was between 06.00 and 18.00 and the dark period between 18.00 and 06.00. Total RNA was isolated from liver and Northern blot analysis was carried out on 20 μg RNA using an α-^{32}P-labeled full-length cDNA probe for CTE-I or a probe for β-actin. Filters were exposed to X-ray film and signals were quantified using Image Master Software 3.0. The mean of CTE-I/actin mRNA ± range for two animals is shown.

Regulation of CTE-I by fat-free diet

Treatment of C57 BL/6 mice with a fat-free diet for 1 week was carried out and CTE-I mRNA expression in liver was repressed by more than half after 1 day and levels were less than 10% of basal level after 2 days of treatment (**Fig. 2A**). The mRNA gradually increased again to about 25% of basal level by day 7 of treatment. The apparent down-regulation of CTE-I expression by treatment with a fat-free diet could not be correlated to changes in PPARα expression, which was found to be unchanged and likewise, expression of ACOX, an apparently strictly PPARα-regulated gene, was also unchanged by this treatment (Fig. 2B). These data suggest that CTE-I expression is regulated in a PPARα-independent manner during treatment of mice with a fat-free diet.

Involvement of the PPARα in acyl-CoA thioesterase expression in liver

PPARα-null mice were used to examine whether the previously reported induction of CTE-I and MTE-I mRNA by fasting is mediated by the PPARα (13). Fasting induced both of these thioesterase transcripts in liver of wild-type animals (**Fig. 3A**), with CTE-I mRNA induced at least 15-fold and MTE-I about 4-fold. Two further thioesterases recently cloned by us (15), peroxisomal acyl-CoA thioesterase Ia and Ib (PTE-Ia and PTE-Ib), showed a doubling of the mRNA transcript levels by fasting. However, an induction of CTE-I was still observed after fasting of PPARα-null mice, which was similar to the level of induction in wild-type animals. MTE-I mRNA levels were also induced by fasting in wild-type animals, with the PPARα-null mice showing a minor induction compared to control PPARα-null animals. In contrast, the induction of the peroxisomal thioesterases was PPARα-dependent during fasting, with no increase in mRNA evident in fasted PPARα-null mice. Acyl-CoA oxidase (ACOX), which is widely used as a marker enzyme of peroxisome proliferator-mediated gene expression, was induced several fold by fasting and this induction was also dependent on the PPARα.

The role of the PPARα in clofibrate-induced increases in CTE-I and MTE-I mRNA in mouse liver was also examined. Treatment for 1 week with clofibrate caused a substantial induction of both CTE-I and MTE-I mRNA in liver (Fig. 3B), and this induction was not found in similarly treated PPARα-null mice, demonstrating that this clofibrate-mediated induction is completely PPARα-dependent in liver. Investigation of CTE-I/MTE-I protein levels in liver homogenates of clofibrate treated mice showed a 7.3-fold induction in wild-type mice after 1 week, whereas protein levels in PPARα-null animals were not increased by this treatment (Fig. 3C). Notably, the constitutive expression of CTE-I/MTE-I in liver of PPARα-null mice is about

