Symposium: The Role of Long Chain Fatty Acyl-CoAs as Signaling Molecules in Cellular Metabolism

Functional Characterization of Mammalian Mitochondrial Carnitine Palmitoyltransferases I and II Expressed in the Yeast *Pichia pastoris*[1,2]

Gebre Woldegiorgis,[3] Jianying Shi, Hongfa Zhu and Dennis N. Arvidson

Department of Biochemistry and Molecular Biology, Oregon Graduate Institute of Science and Technology, Beaverton, OR 97006-8921

ABSTRACT Mitochondrial carnitine palmitoyltransferases I and II (CPTI and CPTII), together with the carnitine carrier, transport long-chain fatty acyl-CoA from the cytosol to the mitochondrial matrix for β-oxidation. Recent progress in the expression of CPTI and CPTII cDNA clones in *Pichia pastoris*, a yeast with no endogenous CPT activity, has greatly facilitated the characterization of these important enzymes in fatty acid oxidation. It is now well established that yeast-expressed CPTI is a catalytically active, malonyl CoA-sensitive, distinct enzyme that is reversibly inactivated by detergents. CPTII is a catalytically active, malonyl CoA-insensitive, distinct enzyme that is detergent stable. Reconstitution studies with yeast-expressed CPTI have established for the first time that detergent inactivation of CPTI is reversible, suggesting that CPTI is active only in a membrane environment. By constructing a series of deletion mutants of the N-terminus of liver CPTI, we have mapped the residues essential for malonyl CoA inhibition and binding to the conserved first six N-terminal amino acid residues. Mutation of glutamic acid 3 to alanine abolished malonyl CoA inhibition and high affinity malonyl CoA binding, but not catalytic activity, whereas mutation of histidine 5 to alanine caused partial loss in malonyl CoA inhibition. Our mutagenesis studies demonstrate that glutamic acid 3 and histidine 5 are necessary for malonyl CoA inhibition and binding to liver CPTI, but not catalytic activity. J. Nutr. 130: 310S–314S, 2000.

KEY WORDS: • *carnitine palmitoyltransferases* • Pichia pastoris *expression* • *malonyl CoA inhibition* • *binding residues*

Transport of long-chain fatty acids from the cytosol to the mitochondrial matrix for β-oxidation involves the conversion of long-chain fatty acyl-CoA to acylcarnitines by carnitine palmitoyltransferase I (CPTI),[4] translocation across the inner mitochondrial membrane by the carnitine carrier and reconversion to long-chain fatty acyl-CoA by carnitine palmitoyltransferase II (CPTII) (Bieber 1988, McGarry et al. 1989). CPTI is an integral membrane enzyme located on the outer mitochondrial membrane, and CPTII is a membrane-associated enzyme that is loosely bound to the matrix side of the inner mitochondrial membrane (**Fig. 1**). A current model for the membrane topology of CPTI predicts exposure of the CPTI N- and C-terminal domains crucial for activity and malonyl CoA sensitivity on the cytosolic side of the outer mitochondrial membrane (**Fig. 2**) (Fraser et al. 1997).

As an enzyme that catalyzes the rate-limiting step in fatty acid oxidation, CPTI is regulated tightly by its physiologic inhibitor, malonyl CoA, the first intermediate in fatty acid synthesis. This is an important regulatory mechanism in fatty acid oxidation and suggests coordinated control of fatty acid oxidation and synthesis. In heart, more recent studies suggest that high levels of long-chain fatty acids activate muscle CPTI expression via the peroxisome proliferator α, resulting in increased fatty acid oxidation (Brandt et al. 1998, Mascaro et al. 1998, Yu et al. 1998a). Long-chain fatty acids are thus regulators of their own metabolism. Understanding the molecular mechanism of the regulation of the CPT system by malonyl CoA and long-chain fatty acids is crucial in the design of drugs for control of excessive fatty acid oxidation in diabetes mellitus (Prentki and Corkey 1996), in myocardial ischemia in which accumulation of acylcarnitines has been associated with arrhythmias (Corr and Yamada 1995) and in human inherited CPT deficiency diseases (Bonnefont et al. 1996).

The role of the CPT system in cellular fatty acid metabolism

Mammalian tissues express two isoforms of CPTI, i.e., a liver isoform (L-CPTI) and a heart/muscle isoform (M-CPTI)

[1] Presented at the symposium entitled "The Role of Long Chain Fatty Acyl-CoAs as Signaling Molecules in Cellular Metabolism" as part of the Experimental Biology 99 meeting held April 17–21 in Washington, DC. This symposium was part of the metabolic and disease processes theme sponsored by the American Society for Nutritional Sciences. Symposium proceedings are published as a supplement to *The Journal of Nutrition*. Guest editors for this supplement were Earl Shrago, University of Wisconsin, Madison, WI and Gebre Woldegiorgis, Oregon Graduate Institute of Science and Technology, Beaverton, OR.

