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Research Article

Short- and medium-chain carnitine acyltransferases and acyl-CoA thioesterases in mouse provide complementary systems for transport of β-oxidation products out of peroxisomes

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Abstract. Peroxisomes metabolize a variety of lipids, acting as a chain-shortening system that produces acyl-CoAs of varying chain lengths, including acetyl-CoA and propionyl-CoA. It is, however, still largely unknown how β-oxidation products exit peroxisomes and where they are further metabolized. Peroxisomes contain carnitine acetyltransferase (CRAT) and carnitine octanoyltransferase (CROT) that produce carnitine esters for transport out of peroxisomes, together with recently characterized acyl-CoA thioesterases (ACOTs) that produce free fatty acids. Here we have performed tissue expression profiling of the short- and medium-chain carnitine acyltransferases *Crat*, *Crot* and the short- and medium-chain thioesterases (*Acot12*) and (*Acot5*), and show that they are largely expressed in different tissues, suggesting that they do not compete for the same substrates but rather provide complementary systems for transport of metabolites across the peroxisomal membrane. These data also explain earlier observed tissue differences in peroxisomal production of acetyl-CoA/acetyl-carnitine/acetate and underscores the differences in peroxisome function in various organs.

Keywords. Metabolite transport, carnitine acetyltransferase, carnitine octanoyltransferase, acyl-CoA thioesterase 5, acyl-CoA thioesterase 12, β-oxidation, peroxisomes.

Introduction

Peroxisomes are essential organelles with various functions in lipid metabolism. The importance of peroxisomal lipid metabolism in man has been clearly demonstrated through the occurrence of severe diseases caused by deficiencies affecting one or more of the peroxisomal enzymes (for reviews see [1–3]). In addition to α-oxidation and β-oxidation of various lipids, peroxisomes also catalyze the first steps in etherphospholipid synthesis. Peroxisomes catalyze α-oxidation of 3-methyl-branched fatty acids, and β-oxidation of a wide variety of lipids including very-long-chain fatty acids, dicarboxylic fatty acids, 2-methyl-branched fatty acids, bile acid intermediates as well as eicosanoids and xenobiotic carboxylic acids (for review see [4]). The main function for β-oxidation

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of these lipids is chain shortening for transport to mitochondria for further β-oxidation, or alternatively excretion in urine or bile. β-Oxidation of most lipid substrates is initiated by activation to the corresponding CoA-ester outside the peroxisome, followed by transport across the peroxisomal membrane and subsequent β-oxidation in the peroxisomal matrix. Following β-oxidation, it is not clear how chain-shortened metabolites are transported out of the peroxisome, although it is generally believed that it is not in the form of the CoA-ester. Acyl-CoAs can either be converted to carnitine esters by carnitine acyltransferases or they can be hydrolyzed to the corresponding free fatty acid by an acyl-CoA thioesterase, and these products may be transported across the peroxisomal membrane (for review see [5–8]). The import of fatty acids into peroxisomes in the form of acyl-CoAs and export in the form of free fatty acids or carnitine esters suggests that CoASH would accumulate inside peroxisomes. However, two recently identified peroxisomal nudix hydrolases that metabolize CoASH or CoA esterified to fatty acids [9, 10] may provide a mechanism to maintain intraperoxisomal CoASH homeostasis. Peroxisomes contain short- and medium-chain carnitine acyltransferase activities [11, 12], catalyzed by carnitine acetyltransferase (CRAT), which is active on short-chain acyl-CoAs, and carnitine octanoyltransferase (CROT), which is active on straight and branched medium-chain acyl-CoAs [7, 13, 14]. These carnitine esters may be transported across the peroxisomal membrane and be further metabolized in mitochondria. Peroxisomes also contain several acyl-CoA thioesterase activities that hydrolyze a wide range of acyl-CoAs [15]. The peroxisomal acyl-CoA thioesterase activity is due to several enzymes that have recently been characterized at the molecular level. Although ACOT8 was recently shown to act as a general acyl-CoA thioesterase [16], peroxisomes also contain several acyl-CoA thioesterases with rather specific substrate specificities. Four of these thioesterases belong to a gene family containing a medium-chain acyl-CoA thioesterase (ACOT5), a long-chain acyl-CoA thioesterase (ACOT3), as well as a succinyl-CoA-specific thioesterase (ACOT4) and a phytanoyl-CoA/pristanoyl-CoA-specific thioesterase (ACOT6) [17–19]. Several studies have suggested that peroxisomes may also contain an acetyl-CoA thioesterase [15, 20, 21]. Indeed, a previously characterized extramitochondrial acetyl-CoA thioesterase (which also hydrolyzes propionyl-CoA, butyryl-CoA and acetoacetyl-CoA), referred to as ACOT12 according to a new nomenclature [22], was purified from the cytosol of rat liver [23], and has been detected in isolated peroxisomes by Western blotting and measurement of enzyme activity [24]. Interestingly, ACOT12 contains a putative, non-consensus, peroxisomal targeting signal (PTS1) of (K)SVL at the C-terminal end of the protein, which may function as a PTS1 signal [25]. Recently, ACOT12 has been identified in mouse kidney peroxisomes using proteomics, confirming the presence of this short-chain acyl-CoA thioesterase in peroxisomes [26]. The presence of short-chain acyl-CoA thioesterase (ACOT12) and short-chain carnitine acyltransferase (CRAT) in peroxisomes suggests the presence of competing enzyme systems to handle acetyl-CoA and propionyl-CoA formed during peroxisomal β-oxidation. Similarly, the presence of a medium-chain acyl-CoA thioesterase (ACOT5) and a medium-chain carnitine acyltransferase (CROT) may provide a similar competitive enzyme system to metabolize medium-chain acyl-CoAs produced in peroxisomal β-oxidation. We hypothesized that if the tissue expression of these enzymes is different, they may in fact complement each other and provide alternative functions for peroxisomes in different tissues. In this study we have therefore mapped the tissue expression of these genes at the mRNA level, and shown that these enzymes are in fact differently expressed and that these two routes for metabolism/transport operate in different tissues.