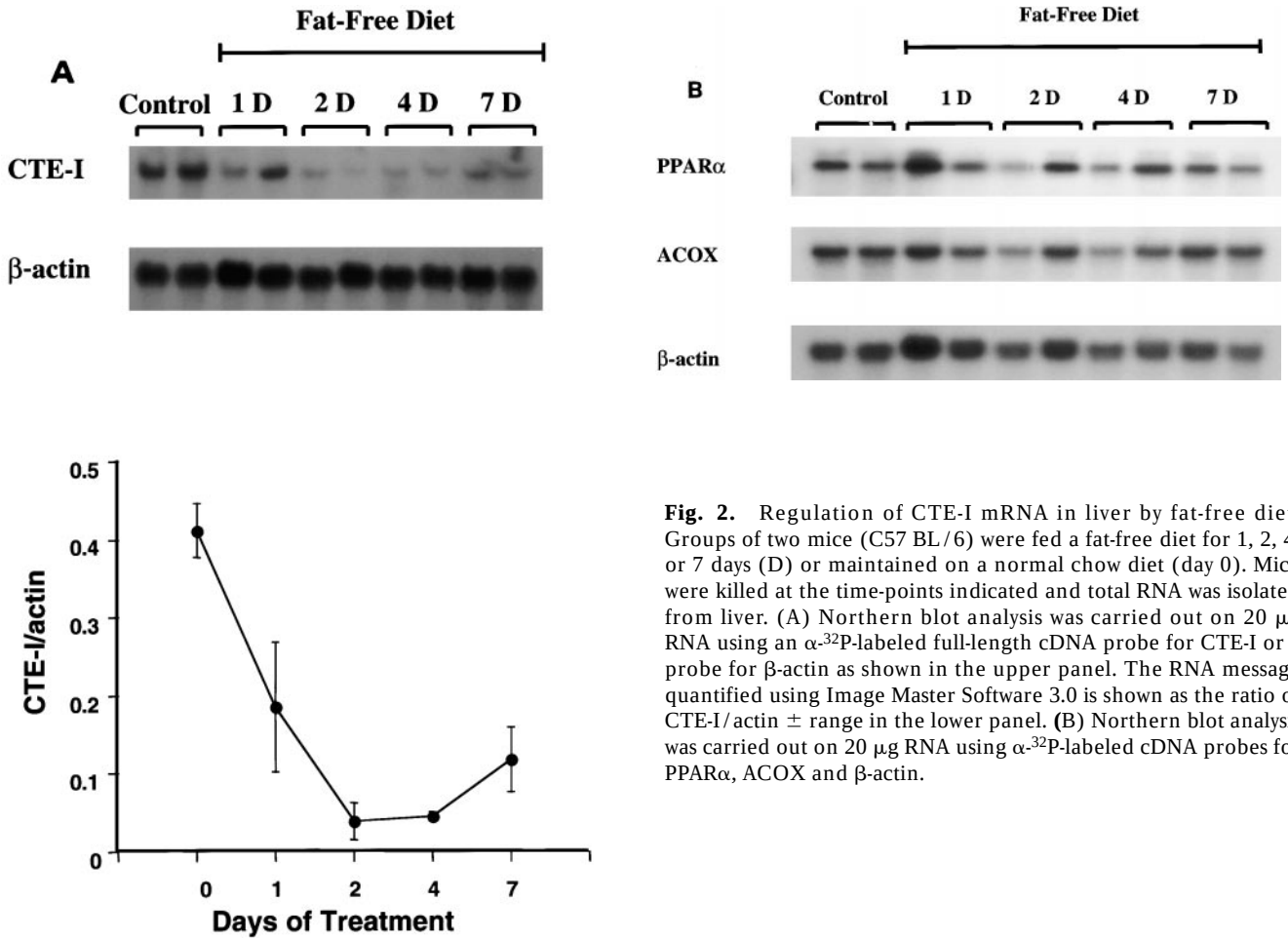

Fig. 2. Regulation of CTE-I mRNA in liver by fat-free diet. Groups of two mice (C57 BL/6) were fed a fat-free diet for 1, 2, 4, or 7 days (D) or maintained on a normal chow diet (day 0). Mice were killed at the time-points indicated and total RNA was isolated from liver. (A) Northern blot analysis was carried out on 20 μg RNA using an α-^{32}P-labeled full-length cDNA probe for CTE-I or a probe for β-actin as shown in the upper panel. The RNA message quantified using Image Master Software 3.0 is shown as the ratio of CTE-I/actin ± range in the lower panel. **(**B) Northern blot analysis was carried out on 20 μg RNA using α-^{32}P-labeled cDNA probes for PPARα, ACOX and β-actin.

2-fold higher at the mRNA and protein levels (Fig. 3A and Fig. 3C) compared to wild-type animals, indicating an involvement of this receptor in the basal expression of CTE-I/MTE-I in liver.

Regulation of CTE-I by fasting

The dramatic increase in thioesterase mRNA levels by fasting, together with the fact that this induction is partially independent of the PPARα for CTE-I, was surprising and was further investigated. Male C57 BL/6 mice were fasted for 6, 12, 18, and 24 h and Western blot analysis showed that CTE-I protein level in cytosol was increased already after 6 h and peaked after 18 h fasting (**Fig. 4**). The increase in CTE-I was even more evident at the mRNA level (data not shown). Although CTE-I enzyme activity represents only a fraction of the total activity in cytosol in untreated mice, the activity in cytosol increased from 7.3 to 10.1 nmol/min × mg protein (about 38% increase) after 24 h fasting. Fasting induced a dramatic and rapid increase in hepatic CTE-I mRNA and protein levels, which suggested a sensitive regulation of this enzyme in response to nutritional changes. To examine the regulation of CTE-I mRNA levels after restoring access to food, mice were fasted for 24 h and then refed a normal chow diet for the time-points indicated (**Fig. 5**). The CTE-I cDNA probe hybridized to the CTE-I transcript of 1.8 kb and also to a second transcript of 2.6 kb, the latter which we have identified as MTE-I (15). Consistent with previous results, CTE-I mRNA levels were induced about 17-fold after mice were fasted for 24 h. However, within 3 h of replacement of the diet, the mRNA levels returned to almost control value. CTE-I mRNA levels continued to decline further to only 20% of original control values after 9 h of refeeding, with some slight increase in the RNA message visible after 30 h refeeding,