[2] Supported by a grant from the National Institutes of Health (HL52571).

[3] To whom correspondence should be addressed.

[4] Abbreviations used: CPT, carnitine palmitoyltransferase; PPAR, peroxisome proliferator-activated receptor; RLM, rat liver mitochondria(l).

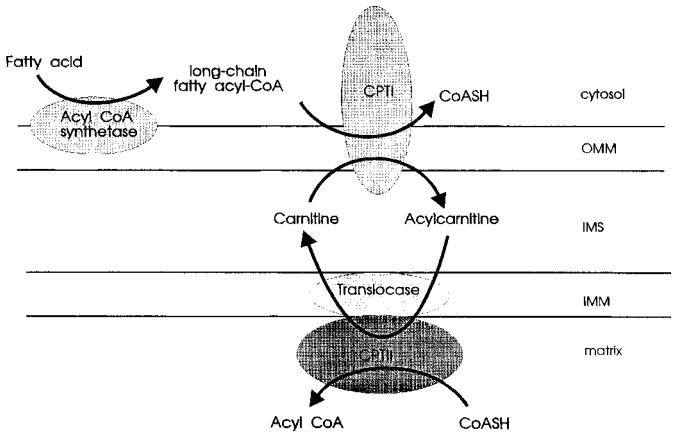

FIGURE 1 Reactions catalyzed by mitochondrial carnitine palmitoyltransferase (CPT)I and CPTII. Abbreviations: OMM, outer mitochondrial membrane; IMS, intermembrane space; IMM, inner mitochondrial membrane.

that are 62% identical in amino acid sequence. L-CPTI is expressed predominantly in liver and the pancreatic islets, heart and, to a lesser extent, in a wide variety of other tissues (McGarry and Brown 1997, Weis et al. 1994, Zhu et al. 1997b). Compared with heart, liver has a much higher affinity for carnitine but is less sensitive to malonyl CoA inhibition. Metabolic conditions that increase cellular long-chain fatty acid oxidation, such as starvation, diabetes and consumption of a high fat diet, have been reported to increase L-CPTI activity and decrease its malonyl CoA sensitivity (Bremer 1981, Cook and Gamble 1987).

Heart and adult testis express both the L- and M-forms of CPTI, but only M-CPTI is expressed in skeletal muscle (Adams et al. 1998, Zhu et al. 1997b). In addition, two novel CPTI isoforms generated by alternative splicing of the M-CPTI transcript have been reported for M-CPTI (Yu et al. 1998b). It is not yet known if these isoforms, which are expressed at very low levels in most tissues compared with M-CPTI, are catalytically active enzymes that play a role in fatty acid oxidation.

In heart M-CPTI, the K_m for carnitine is ~20-fold higher than that of L-CPTI, which is ascribed to the higher tissue levels of carnitine (Brown et al. 1995). Heart M-CPTI is also much more sensitive to malonyl CoA inhibition than liver, although there is no significant difference in the malonyl CoA concentration between the two tissues. The activity and malonyl CoA sensitivity of M-CPTI are also regulated by the fatty acid composition of the diet (Power and Newsholme 1997).

The important question of how fatty acid oxidation can proceed in heart in the presence of high tissue levels of malonyl CoA appears to be resolved in part by the more recent reports of the transcriptional regulation of the M-CPTI gene expression by long-chain fatty acids via the peroxisome proliferator-activated receptor α (PPARα) (Brandt et al. 1998). High levels of long-chain fatty acids stimulate their own metabolism by activation of PPARα, which in turn induces fatty acid oxidation by transcriptional activation of both the L- and M-CPTI genes. It is estimated that ~60–80% of the energy requirement of the heart is derived from fatty acid oxidation (Whitmer et al. 1978). In a normal nonlipogenic tissue such as heart, which is dependent primarily on long-chain fatty acids as an energy source, high rates of fatty acid oxidation should increase cellular long-chain fatty acyl-CoA levels. Long-chain fatty acyl-CoA compete for the malonyl CoA binding site on M-CPTI, effectively making M-CPTI insensitive to malonyl CoA inhibition.