Materials and methods

Elucidation of *Crat* splice variants. The cDNAs for mouse mitochondrial *Crat* was used to blast against the mouse genomic database. Alignment of sequences was carried out using Lasergene DNA and protein analysis software (DNASTAR, Inc).

Animals. Male Sv/129 mice were maintained on a standard chow diet and had access to water and chow *ad libitum.* Animals were killed by CO_2 asphyxiation followed by cervical dislocation and tissues were excised, frozen in liquid nitrogen and stored at –70°C. All animal experiments were carried out with ethical permission obtained from the Animal Experimental Ethical Committee, Stockholm.

RNA isolation and cDNA synthesis. Total RNA was isolated from liver, kidney, heart, lung, spleen, proximal intestine (first 10 cm of small intestine), distal intestine (last 10 cm of small intestine), brown adipose tissue (BAT), white adipose tissue (WAT), and brain from three individual animals. For some experiments, the intestinal segments from three individual animals were rinsed with 0.9% NaCl, cut open with scissors and the endothelial layer was separated from the smooth muscle by scraping. Total RNA isolation was

carried out using TRIzol reagent (Invitrogen) and DNase-treated using RQ1 RNase-free DNase (Promega). The quality of RNA samples was analyzed on a 1% agarose/formaldehyde gel. cDNA was synthesized from 1 μg total RNA pooled from three animals for each tissue using iScript cDNA synthesis kit (Bio-Rad), which was then used for real-time PCR.

Real-time PCR. Specific primers for each gene were designed to span over an exon/exon boundary, except for *Acot5* for which no specific primers could be designed over an exon/exon boundary due to its high sequence similarity to *Acot3*. The primers are listed in Table 1 and were used at a final concentration of 900 nM. The real-time PCR from each tissue was run twice in triplicate in single-plex, using SYBRGreen Power master mix (Applied Biosystems) in an ABI Prism 7000 sequence detection system. The specificity of the PCR was checked by analyzing the dissociation curve after each run (to make sure only one single specific product had been amplified), and the efficacy of all primer pairs were tested by running real-time PCR on a series of dilutions of the template cDNA to verify that tissue expression of the different genes were analyzed in the linear range of the PCR. The average threshold (CT) values per triplicate were used to calculate the relative amounts of mRNA using the $2^{-\Delta\Delta CT}$ method. *Hprt1* was used as an endogenous control, and all data were normalized to *Hprt1*.

Calculation of peroxisomal *Crat* mRNA levels. Total *Crat* mRNA was measured in different tissues and the tissue with the highest expression was set to 100% (this was BAT). Specific primers were then used to detect peroxisomal (Px)-*Crat* mRNA, and the % of Px-*Crat* relative to total *Crat* was calculated (the % figures are shown in Fig. 2A). Although BAT only has 7% Px-*Crat* and liver has 38% Px-*Crat*, this still leaves the Px-*Crat* mRNA in BAT at a much higher level than in liver [Fig. 2A, compare 7% of the BAT bar (100%) with 38% of the liver bar]. Therefore, magnifying this Px-*Crat* data into Figure 2B, BAT is the tissue with the highest Px-*Crat* mRNA expression.

Table 1. Sequences of primers used for real-time PCR with SYBRGreen detection.