Involvement of the PPARα in regulation of acyl-CoA thioesterases in heart and other tissues by fasting

Determination of the expression of CTE-I and MTE-I mRNAs in tissues other than liver where β-oxidation of fatty acids is important as an energy source (heart, kidney, and brown adipose tissue (BAT)) was investigated (**Fig. 6A**). After 24 h fasting, CTE-I mRNA was increased in both heart and kidney, by about 10-fold and 3.4-fold respectively, while MTE-I was increased at least 2-fold in heart and 3.2-fold in kidney. CTE-I and MTE-I mRNAs in BAT were unchanged after this treatment. ACOX, an indicator of PPARα-dependent peroxisome proliferator-induced changes in gene expression, was also examined in these tissues. ACOX mRNA was 3-fold higher in heart and 1.6-fold higher in kidney after fasting. Quantitation of signals showed no apparent induction of this mRNA in BAT.

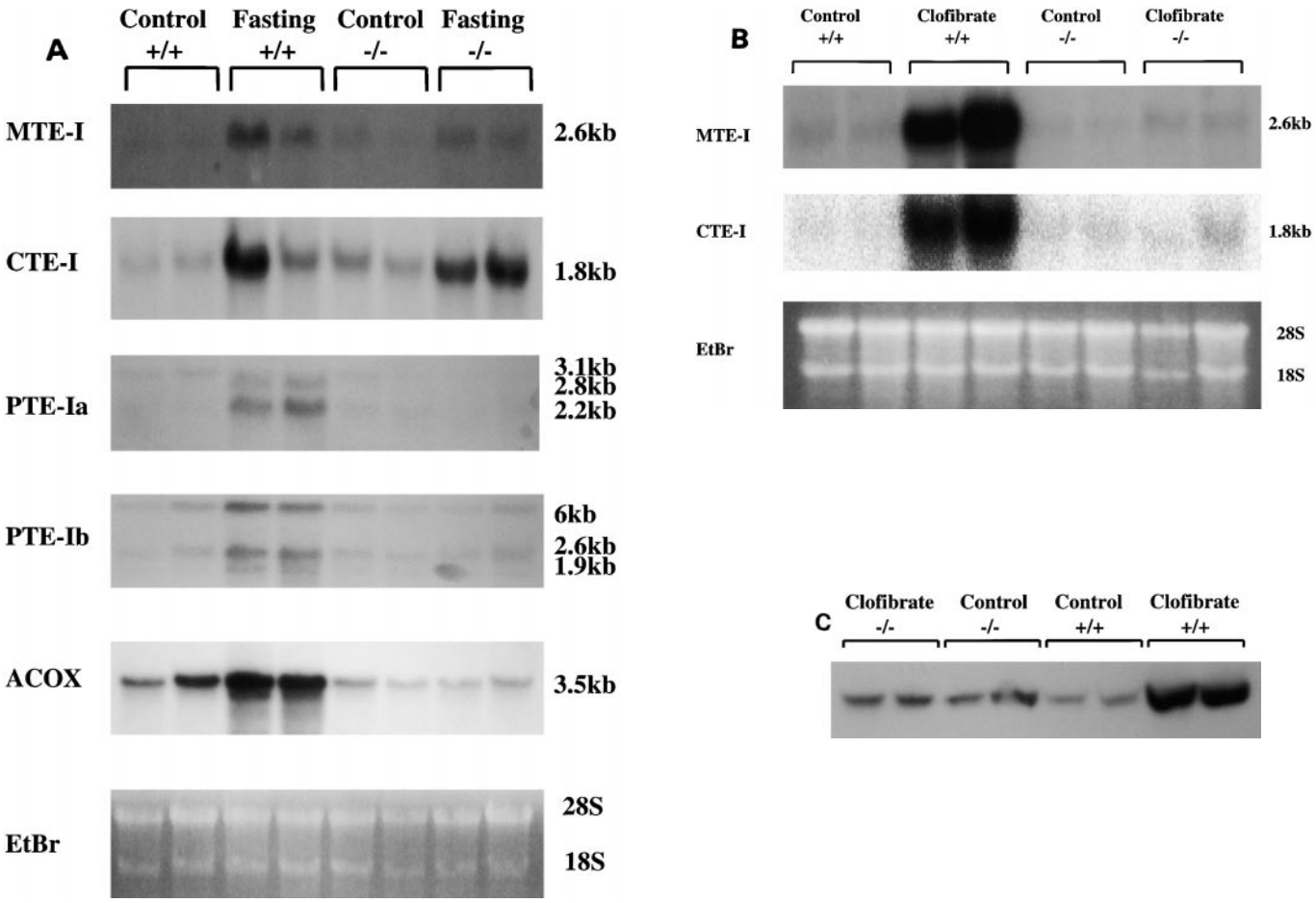

Fig. 3. Regulation of acyl-CoA thioesterase expression in liver. (A) Groups of six PPARα-null mice (−/−) or age-matched wild-type mice (+/+) were fasted for 24 h, while control animals had access to normal chow diet ad libitum. Mice were killed and total RNA was isolated from liver. Northern blot analysis was carried out on 20 μg RNA using α-^{32}P-labeled cDNA probes for CTE-I, MTE-I, PTE-Ia, PTE-Ib, and ACOX as described in Materials and Methods. A representative blot with two samples per group is shown together with the ethidium bromide staining of the blot with positions of the 28S and 18S bands indicated. (B) Groups of six PPARα-null mice (−/−) or age-matched wild-type mice (+/+) were fed a 0.5% clofibrate-containing diet for 1 week, while control animals had access to normal chow diet ad libitum. Mice were killed and total RNA was isolated from liver. Northern blot analysis was carried out on 20 μg RNA using α-^{32}P-labeled cDNA probe for CTE-I and MTE-I. A representative blot with two samples per group is shown together with