Cloning and expression of the cDNAs for the CPT system

CPTII. Rat and human L-CPTII cDNAs have been cloned and sequenced (Finocchiaro et al. 1991, Woeltje et al. 1990). The cDNA sequences predicted proteins of 658 amino acid residues (71 kDa) that had 82 and 85% identity at the amino acid and nucleotide levels, respectively. The size of the mRNA in different rat tissues was identical, and CPTII appears to be the product of a single-copy gene that is expressed uniformly in every tissue examined to date (McGarry and Brown 1997). CPTII is a catalytically active, malonyl CoA-insensitive, distinct enzyme because a rat liver cDNA encoding CPTII synthesizes an active protein when expressed in *Escherichia coli* (Brown et al. 1993) and in the yeasts *Saccharomyces cerevisiae* (Brown et al. 1994a) and *Pichia pastoris* (de Vries et al. 1997), in baculovirus (Johnson et al. 1995) or in COS cells (Woeltje et al. 1990). Detergent-solubilized, yeast-expressed CPTII showed saturation kinetics when the carnitine concentration was varied relative to a constant second substrate, palmitoyl CoA. The calculated K_m for carnitine was 105 μmol/L, which is similar to the calculated K_m obtained for the *S. cerevisiae*–expressed enzyme. However, the yeast-expressed CPTII showed abnormal nonsaturation kinetics with respect to palmitoyl CoA at a fixed albumin concentration of 1% (wt/v). This was due to the high albumin concentration used to buffer the detergent effect of palmitoyl CoA. With the *S. cerevisiae*–expressed enzyme, mutations of the conserved residues H372A, D376A and D464A resulted in complete loss of CPTII activity, suggesting that these residues may be required for catalysis. However, although chemical modification of *P. pastoris*–expressed CPTII with the histidine-specific reagent diethylpyrocarbonate resulted in >90% loss of CPTII activity, it did not have any effect on the malonyl CoA-sensitive L-CPTI and M-CPTI activity, suggesting that the histidine residue in CPTI (H473) that corresponds to H372 of CPTII is not essential for catalysis and/or may not be accessible to the reagent (Shi and Woldegiorgis, unpublished).

L-CPTI. The cDNAs for rat and human L-CPTI have been cloned and sequenced (Britton et al. 1995, Esser et al. 1993). The cDNAs predicted proteins of 773 amino acid residues (88 kDa) with an overall identity of ~30% to CPTII. The rat and the human L-CPT showed an 82 and 88% identity at the amino acid and nucleotide levels, respectively. We were the first to report high level expression of rat L-CPTI cDNA

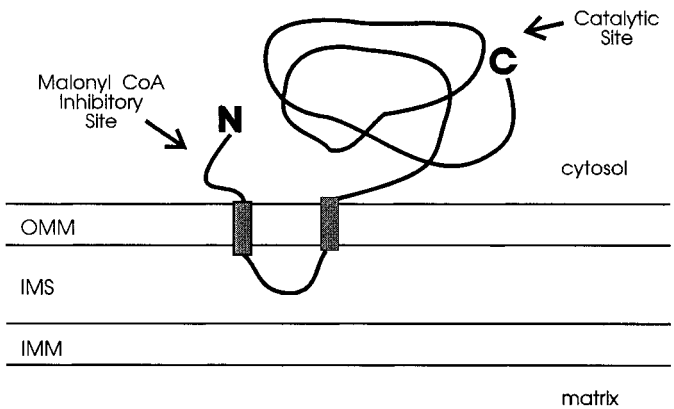

FIGURE 2 Model for carnitine palmitoyltransferase (CPT)I membrane topology. (See Fig. 1 for abbreviations.)

in *P. pastoris*, a yeast with no endogenous CPT activity, and to demonstrate conclusively that L-CPTI is a catalytically active, malonyl CoA-sensitive, distinct enzyme that is reversibly inactivated by detergents (de Vries et al. 1997). Previous reports on the expression of the L-CPTI cDNA concerned results either in COS cells (Esser et al. 1993), which have endogenous CPT activity, or in *S. cerevisiae* (Brown et al. 1994b). In the latter, the expression levels were too low to study the enzyme and reach a definite conclusion whether CPTI is a distinct, catalytically active, malonyl CoA-sensitive enzyme or a regulatory protein that confers malonyl CoA sensitivity to CPTII. *P. pastoris*–expressed L-CPTI was localized to the mitochondria of the L-CPTI expression strain. Immunoblots with L-CPTI–specific C-terminal polyclonal antibodies showed the presence of an 88-kDa protein species corresponding to L-CPTI in the mitochondria of the strain that expressed L-CPTI and rat liver mitochondria (RLM), but not in the control strain with the vector but without the L-CPTI cDNA insert. After our successful expression of the L-CPTI cDNA in the yeast *P. pastoris*, an improved method for expression of L-CPTI cDNA in *S. cerevisiae* was reported recently (Prip-Buus et al. 1998). Furthermore, the yeast-expressed L-CPTI, like RLM CPTI, is located in the outer mitochondrial membrane of the expression strain.