Gene	Primer sequence
Acot5	Fwd 5′-TCAGGATGGTGCCAACAGTA-3′ Rev 5′-GATGCTCAGCGGTTCGTC-5′
Acot8	Fwd 5′-GATGCTGTACGAGTGTGAGAGC-3′ Rev 5′-ACAGCAAGGACCCCATCC-3′
Acot12	Fwd 5′-GGAGATTACCACCACCTTGG-3′ Rev 5′-TTCAACCTTAACAGATATGGCATC-3′
Acox1	Fwd 5′-GCCCAACTGTGACTTCCATC-3′ Rev 5′-GCCAGGACTATCGCATGATT-3′
Cat	Fwd 5′-GCGACCAGATGAAGCAGTG-3′ Rev 5′-GTGGTCAGGACATCAGGTCTC-3′
Crat (mito + Px)	Fwd 5′-GCCATTGCTATGCACTTCAAC-3′ Rev 5′-GGTCCGAAGAACATGACACA-3′
Crat (Px)	Fwd 5′- ACG GTC AAG AAA ACC CCA GA-3′ Rev 5′- TCC ACC TTG CAG ATC GCA C-3′
Crot	Fwd 5′-GAAAGGGCTAGAGGAAAACGA-3′ Rev 5′-CCAGGTAGGCAACATTGAGC-3′
Mfp2	Fwd 5′-GCAAGCTTGACCCACAGAA-3′ Rev 5′-TCAGCATGATGTTCCCTCTG-3′
Hprt1	Fwd 5′-GGTGAAAAGGACCTCTCGAAGTG-3′ Rev 5′-ATAGTCAAGGGCATATCCAACAAC-3′

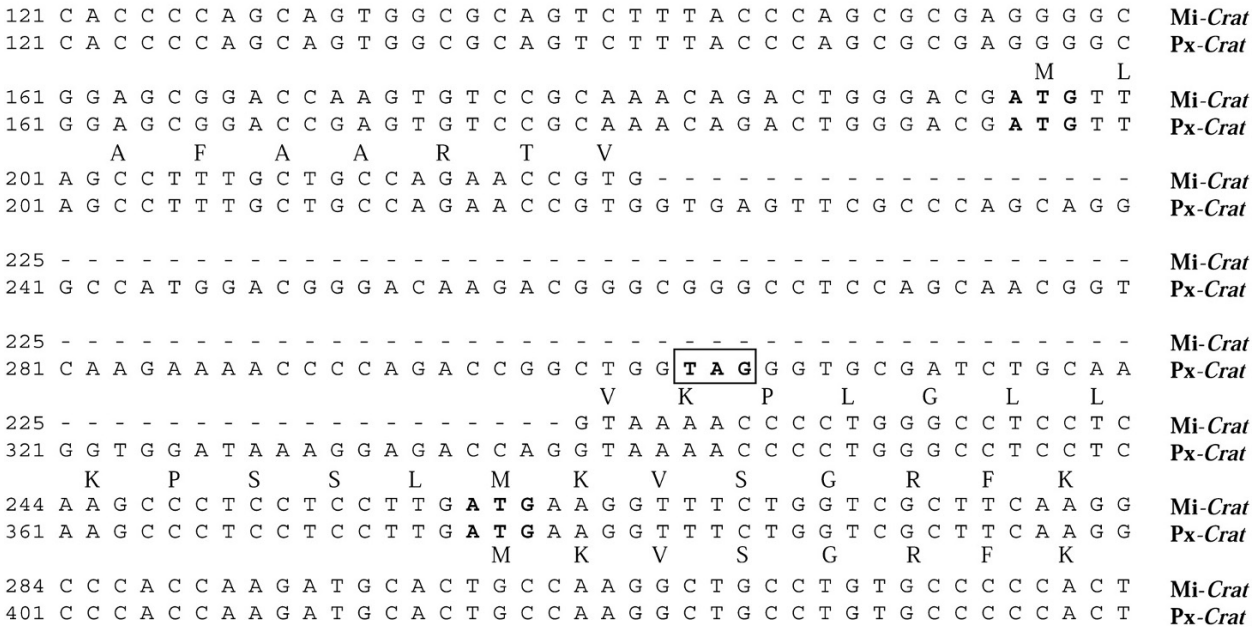
Acot5, acyl-CoA thioesterase 5; *Acot8*, acyl-CoA thioesterase 8; *Acot12*, acyl-CoA thioesterase 12; *Acox1*, acyl-CoA oxidase 1; *Cat*, catalase; *Crat*, carnitine acetyltransferase; *Crot*, carnitine octanoyltransferase; *Hprt1*, hypoxanthine guanine phosphoribosyl transferase 1; *Mfp2*, multifunctional protein 2; mito, mitochondrial; Px, peroxisomal.