the ethidium bromide staining of the blot with positions of the 28S and 18S bands indicated. (C) Western blot analysis was carried out on 25 μg liver protein from two PPARα-null mice (−/−) or age-matched wild-type mice (+/+) treated with a 0.5% clofibrate-containing diet for 1 week, using an anti-MTE-I antibody as described in Materials and Methods.

Tissue-specific expression of PPARα was examined in various tissues of untreated mice (Fig. 6B). The expression of PPARα was highest in BAT, liver, heart, kidney, and adrenal gland, and correlated well with the expression of CTE-I, MTE-I, and ACOX mRNAs in these tissues. Using the PPARα-null mice, the induction of CTE-I and MTE-I mRNAs in heart by fasting was further examined. In contrast to liver, the basal mRNA level for CTE-I and MTE-I was significantly lower in cardiac tissue of the PPARα-null mice compared to wild-type animals, suggesting that PPARα influences the basal expression of these genes also in heart (Fig. 6C). Fasted wild-type animals showed at least a 10-fold induction of expression of CTE-I and a 2-fold induction of MTE-I after 24 h, consistent with previous results. However, CTE-I mRNA was 2.4-fold higher in fasted PPARα-null mice compared to control PPARα-null mice, but no significant increase in MTE-I mRNA levels was detected by this treatment compared to controls. CTE-I mRNA in heart from fasted PPARα-null mice was still 1.8-fold higher than in wild-type controls. These data suggest that the regulation of MTE-I in heart by fasting is mediated by the PPARα, while also factors other than the PPARα regulate CTE-I mRNA in heart during fasting.

DISCUSSION

We have examined the regulation of various members of the peroxisome proliferator-induced long-chain acyl-CoA thioesterase multi-gene family, with particular emphasis on the cytosolic and mitochondrial enzymes. As expected, the peroxisome proliferator-induced up-regulation was completely PPARα-dependent, identifying a signifi-