Kinetic analysis of the *P. pastoris*, *S. cerevisiae* and RLM L-CPTI showed similar calculated K_m for carnitine, but the V_{max} for the yeast-expressed L-CPTI were higher than the corresponding RLM L-CPTI. The yeast-expressed L-CPTI showed nonsaturable kinetics when palmitoyl CoA was varied relative to a constant second substrate, i.e., carnitine at high albumin concentration (1% wt/v). Normal saturation kinetics were obtained when the fixed molar ratio of palmitoyl CoA to albumin was raised from 0.53:1 to 6.1:1, suggesting that the decrease in substrate availability due to the high albumin concentration was the cause of the nonsaturation kinetic behavior observed. The calculated K_m and V_{max} for palmitoyl CoA were similar for the two yeast-expressed L-CPTI, but were higher compared with the values obtained for RLM L-CPTI. This could be due to the differences in the membrane lipid environment or to one or more protein-protein interactions that occur in RLM but not in yeast, and/or to higher expression levels in yeast. Yeast-expressed L-CPTI is malonyl CoA sensitive, detergent inactivated and reconstitutable (de Vries et al. 1997).

Heart M-CPTI. The rat brown adipose tissue (Yamazaki et al. 1995) and the human M-CPTI cDNAs have been cloned and sequenced (Adams et al. 1996, Yamazaki et al. 1996, Zhu et al. 1997b). These cDNAs encode a protein of 772 amino acid residues (88 kDa) with 62% identity to the rat L-CPTI at the amino acid level. In human or rat tissues, M-CPTI is expressed only in heart, skeletal muscle and testis. The genomic DNA for both the human M-CPTI and the rat M-CPTI have also been isolated and characterized (Adams et al. 1996, Wang et al. 1998). The genes for human muscle M-CPTI and L-CPTI are located on chromosomes 22q and 11q, respectively (Adams et al. 1996, Britton et al. 1995). Thus, human liver and skeletal muscle CPTI are different proteins encoded by separate genes. Expression of the human M-CPTI cDNA in *P. pastoris*, a yeast with no endogenous CPT activity, produced an 80-kDa protein that was located in the mitochondria (Zhu et al. 1997b). Isolated mitochondria from the M-CPTI expression strain exhibited malonyl CoA-sensitive CPT activity that was detergent labile. Furthermore, yeast-expressed M-CPTI had a high K_m for carnitine and low I_{50} for malonyl CoA inhibition, characteristics similar to those observed with rat heart mitochondrial CPTI, but different from those for the yeast-expressed L-CPTI. This is the first report of the expression of human heart M-CPTI in a system devoid of endogenous CPT activity and the functional characterization of human heart M-CPTI in the absence of the liver isoform and CPTII. However, the level of expression of human heart M-CPTI was lower than that obtained with rat L-CPTI. As a result, we were unable to demonstrate restoration of malonyl CoA-sensitive CPT activity in detergent-inactivated M-CPTI by reconstitution.

A high level of expression of human heart M-CPTI was obtained using a multicopy *P. pastoris* expression strain containing ~24 copies of the expression vector integrated into its genome (Zhu et al. 1997a). Levels of M-CPTI activity were >10-fold higher than previously reported by us with a single-copy strain and were sufficient to perform reconstitution studies on the membrane protein, a key step in purification and structural analysis of the enzyme. M-CPTI in the multicopy strain was malonyl CoA sensitive and detergent labile. Immunoblots with the M-CPTI–specific polyclonal antibodies showed the presence of an 80-kDa protein species corresponding to M-CPTI in the mitochondria of the multicopy transformant strain that expressed M-CPTI, but not in the non-CPTI cDNA containing the control strain. The rat heart cDNA for M-CPTI was also expressed in COS cells, which have endogenous L-CPTI activity that may be induced (Esser et al. 1996). Because of the high endogenous L-CPTI present in COS cells, it was not possible to determine the I_{50} for malonyl CoA inhibition of M-CPTI or the K_m for the substrates.

Reconstitution of yeast-expressed L- and M-CPTI. Previous studies from our laboratory and others have demonstrated that detergent solubilization of RLM and rat heart mitochondria, but not CPTII, abolishes malonyl CoA-sensitive CPT activity, but not malonyl CoA binding (Bremer et al. 1985, Chung et al. 1992, Lund and Woldegiorgis 1987, McGarry et al. 1989, Woldegiorgis et al. 1985 and 1992). Until recently, it was presumed that CPTI was irreversibly inactivated by detergents and hence not recoverable as an active enzyme; however, our reconstitution studies with yeast-expressed L-CPTI and M-CPTI established for the first time that detergent inactivation of CPTI is reversible (de Vries et al. 1997, Zhu et al. 1997a). Removal of CPTI from its membrane environment inactivates the enzyme, and reconstitution via detergent removal in the presence of phospholipids reactivates the enzyme, suggesting that CPTI is active only in a membrane environment. The ability to reconstitute yeast-expressed CPTI is critical to its purification and further studies on the structure and function of this important enzyme. Thus, *P. pastoris* appears to be an excellent model system with which to investigate the CPT enzymes.