Results

Peroxisomal and mitochondrial *Crat* are produced by alternative splicing. The CRAT protein is present in both mitochondria and peroxisomes with two- to threefold higher specific activity in peroxisomes than mitochondria in rat and pig liver [11, 12]. In man, this dual localization is due to alternative splicing of the *Crat* mRNA, resulting in proteins that either contain a peroxisomal PTS1 signal or a protein that additionally contains an N-terminal mitochondrial targeting signal [27]. The mouse *Crat* cDNA was previously cloned by Brunner et al. [28] and we have now elucidated the gene structure of mouse *Crat* (Fig. 1A). This gene contains two alternative start ATGs. Translation from the first ATG results in a protein of 626 amino acids, with a 21-amino acid leader sequence that targets the CRAT protein to mitochondria (Fig. 1B). However, in the peroxisomal *Crat* mRNA, there is an in-frame stop codon and translation starts at a downstream second ATG, resulting in a protein that contains 605 amino acids, with a C-terminal peroxisomal targeting tripeptide of -AKL.

Peroxisomal carnitine acyltransferases and acyl-CoA thioesterases are differently expressed to function as complementary enzymes for transport of β-oxidation metabolites. Peroxisomes contain carnitine acyltransferases and acyl-CoA thioesterases with similar substrate specificities, which raises the question as to the function of these enzymes. We hypothesized that the carnitine acyltransferases and the acyl-CoA thioesterases may be differently expressed and we therefore set out to investigate the tissue expression of these enzymes using real-time PCR, as well as the tissue expression of other peroxisomal enzymes involved in

A



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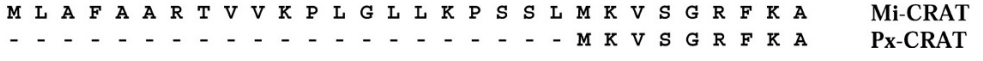

Figure 1. Structure of the mouse carnitine acetyltransferase (*Crat*) gene. (*A*) The mitochondrial *Crat* splice variant is translated from a start ATG resulting in a protein with a 21-amino acid leader peptide. The un-spliced variant contains an in-frame stop codon (which is spliced out in the mitochondrial variant), and in the case of the peroxisomal variant the translation starts at a methionine after this stop codon. Therefore, the resulting protein lacks the mitochondrial leader peptide and instead only contains a C-terminal tripeptide (-AKL), which targets the protein to peroxisomes. (*B*) The translated N-terminal sequences of mitochondrial and peroxisomal CRAT. Mi, mitochondrial; Px, peroxisomal.

fatty acid degradation. We designed real-time PCR primers that specifically amplify the peroxisomal *Crat,* and primers that recognize both the mitochondrial and peroxisomal variants, thus allowing us to calculate the quantity of both mitochondrial and peroxisomal *Crat* mRNA. When total *Crat* mRNA (both mitochondrial and peroxisomal) was measured, the *Crat* transcript showed very high expression in BAT, followed by heart and WAT (Fig. 2A). Interestingly, the majority of *Crat* mRNA in mouse is mitochondrial, with only about 1–20% of *Crat* mRNA being peroxisomal, except for liver, where peroxisomal *Crat* represents about 38% of total *Crat* mRNA. Thus, a similar pattern of tissue expression was evident for peroxisomal *Crat* as for total *Crat*, although at much lower levels, with highest expression in BAT, followed by WAT, heart and liver (Fig. 2B). Based on previous findings that ACOT12 was detected in isolated peroxisomes by Western blotting and enzyme activity (ATP-stimulated/ADP inhibited activity), the presence of a putative peroxisomal targeting signal, and the recent identification of this protein in peroxisomes using proteomics [26], we consider ACOT12 to be a peroxisomal enzyme in mouse and rat. The tissue expression of *Acot12*, which is a short-chain (mainly acetyl-CoA) acyl-CoA thioesterase, was compared to the tissue expression of peroxisomal *Crat,* the short-chain carnitine acyltransferase. As shown in Figure 2B, peroxisomal *Crat* and *Acot12* mRNAs show very different expression patterns, with *Acot12* being most highly expressed in proximal intestinal epithelium, liver and kidney, and *Crat* instead being most highly expressed in BAT, WAT and heart. Although about 40% of liver *Crat* is peroxisomal, comparison of the expression levels showed that *Acot12* is expressed at about tenfold higher mRNA level than peroxisomal *Crat* in liver (data not shown). The very different tissue expression pattern thus suggests that these two enzymes do not compete for the same substrates, and that the carnitine ester and free acid export routes operate in different tissues.