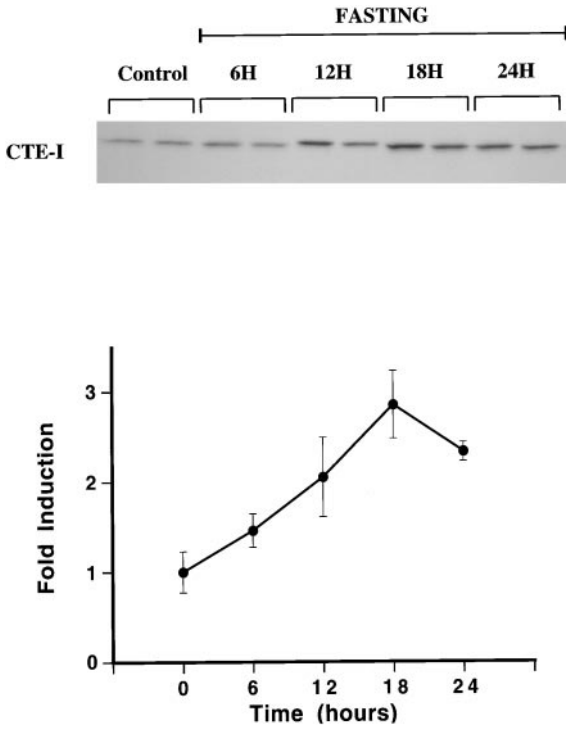

Fig. 4. Regulation of acyl-CoA thioesterase protein in liver by fasting. Groups of two animals (C57 BL/6) were fasted for the indicated times. Western blot analysis was carried out using 25 μg liver cytosolic protein from mice fasted for the time-points indicated. The filter was hybridized with an anti-MTE-I antibody as described in Materials and Methods (upper panel). The signal was quantified using Image Master Software 3.0 as shown in the lower panel (mean ± range of two animals at each time-point).

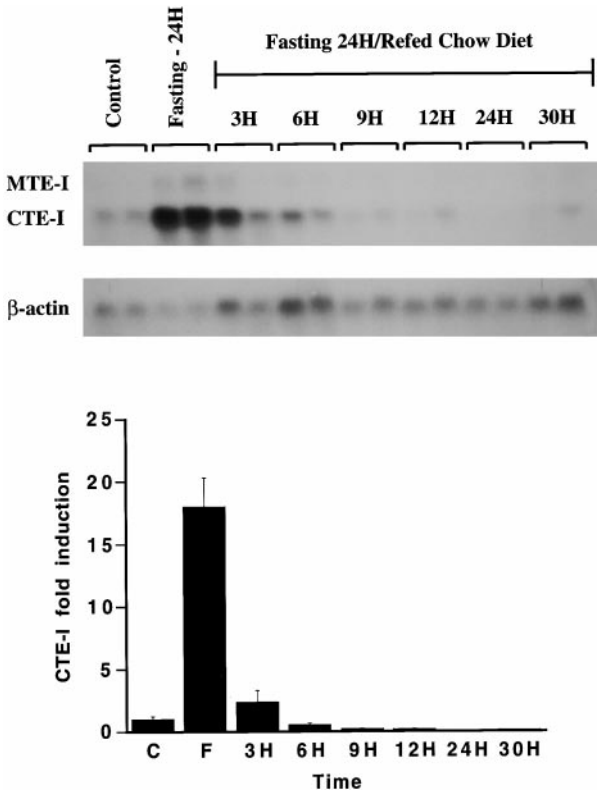

Fig. 5. Regulation of CTE-I mRNA expression in liver by fasting and refeeding. Groups of two animals (C57 BL/6) were fasted for 24 h and then refed a normal chow diet for 3, 6, 9, 12, 24, and 30 h (H). Mice were killed and total RNA was isolated from liver. Northern blot analysis was carried out on 20 μg RNA using an α-^{32}P-labeled full-length cDNA probe for CTE-I or a probe for β-actin as shown in the upper panel. The signals were quantified using Image Master Software 3.0 and the lower panel shows the fold induction of CTE-I mRNA as mean and range of two animals (C: control; F: fasting 24 h).

cant involvement of the PPARα in modulating the expression of these genes at the transcriptional level. The PPARα has been shown to play a key role in controlling the expression of many enzymes involved in liver fatty acid metabolism (19, 20, 28). Recent reports by several groups have identified the PPARα as the mediator in the adaptive response to fasting (21–23). Using the PPARα-null mouse model, the up-regulation of many genes involved in fatty acid oxidation was shown to be PPARα-dependent during fasting, such as medium chain acyl-CoA dehydrogenase and ACOX (23) and the CYP4A genes (21). Interestingly two genes were identified that were highly induced in the fasted state, but this induction was not dependent on the PPARα signaling pathway, namely carnitine palmitoyltransferase I (CPT-I) and short chain acyl-CoA dehydrogenase (22, 23). Our findings show that the cytosolic thioesterase CTE-I is also induced in a PPARα-independent manner during fasting, suggesting that other signaling pathways may be activated in the fasting state that control the expression of some genes involved in lipid metabolism. This activation pathway may involve other lipid-activated nuclear receptors such as PPARδ or ARP-1 or a yet unidentified fatty acid-activated receptor, which could be involved in regulating CTE-I at the basal level. PPARδ is ubiquitously expressed and although a function for PPARδ remains to be established, it can be activated by a similar set of ligands to PPARα, indicating that it may respond to similar endogenous signals (4). The induction of CTE-I during fasting could be explained by hormonal changes, such as an increase in glucocorticoids, but the activation of CTE-I by a lipid-activated receptor is supported by our data after treatment with a fat-free diet which caused a slow reduction in CTE-I mRNA expression (after 1 to 2 days), suggesting that a decrease in fatty acids rather than hormonal changes may be responsible for the reduced expression.