Structure-function studies

Our development of a high level of expression for CPTI in *P. pastoris*, a yeast with no endogenous CPT activity, has enabled us to begin to map the malonyl CoA and substrate binding sites by mutational analysis (de Vries et al. 1997, Zhu et al. 1997a and 1997b). Amino acid sequence alignment of both human and rat heart M-CPTI, L-CPTI and CPTII reveals the presence of a conserved N-terminal sequence of 124 residues with two putative transmembrane domains in all CPTI, which is absent from CPTII, the malonyl CoA-insensitive enzyme (**Fig. 3**). It has been hypothesized that malonyl CoA sensitivity of CPTI may reside in these 124 N-terminal amino acid residues. By systematically constructing a series of deletion mutants to the N-terminus of L-CPTI, we showed

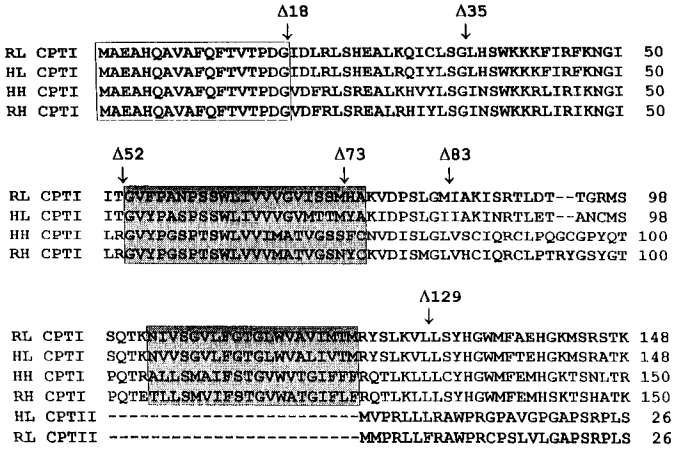

FIGURE 3 N-Terminal amino acid sequence line-up of human and rat carnitine palmitoyltransferases (CPT). Shaded areas represent the position of the two predicted membrane-spanning domains of all known CPTI. The position of the conserved 18 N-terminal amino acid residues within the CPTI is boxed. The position of each of the deletion mutants is shown by an arrow. Abbreviations: HL, human liver; RL, rat liver; HH, human heart; RH, rat heart (Shi et al. 1999 with permission of the ACS).

that residues necessary for malonyl CoA inhibition and binding are located within the conserved first 18 N-terminal amino acids (Shi et al. 1998). To identify specific residue(s) involved in malonyl CoA binding and inhibition of L-CPTI, we constructed two more deletion mutants Δ12 and Δ6, that resulted in loss of malonyl CoA inhibition and binding similar to that of the Δ18 mutant.

Three substitution mutations (E3A, H5A and Q6A) within the conserved first six N-terminal amino acid residues were then constructed and tested for malonyl CoA inhibition and binding. A change of glutamic acid 3 to alanine caused a 100-fold decrease in malonyl CoA inhibition and loss of high affinity malonyl CoA binding, but not catalytic activity (Shi et al. 1999). A mutant L-CPTI with a change of histidine 5 to alanine caused partial loss of malonyl CoA inhibition, whereas a mutant L-CPTI with a change of glutamine 6 to alanine had wild-type properties. Our results demonstrate that glutamic acid 3 and histidine 5 are necessary for malonyl CoA inhibition and binding to L-CPTI, but not catalytic activity (Shi et al. 1999).

Similar deletion mutation analysis with human M-CPTI, which is 30-fold more sensitive to malonyl CoA inhibition, revealed loss of malonyl CoA inhibition and binding when the first 28 N-terminal residues were deleted (Shi et al., unpublished data). Unlike L-CPTI, deletion of the conserved first 18 N-terminal residues of the heart enzyme causes only moderate loss in malonyl CoA inhibition. To identify the specific residue(s) in this region of M-CPTI that are necessary in malonyl CoA inhibition and binding, we are currently constructing and testing substitution mutations of the N-terminal 19–28 amino acids in M-CPTI.