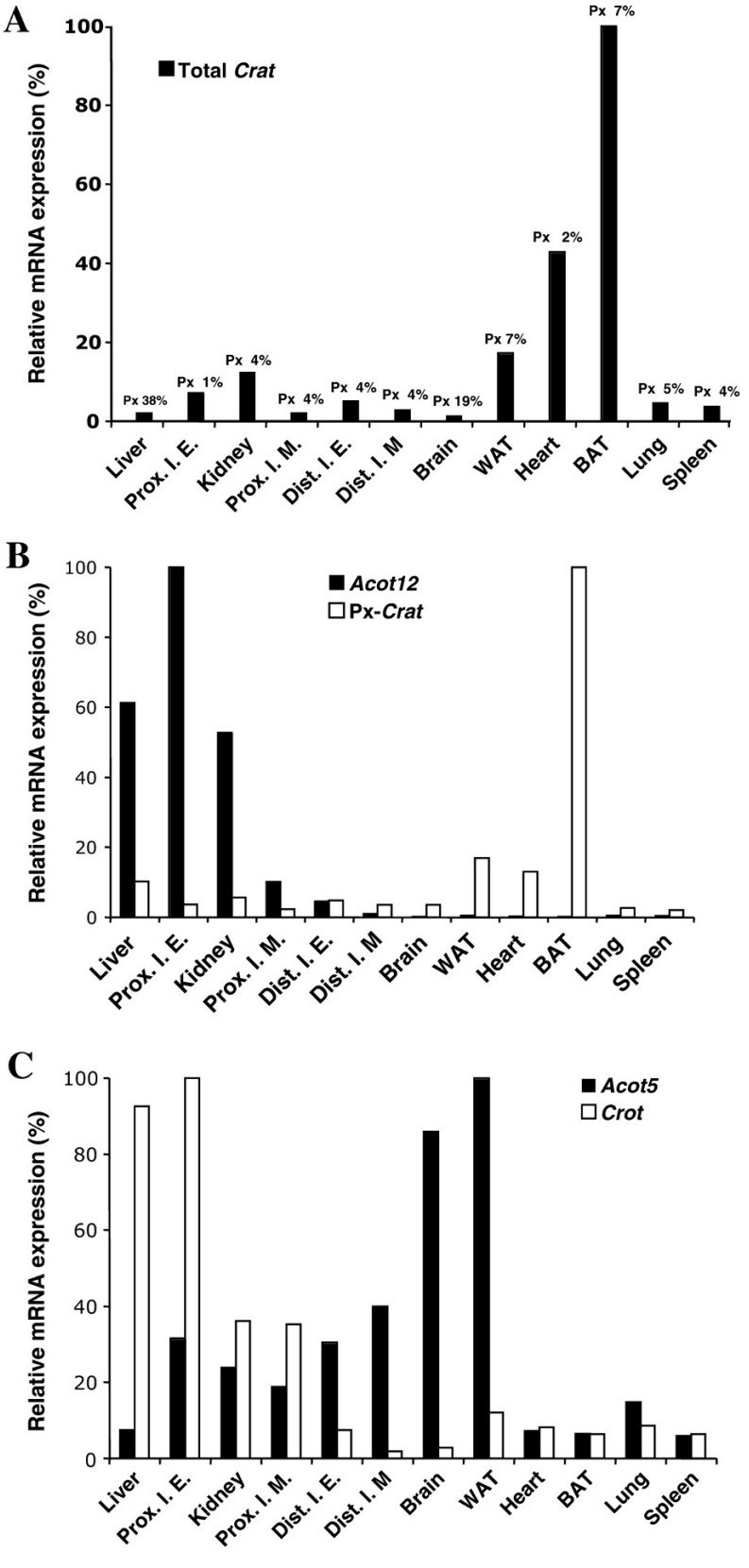

Figure 2 Carnitine acyltransferases and acyl-CoA thioesterases are differently expressed. mRNA expression was investigated by real-time PCR using SYBRGreen for detection, and an amplicon for *Hprt1* was used as an endogenous control. The $2^{-\Delta\Delta Ct}$ method was used to calculate the expression levels, normalized to *Hprt1*, which are presented as percent of the tissue with highest expression for each gene. (*A*) Expression of total carnitine acetyltransferase mRNA (mitochondrial and peroxisomal *Crat*) is shown, and the numbers on top of the bars refer to the expression of the peroxisomal (Px)-*Crat* mRNA as percent of total *Crat.* The tissue with the highest expression (BAT) is shown as 100% and relative mRNA levels in other tissues were calculated. (*B*) The expression levels of peroxisomal *Crat* (using specific primers) and *Acot12* at mRNA level using real-time PCR as described above. (*C*) The expression levels of *Crot* and *Acot5* were investigated by real-time PCR as described above. Prox. I.E., proximal intestine epithelium; Prox. I. M., proximal intestine muscle; Dist. I.E., distal intestine epithelium; Dist. I. M., distal intestine muscle; BAT, brown adipose tissue; WAT, white adipose tissue.

Interestingly, the medium-chain enzymes *Acot5* and *Crot* also show very different expression patterns, with *Crot* being most highly expressed in proximal intestinal epithelium, liver and kidney (similar to the short-chain thioesterase *Acot12*), while *Acot5* is most highly expressed in WAT and brain (Fig. 2C). The expression data for *Crot* and *Acot5* suggest that the enzymes responsible for the production of carnitine esters and free acids for export from medium-chain acyl-CoAs are differently expressed, again indicating that they operate in different tissues rather than competing for the same substrates in the same tissue. A further notable difference is that the short- and medium-chain carnitine acyltransferases are not co-expressed in tissues, as peroxisomal *Crat* shows highest expression in BAT, heart and WAT, while *Crot* is highest in liver and proximal intestine. This suggests that transfer of acetyl- and propionyl-carnitine (produced by CRAT) to mitochondria is prevalent in different tissues than the transfer of medium-chain acylcarnitines (produced by CROT).