The in vivo functions for long-chain acyl-CoA thioesterases have not been elucidated, although it has been suggested that they may be involved in lipid metabolism by modulating cellular non-esterified fatty acids and acyl-CoA concentrations. Because several pathophysiological conditions like starvation, diabetes, and some other metabolic diseases are accompanied by elevated tissue levels of fatty acids, these fatty acids may have an important role in activating the PPARα and thereby increase the transcription of genes encoding lipid metabolizing enzymes. It has been demonstrated that fatty acids can activate the PPARα in reporter gene systems (24) and that fatty acids bind to the

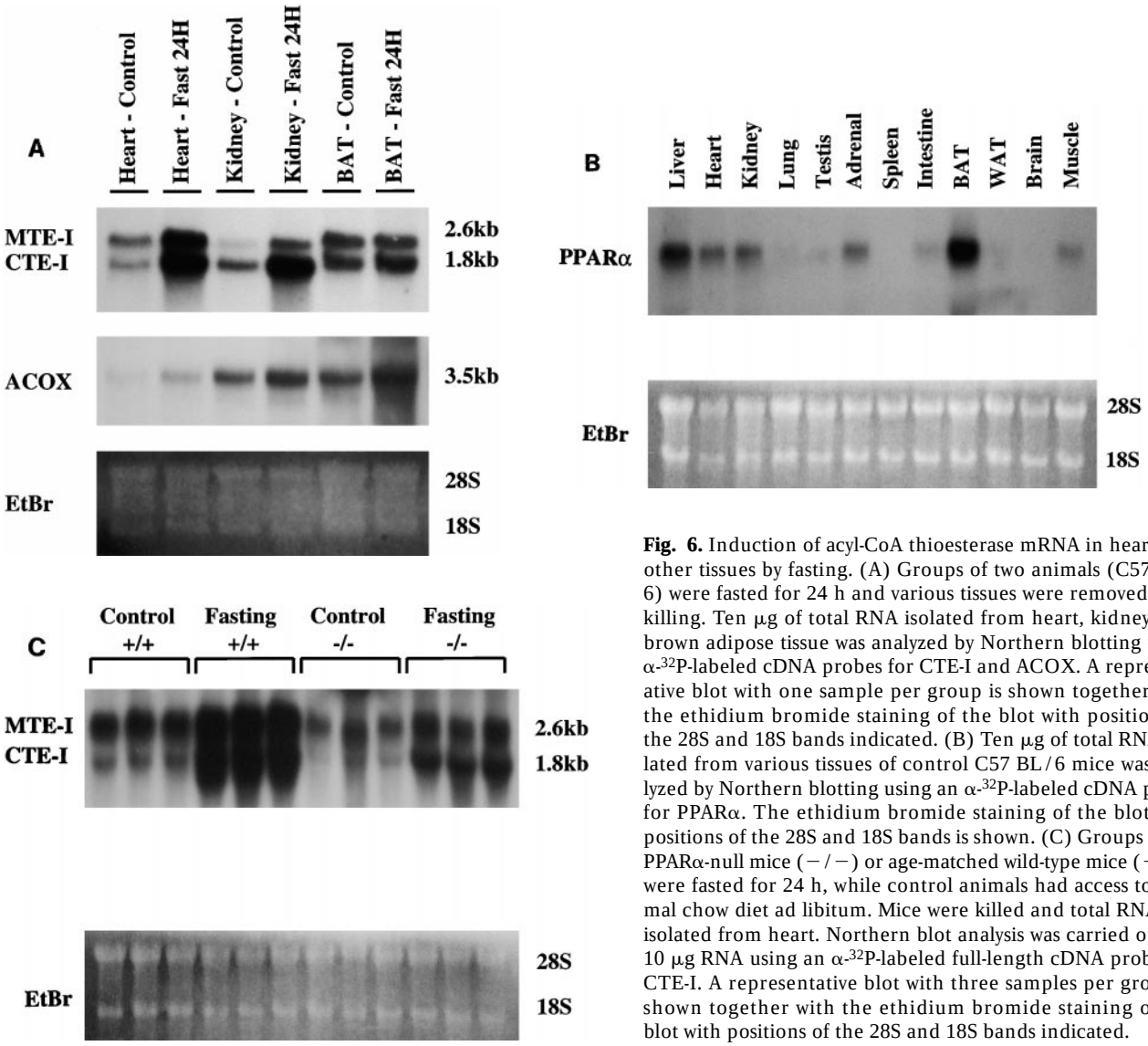

Fig. 6. Induction of acyl-CoA thioesterase mRNA in heart and other tissues by fasting. (A) Groups of two animals (C57 BL/6) were fasted for 24 h and various tissues were removed after killing. Ten μg of total RNA isolated from heart, kidney, and brown adipose tissue was analyzed by Northern blotting using α-^{32}P-labeled cDNA probes for CTE-I and ACOX. A representative blot with one sample per group is shown together with the ethidium bromide staining of the blot with positions of the 28S and 18S bands indicated. (B) Ten μg of total RNA isolated from various tissues of control C57 BL/6 mice was analyzed by Northern blotting using an α-^{32}P-labeled cDNA probe for PPARα. The ethidium bromide staining of the blot with positions of the 28S and 18S bands is shown. (C) Groups of six PPARα-null mice (−/−) or age-matched wild-type mice (+/+) were fasted for 24 h, while control animals had access to normal chow diet ad libitum. Mice were killed and total RNA was isolated from heart. Northern blot analysis was carried out on 10 μg RNA using an α-^{32}P-labeled full-length cDNA probe for CTE-I. A representative blot with three samples per group is shown together with the ethidium bromide staining of the blot with positions of the 28S and 18S bands indicated.

PPARα, and thus may act as the natural ligands for this receptor (4, 5). It was also recently reported that the muscle CPT-I gene is activated by fatty acids via the PPARα in cardiac myocytes (29). Thus, the hydrolysis of long-chain acyl-CoAs to free fatty acids and CoA by the cytosolic thioesterase CTE-I may provide free fatty acids as ligands for the PPARα. As CTE-I is rapidly induced during fasting (protein is increased already after 6 h), we hypothesize that in the fasted state, when levels of fatty acids and acyl-CoA esters in liver are high, CTE-I is rapidly induced in a PPARα-independent manner and could act by supplying ligand for this receptor. This