The N-terminal domain of L-CPTI (residues 1–150), which contains two transmembrane segments, was recently shown to contain all of the information required for mitochondrial targeting and insertion into the outer mitochondrial membrane (Cohen et al. 1998). When the N-terminal domain of L-CPTI was fused to the N-terminal of malonyl CoA-insensitive CPTII, a membrane-associated protein, the active chimeric CPTII was anchored at the outer mitochondrial membrane but was insensitive to malonyl CoA inhibition, indicating that malonyl CoA sensitivity is an intrinsic property of L-CPTI and that its N-terminal domain cannot confer malonyl CoA sensitivity to CPTII. Similarly, replacement of the N-terminal domain of L-CPTI with the N-terminal domain of M-CPTI and vice versa does not change the malonyl CoA sensitivity of the chimeric L-CPTI or M-CPTI, suggesting that the amino acid residues responsible for the differing sensitivity to malonyl CoA are not located in this N-terminal region (Shi et al. unpublished data, Swanson et al. 1998). Replacement of the N-terminal domain of L-CPTI by a specific outer mitochondrial membrane signal anchor sequence (Cohen et al. 1998) or removal of the two transmembrane domains and the linker region (Zhu et al., unpublished data) results in a less active protein that is malonyl CoA insensitive. Thus, the N-terminal domain of L-CPTI is essential to maintain an optimal conformation for both catalytic function and malonyl CoA sensitivity. Future structure-function studies of the CPT system should unravel the molecular architecture of this important enzyme system in fatty acid metabolism.

In summary, our functional high level expression of rat L-CPTI and human M-CPTI in *P. pastoris*, a yeast with no endogenous CPT activity, establishes that CPTI is a catalytically active, malonyl CoA-sensitive, distinct enzyme that is reversibly inactivated by detergents. Our site-directed mutagenesis studies with the *P. pastoris*–expressed rat L-CPTI demonstrate for the first time that glutamic acid 3 and histidine 5 on the N-terminal domain of L-CPTI are necessary for malonyl CoA inhibition and binding to L-CPTI, but not for catalysis.

ACKNOWLEDGMENT

We are grateful to James M. Cregg (Oregon Graduate Institute of Science and Technology) for advice, helpful suggestions and encouragement throughout these studies.

LITERATURE CITED

Adams, M. D., Kerlavage, A. R., Fuldner, R. A., Philips, C. A. & Venter, J. C. (1996) Complete sequence of a chromosome 22q subtelomeric BAC. Unpublished, Genbank Accession #U62317.

Adams, S. H., Esser, V., Brown, N. F., Ing, N. H., Johnson, L., Foster, D. W. & McGarry, J. D. (1998) Expression and possible role of muscle-type carnitine palmitoyltransferase I during sperm development in the rat. Biol. Reprod. 59: 1399–1405.

Bieber, L. L. (1988) Carnitine. Annu. Rev. Biochem. 57: 261–283.

Bonnefont, J. P., Taroni, F., Cavadini, P., Cepanec, C., Brivet, M., Saudubray, J. M., Leroux, J. P. & Demaugre, F. (1996) Molecular analysis of carnitine palmitoyltransferase II deficiency with hepatocardiomuscular expression. Am. J. Hum. Genet. 58: 971–978.

Brandt, J. M., Djouadi, F. & Kelly, D. P. (1998) Fatty acids activate transcription of the muscle carnitine palmitoyltransferase I gene in cardiac myocytes via the peroxisome proliferator activated receptor α. J. Biol. Chem. 273: 23786–23792.

Bremer, J. (1981) The effect of fasting on the activity of liver carnitine palmitoyltransferase and its inhibition by malonyl CoA. Biochim. Biophys. Acta 665: 628–631.

Bremer, J., Woldegiorgis, G., Schalinske, K. & Shrago, E. (1985) Carnitine palmitoyltransferase: activation by palmitoyl CoA and inactivation by malonyl CoA. Biochim. Biophys. Acta 833: 9–16.

Britton, C. H., Schultz, R. A., Zhang, B., Esser, V., Foster, D. W. & McGarry, J. D. (1995) Human liver mitochondrial carnitine palmitoyltransferase I: characterization of its cDNA and chromosomal localization and partial analysis of the gene. Proc. Natl. Acad. Sci. U.S.A. 92: 1984–1988.

Brown, N. F., Anderson, R. C., Caplan, S. L., Foster, D. W. & McGarry, J. D. (1994a) Catalytically important domains of rat carnitine palmitoyltransferase II as determined by site-directed mutagenesis and chemical modification. J. Biol. Chem. 269: 19157–19162.

Brown, N. F., Esser, V., Foster, D. W. & McGarry, J. D. (1994b) Expression of a cDNA for rat liver carnitine palmitoyltransferase I in yeast establishes that the catalytic activity and malonyl-CoA sensitivity reside in a single polypeptide. J. Biol. Chem. 269: 26438–26442.