It should be noted that the tissue expression data for *Acot5* differs somewhat from the data previously obtained by semi-quantitative PCR, where *Acot5* had highest expression in spleen and a lower expression in WAT [17].

Expression of the 'general' peroxisomal acyl-CoA thioesterase *Acot8*. ACOT8 is a 'general' acyl-CoA thioesterase that hydrolyzes all acyl-CoA substrates tested including straight chain acyl-CoAs of varying chain length, dicarboxylic fatty acids and branched-chain fatty acids, although to varying extents [16, 29]. ACOT8 is suggested to have a function in the regulation of intra-peroxisomal CoASH levels since this enzyme is negatively regulated by CoASH. The tissue expression of *Acot8* mRNA is more general than expression of the other acyl-CoA thioesterases and carnitine acyltransferases, with the highest expression in proximal intestine (combined epithelium and muscle), kidney and liver, and lower expression in BAT (Fig. 3).

Expression of catalase (*Cat*) and some genes involved in β-oxidation of straight-chain acyl-CoAs. The expression of *Cat* was analyzed as a general marker for peroxisomes. As shown in Figure 4, *Cat* is most highly expressed in liver, followed by BAT, kidney and proximal intestine. As a measure of β-oxidation capacity we also measured the expression of the rate-limiting enzyme of straight-chain β-oxidation, the acyl-CoA oxidase 1 (*Acox1*), together with the multifunctional protein 2 (*Mfp2*), which catalyses the second and third step of straight-chain β-oxidation (Fig. 4). Both *Acox1* and *Mfp2* are expressed in a very similar fashion to *Cat*, with highest expression in liver, BAT, kidney and proximal intestine. The high degree of co-expression of *Cat*, *Acox1* and *Mfp2* suggest that *Cat* is a good marker for peroxisome number, and that straight-chain fatty acid β-oxidation is a general function of peroxisomes. It is striking that the expression of all analyzed genes is low in brain, suggesting very low amounts of peroxisomes and peroxisomal β-oxidation in this tissue in spite of their importance in brain function, although the expression may be restricted to certain cells in the brain. Interestingly, the combined expression of *Acot12* and *Crat* (see Fig. 2B) coincides well with the expression of peroxisomal β-oxidation, indicating that these two enzymes indeed participate in export of short-chain β-oxidation products.

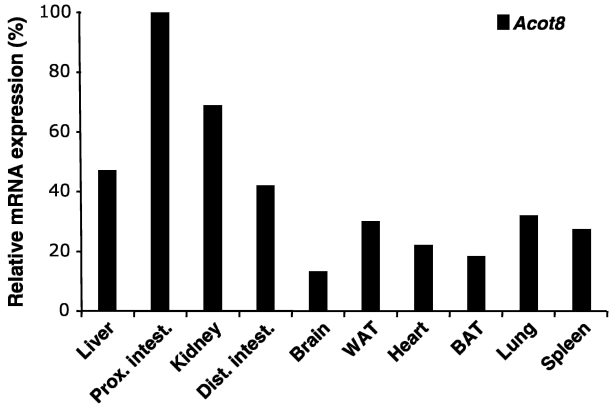

Figure 3. Tissue expression of acyl-CoA thioesterase 8 (*Acot8*). The expression levels of *Acot8* were investigated by real-time PCR as described in Figure 2. Prox. intest, intact proximal intestine (first 10 cm of small intestine); Dist. intest, intact distal intestine (last 10 cm of small intestine).

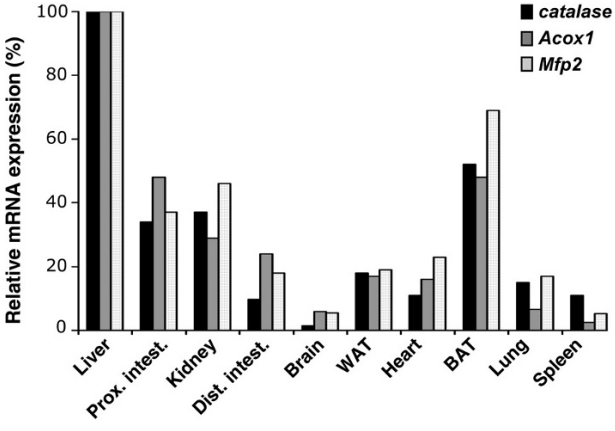

Figure 4. Tissue expression of the peroxisomal enzymes *Cat*, *Acox1* and *Mfp2*. The expression level of *Cat*, *Acot1* and *Mfp2* was investigated by real-time PCR as described in Figure 2.