will result in activation of PPARα-regulated genes, including CTE-I, which itself will be further up-regulated. This cycle of events may act as an amplifier of the PPARα signaling pathway. It is likely that a factor or enzyme involved in nutritional regulation of gene expression would be rapidly regulated and, in this respect, the increase in CTE-I mRNA by fasting is very rapidly repressed by refeeding, with mRNA levels decreased to less than 20% of control within 9 h.

It has been speculated that the CYP4A enzymes may have a regulatory role in control of hepatic concentrations of putative endogenous fatty acid activators of the PPARα. A recent report showed that hepatic CYP4A mRNA is induced in response to fasting and diabetes in rats and mice (21). Using PPARα-null mice, this response was shown to be mediated by the PPARα in mouse liver. However, the induction of CYP4A protein and activity levels required at least 24 h before detection, indicating that the induction of CYP4A activity is unlikely to be a prerequisite for PPARα activation and induction of PPARα-inducible enzymes.

Acyl-CoA thioesters have been implicated in regulation of gene transcription as it was recently demonstrated that various acyl-CoAs bind to the HNF-4α and could modulate the transcriptional activity in a fatty acyl-specific manner (3). Thus, it is possible that the cytosolic acyl-CoA thioesterase may be of importance in modulating the transcriptional activation via PPARα versus HNF-4α by affecting cellular free fatty acid and acyl-CoA levels. This modulation

may be important especially under conditions of fasting when the levels of other proteins that interact with free fatty acids and acyl-CoAs, such as fatty acid binding protein (FABP) and acyl-CoA binding protein (ACBP), are not changed significantly. Studies on ACBP (which has been suggested to act as a "sink" for acyl-CoAs (30)), show that the mRNA level was reduced by 50% after mice were fasted for 24 h (31). The ACBP binds fatty acyl-CoAs with very high affinity, indicating that the free concentrations of acyl-CoA are very low in vivo. Liver FABP, on the other hand, binds free fatty acids with 3–4 orders of magnitude lower affinity ($K_D \sim 1$ μm), suggesting that the concentrations of free fatty acids may be much higher during certain physiological conditions. Liver FABP shows a slow rate of turnover and the level is not acutely modulated by dietary or diurnal changes (32). Thus, it appears that neither ACBP nor liver FABP is regulated to any large extent during acute changes in nutritional status, suggesting that fasting may lead to a large increase in free fatty acid levels in the liver.