Brown, N. F., Sen, A., Soltis, D. A., Jones, B., Foster, D. W. & McGarry, J. D. (1993) Expression of precursor and mature carnitine palmitoyltransferase II

in *Escherichia coli* and *in vitro*: differential behaviour of rat and human isoforms. Biochem. J. 294: 79–86.
Brown, N. F., Weis, B. C., Husti, J. E., Foster, D. W. & McGarry, J. D. (1995) Mitochondrial carnitine palmitoyltransferase I isoform switching in the developing rat heart. J. Biol. Chem. 270: 8952–8957.
Chung, C. H., Woldegiorgis, G., Dai, G., Shrago, E. & Bieber, L. L. (1992) Conferral of malonyl coenzyme A sensitivity to purified rat heart mitochondrial carnitine palmitoyltransferase. Biochemistry 31: 9777–9783.
Cohen, I., Kohl, C., McGarry, J. D., Girard, J. & Prip-Buus, C. (1998) The N-terminal domain of rat liver carnitine palmitoyltransferase I mediates import into the outer mitochondrial membrane and is essential for activity and malonyl CoA sensitivity. J. Biol. Chem. 273: 29896–29904.
Cook, G. A. & Gamble, M. S. (1987) Regulation of carnitine palmitoyltransferase by insulin results in decreased activity and decreased apparent K_i values for malonyl CoA. J. Biol. Chem. 262: 2050–2055.
Corr, P. B. & Yamada, K. A. (1995) Selected metabolic alterations in the ischemic heart and their contributions to arrhythmogenesis. Herz 20: 156–168.
de Vries, Y., Arvidson, D. N., Waterham, H., Cregg, J. M. & Woldegiorgis, G. (1997) Functional characterization of mitochondrial carnitine palmitoyltransferases I and II expressed in the yeast *P. pastoris.* Biochemistry 36: 5285–5292.
Esser, V., Britton, C. H., Weis, B. C., Foster, D. W. & McGarry, J. D. (1993) Cloning, sequencing, and expression of a cDNA encoding rat liver carnitine palmitoyltransferase I. J. Biol. Chem. 268: 5817–5822.
Esser, V., Brown, N. F., Cowan, A. T., Foster, D. W. & McGarry, J. D. (1996) Expression of a cDNA isolated from rat brown adipose tissue and heart identifies the product as the muscle isoform of carnitine palmitoyltransferase I (M-CPTI). J. Biol. Chem. 271: 6972–6977.
Finocchiaro, G., Taroni, F., Rocchi, M., Martin, A. L., Irma, C., Tarelli, G. T. & Didonato, S. (1991) cDNA cloning, sequence analysis, and chromosomal localization of the gene for human carnitine palmitoyltransferase. Proc. Natl. Acad. Sci. U.S.A. 88: 661–665.
Fraser, F., Corstorphine, C. G. & Zammit, V. A. (1997) Topology of carnitine palmitoyltransferase I in the mitochondrial outer membrane. Biochem. J. 323: 711–718.
Johnson, T. J., Mann, W. R., Dragland, C. J., Anderson, R. C., Nemecek, G. M. & Bell, P. A. (1995) Over-expression and characterization of active recombinant rat liver carnitine palmitoyltransferase II using baculovirus. Biochem. J. 309: 689–693.
Lund, H. & Woldegiorgis, G. (1987) Carnitine palmitoyltransferase: separation of enzyme activity and malonyl CoA binding in rat liver mitochondria. Biochim. Biophys. Acta 878: 243–249.
Mascaro, C., Acosta, E., Ortiz, J. A., Marrero, P. F., Hegardt, F. G. & Haro, D. (1998) Control of human muscle type carnitine palmitoyltransferase I gene transcription by peroxisome proliferator activated receptor. J. Biol. Chem. 273: 8560–8563.
McGarry, J. D. & Brown, N. F. (1997) The mitochondrial carnitine palmitoyltransferase system: from concept to molecular analysis. Eur. J. Biochem. 244: 1–14.
McGarry, J. D., Woeltje, K. F., Kuwajima, M. & Foster, D. W. (1989) Regulation of ketogenesis and the renaissance of carnitine palmitoyltransferase. Diabetes Metab. Rev. 5: 271–284.
Power, G. W. & Newsholme, E. A. (1997) Dietary fatty acids influence the activity and metabolic control of mitochondrial carnitine palmitoyltransferase I in rat heart and skeletal muscle. J. Nutr. 127: 2142–2150.
Prentki, M. & Corkey, B. E. (1996) Are the β-cell signaling molecules malonyl-CoA and cytosolic long-chain acyl-CoA implicated in multiple tissue defects of obesity and NIDDM? Diabetes 45: 273–283.