Discussion

In this study we examined the expression of genes directly involved in peroxisomal straight-chain fatty acid oxidation (*Acox1* and *Mfp2*), which show the expected high expression in liver and kidney. However, these data also point out the proximal intestine, and in particular BAT, as additional important organs for peroxisomal fatty acid degradation. We have recently characterized novel acyl-CoA thioesterases and several reports in the literature have demonstrated the presence of carnitine acyltransferases (CRAT and CROT) in peroxisomes and mitochondria, which may act as auxiliary enzymes in fatty acid metabolism. While CROT is entirely peroxisomal [13], CRAT is present in both mitochondria and peroxisomes, and both CRAT and CROT are induced in liver by treatment with clofibrate [12]. In contrast to the crucial dependence of mitochondrial long-chain fatty acid β-oxidation on carnitine-dependent transport across the mitochondrial membrane, the peroxisomal β-oxidation system has not been demonstrated to depend on carnitine. However, the presence of CROT and CRAT in peroxisomes has been implicated in transport of acetyl-, propionyl- and medium-chain metabolites of peroxisomal β-oxidation to mitochondria for further metabolism *via* a carnitine-dependent transport mechanism. For example, a carnitine-dependent transport of acetyl-CoA from peroxisomes to mitochondria was demonstrated in yeast [30], and transport of propionyl-CoA from peroxisomes to mitochondria is deficient in fibroblasts lacking the carnitine/acylcarnitine transporter, demonstrating that CRAT is needed for the transfer of propionyl-CoA produced by peroxisomal β-oxidation, e.g., from pristanic acid [31]. Also, β-oxidation of pristanic acid proceeds to the medium-chain product dimethylnonanoyl-CoA (DMN-CoA) in peroxisomes, which is converted to the carnitine ester by CROT and subsequently transported to the mitochondria for complete β-oxidation [14]. The recent identification of acyl-CoA thioesterases in peroxisomes raised new questions concerning their possible functions in termination of β-oxidation at various chain-lengths. Since both the carnitine acyltransferases and the acyl-CoA thioesterases use CoA-esters of fatty acids as substrates, these enzymes would compete for the same substrates. Also, review of the literature available on activities of CRAT, CROT and acyl-CoA thioesterases, show that the K_m (low μM range) and V_{max} values appear to be similar [13, 17, 23], suggesting that they can compete for the same acyl-CoA substrates, if the enzymes are co-expressed in the same tissue. However, the tissue expression analysis of the short- and medium-chain acyltransferases and thioesterases shows that there is minimal competition for substrate between these enzymes since they are differently expressed.

Interestingly, these data also point to different functions for peroxisomal β-oxidation in various tissues. The expression of both peroxisomal and mitochondrial *Crat* is by far the highest in BAT, suggesting that peroxisomes would produce substantial amounts of acetyl-carnitine for transfer to mitochondria for further metabolism. This is in line with previous data showing that indeed BAT peroxisomes have a high capacity to produce acetyl groups for transfer to mitochondria, which were shown to oxidize acetyl-carnitine during saturated palmitate oxidizing conditions and thereby significantly contributing to heat production in this tissue [32]. Our data further suggest that peroxisomes may play a similar function to produce acetyl-carnitine for mitochondrial oxidation in heart and WAT. In contrast, liver, proximal intestinal epithelium and kidney peroxisomes contain high levels of the short-chain (acetyl) acyl-CoA thioesterase (ACOT12) and low levels of CRAT, suggesting that acetate would be the main product in these tissues. In fact, several studies support a role in these tissues for acetate production, presumably by hydrolysis of acetyl-CoA formed in peroxisomes into acetate [21, 33, 34]. It has also been shown that acetate derived from peroxisomal β-oxidation of fatty acids is not transferred to mitochondria, but is used for malonyl-CoA synthesis [35].

In contrast to the liver, it was demonstrated that peroxisomal oxidation of fatty acids in heart does not produce acetate [36], but acetyl units used for malonyl-CoA synthesis in rat heart are partially derived from peroxisomal β-oxidation [37]. These data are compatible with a lack of expression of ACOT12 in heart and high expression of CRAT that could transfer the acetyl moieties to the mitochondria. The acetate produced in the liver can either be metabolized, used in biosynthetic pathways or utilized in peripheral tissues depending on the intracellular acetate levels and physiological/pathological state [33]. Acetate is also produced in the intestine even in the absence of the gut flora [38], in line with the high expression of *Acot12* in the intestine, which may be taken up in the liver. The fate of acetate in the cell probably depends on the concentrations of acetate, CoASH and the cellular activities of the cytosolic acetyl-CoA synthetase (ACS1), acetyl-CoA carboxylase and malonyl-CoA decarboxylase.