The expression of MTE-I is up-regulated by peroxisome proliferators in a PPARα-dependent mechanism, indicating that the gene may contain a functional PPRE. MTE-I is mainly expressed in heart, brown adipose tissue, kidney, and muscle and is strongly induced in liver, kidney, and heart by fasting or in liver and kidney after treatment of mice with peroxisome proliferators, which is consistent with a function in fatty acid degradation. The acyl-CoA chain-length specificity of the MTE-I enzyme is similar to the chain-length specificity of mitochondrial very long-chain acyl-CoA dehydrogenase (11). The similar acyl-CoA chain-length preference of MTE-I to the very long-chain acyl-CoA dehydrogenase may be interpreted as a possible role for MTE-I in the regulation of the relative amounts of acyl-CoA/acyl-carnitine/free fatty acids under various conditions where fatty acid load may differ significantly. Fatty acyl groups are transported into the mitochondria in the form of acylcarnitine via a translocase system. During conditions such as fasting, there is an increased dependence on β-oxidation for energy supply. Fasting is associated with an increased uptake of acylcarnitine into the mitochondria, where the acyl group is transferred back to CoA, thus re-generating acyl-CoA for β-oxidation. The latter reaction is catalyzed by carnitine palmitoyltransferase II (CPT-II). During conditions of high intramitochondrial acyl-CoA, this enzyme may catalyze the reverse reaction to generate acylcarnitine. Acylcarnitine is a much stronger surfactant than acyl-CoA or free fatty acids (33) and could cause membrane damage, therefore the hydrolysis of acyl-CoAs by MTE-I would prevent CPT-II catalyzing the back reaction to form acylcarnitine. Heterologously expressed rat liver CPT-II shows an estimated K_m for palmitoyl-CoA of about 34 μm (34), whereas purified rat liver MTE-I shows a K_m of 6 μm for palmitoyl-CoA (11), indicating that MTE-I, by hydrolysis of acyl-CoA, could prevent catalysis of the back reaction by CPT-II and thus may act in a protective manner to prevent formation of carnitine esters.

Recently, an enzyme involved in steroid synthesis, arachidonic acid-related thioesterase involved in steroidogenesis (ARTISt), was purified (35) and cloned (36). The sequence of this enzyme was found to be identical to the sequence of MTE-I, suggesting that the MTE-I protein may also have an important role in the adrenocorticotropin-stimulated mobilization of arachidonic acid, thus stimulating steroidogenesis in adrenal gland. Consistent with this is the finding of relatively strong expression of MTE-I in mouse adrenal gland (15).

Peroxisomal acyl-CoA thioesterase activity has been characterized in liver and brown adipose tissue and a long-chain acyl-CoA thioesterase activity (maximally active on myristoyl-CoA) was shown to be increased in liver peroxisomes after treatment of rats with peroxisome proliferators (10, 37). We recently cloned two peroxisomal acyl-CoA thioesterases that vary markedly in tissue expression (15). PTE-Ia is expressed in all tissues investigated in mice (liver, kidney, heart, brown adipose tissue, lung, brain, muscle, and adrenal gland), in line with the general tissue expression of peroxisomal β-oxidation enzymes, and was inducible in liver by clofibrate and fasting. Thus, PTE-Ia may have a function in peroxisomes in maintaining long-chain acyl-CoA/CoA levels for optimal β-oxidation. This is supported by a recent study demonstrating that disruption of a gene in *S. cerevisiae* (where fatty acid oxidation is exclusively a peroxisomal process) encoding a peroxisomal thioesterase, impaired growth on oleate (38, 39). Although thioesterase activity has not been considered important in peroxisomal β-oxidation, it appears that intraperoxisomal CoA levels must be closely regulated for efficient β-oxidation in this organelle.

PTE-Ib shows a very different tissue distribution with strong expression only in kidney and liver and weak expression in intestine, adrenal gland, and brown and white adipose tissue. The function of PTE-Ib may therefore be more restricted to regulating liver–kidney specific metabolic pathways, possibly being involved in regulating conjugation of bile acids to glycine and taurine. This activity is catalyzed by a bile acid–CoA:amino acid *N*-acyltransferase which is expressed in liver and kidney (40–42).

All four acyl-CoA thioesterase genes are highly regulated by peroxisome proliferators and by fasting, suggesting functions for these enzymes in lipid metabolism. The different subcellular localizations of these enzymes propose that they have different functions in vivo. It is therefore important to further characterize the regulation of these genes using cloned promoters and reporter gene systems and to investigate their activity towards various types of lipids such as straight chain acyl-CoAs, branched chain acyl-CoAs, 3-hydroxy acyl-CoAs, CoA esters of bile acid intermediates, etc. However, the findings that the nutritional regulation of CTE-I is very rapid and mainly PPARα-independent and that the enzyme is localized in the cytosol suggest a possible role for this enzyme in PPARα signaling. The definitive functions of these enzymes are likely to be revealed using gene targeting, which work is currently in progress.

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