Prip-Buus, C., Cohen, I., Kohl, C., Esser, V., McGarry, J. D. & Girard, J. (1998) Topological and functional analysis of the rat liver carnitine palmitoyltransferase I expressed in *Saccharomyces cerevisiae*. FEBS Lett. 429: 173–178.
Shi, J., Zhu, H., Arvidson, D. N., Cregg, J. M. & Woldegiorgis, G. (1998) Deletion of the conserved first 18 N-terminal amino acid residues in rat liver carnitine palmitoyltransferase I abolishes malonyl CoA sensitivity and binding. Biochemistry 37: 11033–11038.
Shi, J., Zhu, H., Arvidson, D. N. & Woldegiorgis, G. (1999) A single amino acid change (substitution of glutamate 3 with alanine) in the N-terminal region of rat liver carnitine palmitoyltransferase I abolishes malonyl CoA inhibition and high affinity binding. J. Biol. Chem. 274: 9421–9426.
Swanson, S. T., Foster, D. W., McGarry, J. D. & Brown, N. F. (1998) Roles of the N- and C-terminal domains of carnitine palmitoyltransferase I isoforms in malonyl CoA sensitivity of the enzymes: insights from expression of chimaeric proteins and mutation of conserved histidine residues. Biochem. J. 335: 513–519.
Wang, D., Harrison, W., Buja, L. M., Elder, F.F.B. & McMillin, J. B. (1998) Genommic DNA sequence, promoter expression, and chromosomal mapping of rat muscle carnitine palmitoyltransferase I. Genomics 48: 314–323.
Weis, B. C., Cowan, A. T., Brown, N., Foster, D. W. & McGarry, J. D. (1994) Use of a selective inhibitor of liver carnitine palmitoyltransferase I (CPT I) allows quantification of its contribution to total CPT I activity in rat heart: Evidence that the dominant cardiac CPT I isoform is identical to the skeletal muscle enzyme. J. Biol. Chem. 269: 26443–26448.
Whitmer, J. T., Idell-Wenger, J. A., Rovetto, M. J. & Neely, J. R. (1978) Control of fatty acid metabolism in ischemic and hypoxic hearts. J. Biol. Chem. 253: 4305–4309.
Woeltje, K. F., Esser, V., Weis, B. C., Sen, A., Cox, W. F., McPhaul, M. J., Slaughter, C. A., Foster, D. W. & McGarry, J. D. (1990) Cloning, sequencing, and expression of a cDNA encoding rat liver mitochondrial carnitine palmitoyltransferase. J. Biol. Chem. 265: 10720–10725.
Woldegiorgis, G., Bremer, J. & Shrago, E. (1985) Substrate inhibition of carnitine palmitoyltransferase by palmitoyl CoA and activation by phospholipids and proteins. Biochim. Biophys. Acta 837: 135–140.
Woldegiorgis, G., Fibich, B., Contreras, L. & Shrago, E. (1992) Restoration of malonyl CoA sensitivity of soluble rat liver mitochondria carnitine palmitoyltransferase by reconstitution with a partially purified malonyl CoA binding protein. Arch. Biochem. Biophys. 295: 348–351.
Yamazaki, N., Shinhara, Y., Shima, A. & Terada, H. (1995) High expression of a novel carnitine palmitoyltransferase I like protein in rat brown adipose tissue and heart: isolation and characterization of its cDNA clone. FEBS Lett. 363: 41–45.
Yamazaki, N., Shinhara, Y., Shima, A., Yamanaka, Y. & Terada, H. (1996) Isolation and characterization of cDNA and genomic clones encoding human muscle type carnitine palmitoyltransferase I. Biochim. Biophys. Acta 1307: 157–161.
Yu, G.-S., Lu, Y.-C. & Gulick, T. (1998a) Co-regulation of tissue-specific alternative human carnitine palmitoyltransferase Iβ gene promoters by fatty acid enzyme substrate. J. Biol. Chem. 273: 32901–32909.
Yu, G.-S., Lu, Y.-C. & Gulick, T. (1998b) Expression of novel isoforms of carnitine palmitoyltransferase I (CPT-I) generated by alternative splicing of the CPTI-Iβ gene. Biochem. J. 334: 225–231.
Zhu, H., Shi, J., Cregg, J. M. & Woldegiorgis, G. (1997a) Reconstitution of highly expressed human heart muscle carnitine palmitlytransferase I. Biochem. Biophys. Res. Commun. 239: 498–502.
Zhu, H., Shi, J., de Vries, Y., Arvidson, D. N., Cregg, J. M. & Woldegiorgis, G. (1997b) Functional studies of yeast expressed human heart muscle carnitine palmitoyltransferase I. Arch. Biochem. Biophys. 347: 53–61.