An overview of the roles of CRAT, CROT, ACOT5 and ACOT12 are shown in Figure 5. Both ACOT5 and CROT have been characterized as medium-chain enzymes, although ACOT5 shows a wider substrate specificity, being active on C8–C14 acyl-CoAs, while

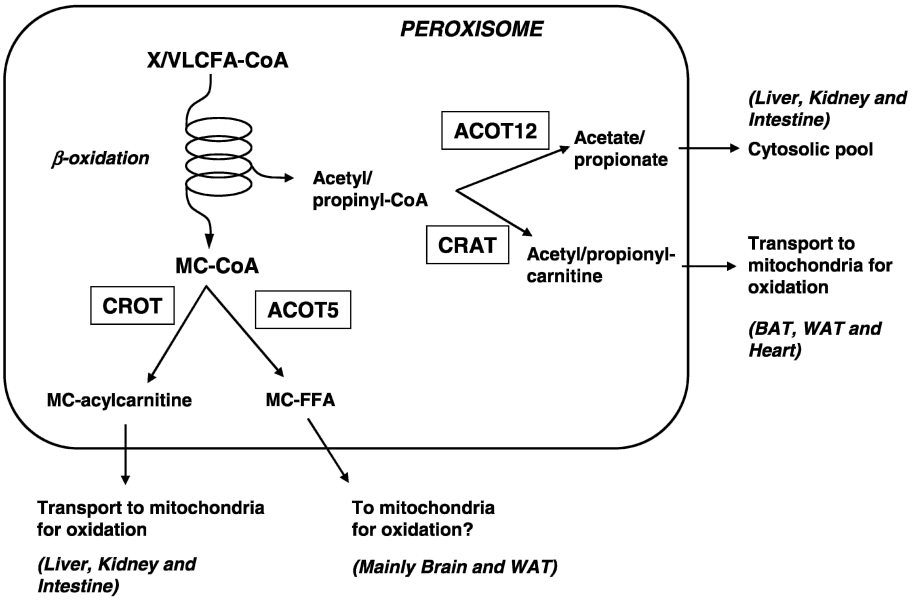

Figure 5. Roles of acyl-CoA thioesterases and carnitine acyltransferases in tissue selective production of carnitine esters or free acids in peroxisomes. In peroxisomes, straight-chain (e.g., VLCFA-CoA) and branched-chain (e.g., bile acid-CoAs or methyl-branched acyl-CoAs, X-CoA) CoA esters undergo β-oxidation to produce shorter (medium chain) acyl-CoAs (MC-CoA) or other chain-shortened CoA esters, with the concomitant production of acetyl-CoA and/or propionyl-CoA. The fate of the acetyl/propionyl-CoA produced depends on the tissue. In liver, kidney and proximal intestine, acetyl/propionyl-CoA would be a substrate for ACOT12, to produce acetate and propionate. However, in BAT, WAT and heart, the acetyl/propionyl-CoA would result in production of acetyl-carnitine and propionyl-carnitine by the action of CRAT. The MC-CoA would be a substrate either for CROT to produce medium chain acyl-carnitine (MC-carnitine) or a substrate for ACOT5 to produce the free fatty acid, again depending on the tissue where these enzymes are expressed.

CROT is instead mainly active on C6–C10 acyl-CoAs, including the branched-chain 4,8-dimethylnonanoyl-CoA (DMN-CoA) [13, 17]. CROT has previously been implicated in the transport of DMN as DMN-carnitine to the mitochondria (*via* carnitine transporters) for complete β-oxidation, since the oxidation of DMN-CoA is severely decreased in carnitine/acylcarnitine transporter-deficient fibroblasts [14]. Also *Acot5* and *Crot* show "opposite" tissue expression patterns, with *Acot5* being most highly expressed in WAT and brain, and *Crot* being most highly expressed in liver, kidney and proximal intestine. Interestingly, these data also show that the carnitine acyltransferases (CRAT and CROT) are not co-localized with each other in tissues, and neither are the acyl-CoA thioesterases (ACOT5 and ACOT12), further underscoring the possible different functions of peroxisomal fatty acid oxidation in different tissues. The fate of the medium-chain free fatty acids produced by ACOT5 is, however, still unknown. Although medium-chain fatty acids generated by ACOT5 may also be transported to the mitochondria since uptake into mitochondria is apparently carnitine independent, the high expression of *Acot5* in brain and WAT suggests a more specific function for this enzyme.

In summary, our data show that the complementary short- and medium-chain carnitine acyltransferases and acyl-CoA thioesterases have distinct tissue expression patterns, suggesting that they function in export of β-oxidation products out of the peroxisome, and that these mechanisms operate in different tissues. The data also suggest that substrates may undergo more cycles of β-oxidation in BAT and heart, tissues that lack medium-chain carnitine acyltransferases and the ACOTs to handle medium-chain β-oxidation products, which would lead to more complete β-oxidation of straight chain fatty acids in these tissues.

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