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Hormonal and nutritional regulation of muscle carnitine palmitoyltransferase I gene expression *in vivo*

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Abstract

Transgenic mice carrying the human heart muscle carnitine palmitoyltransferase I (M-CPTI) gene fused to a CAT reporter gene were generated to study the regulation of M-CPTI gene expression. When the mice were fasted for 48 h, CAT activity and mRNA levels increased by more than 2-fold in heart and skeletal muscle, but not liver or kidney. In the diabetic transgenic mice, there was a 2- to 3-fold increase in CAT activity and CAT mRNA levels in heart and skeletal muscle which upon insulin administration reverted to that observed with the control insulin sufficient transgenic mice. Feeding a high fat diet increased CAT activity and mRNA levels by 2- to 4-fold in heart and skeletal muscle of the transgenic mice compared to the control transgenic mice on regular diet. Overall, the M-CPTI promoter was found to be necessary for the tissue-specific hormonal and dietary regulation of the gene expression.

Keywords: M-CPTI; Promoter; CAT; Gene expression; Transgenic mice; Regulation; Hormonal; Dietary

Carnitine palmitoyltransferase I (CPTI)[1] catalyzes the conversion of long-chain fatty acyl-CoAs to acylcarnitines in the presence of carnitine [1,2]. Mammalian tissues express three isoforms of CPTI, a liver, L-CPTI, and a heart/skeletal muscle, M-CPTI, that are 62% identical in amino acid sequence [3–8] and a brain isoform, CPTIc, that is 54% identical to L- and M-CPTI [9]. As an enzyme that catalyzes the first rate-limiting step in β-oxidation, CPTI is tightly regulated by its physiological inhibitor, malonyl-CoA, the first intermediate in fatty acid synthesis, suggesting coordinated control of fatty acid oxidation and synthesis [1,2,10]. Metabolic conditions that increase cellular long-chain fatty acid oxidation, such as starvation, diabetes, and consumption of a high fat diet increase L-CPTI activity and decrease its malonyl-CoA sensitivity [11,12]. The activity and malonyl-CoA sensitivity of M-CPTI is also regulated by the fatty acid composition of the diet [13], and PPARα is necessary for the fasting-induced expression of the M-CPTI gene [14]. In addition, high levels of long-chain fatty acyl-CoAs stimulate the AMP-activated protein kinase, inhibit acetyl CoA carboxylase, and turn off malonyl-CoA synthesis, thus decreasing M-CPTI inhibition [15]. Because of its central role in fatty acid metabolism, a good understanding of the molecular mechanism of regulation of the CPT system is an important first step in the development of treatments for diseases such as myocardial ischemia [16], heart failure [17], diabetes [18], obesity [19], cancer [20], and in human inherited CPT deficiency diseases [21].

The transcriptional regulators of fatty acid oxidation enzyme genes are PPARα, a member of the ligand-activated nuclear receptor superfamily, and its coactivator, PGC-1α [22–26]. PPARα- and PGC-1α-mediated control of cardiac metabolic gene expression is activated during postnatal development, short-term starvation, diabetes, and in response to exercise training [27,28]. The expression of the

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[1] *Abbreviations used:* CPTI, carnitine palmitoyltransferase I; M-CPTI, muscle carnitine palmitoyltransferase I; FARE-1, fatty acid response element; SZ, streptozotocin.

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M-CPTI gene has been shown to be regulated at the transcription level *in vitro* by long-chain fatty acids via the PPARα. The fatty acid response element (FARE-1) in the M-CPTI gene is located between −775 and −763 bp upstream of the initiator codon [22]. FARE-1 interacts with the PPARα/RxR heterodimers/PGC-1α coactivator complex to confer transcriptional activation of the M-CPTI gene. High circulating levels of long-chain fatty acids thus stimulate their own metabolism by transcriptional activation of PPARα. In addition, PPARα may also regulate fatty acid oxidation in heart in part by transcriptional control of malonyl-CoA decarboxylase [29]. In this study, using transgenic mice that express a CAT reporter gene driven by M-CPTI promoter, we demonstrate for the first time that the 1.1-kb 5′-flanking sequence of the M-CPTI gene with FARE-1 is sufficient for the tissue-specific response to fasting, diabetes and high fat diet.

Experimental procedures

Generation of transgenic mice that express CAT reporter gene driven by M-CPTI promoter

A genomic clone that contains the full-length promoter region of the human M-CPTI gene was isolated from a human Hela cell genomic DNA library using as probes a 5′ end 700 bp EcoRI and a 3′ end 2.0 kb EcoRI fragments of the human heart M-CPTI cDNA. The identity of the human genomic clone, pG22-3 in pUC119 was confirmed by DNA sequencing. A plasmid containing the human heart M-CPTI gene promoter fused to a CAT reporter gene was constructed for the production of transgenic mice as follows: the M-CPTI promoter–reporter construct containing FARE-1 of the M-CPTI gene (p1.1CAT) was constructed using the pCAT-Basic vector (Promega, Madison, WI), a plasmid which contains the CAT structural gene without the promoter as follows: A 1.1-kb fragment with a HindIII site on the 5′ end was PCR amplified using the plasmid pG22-3 as a template and the primers shown in Table 1. The PCR product was digested with HindIII–SalI and subcloned into the HindIII–SalI site of the pCAT-Basic vector to produce the plasmid, p1.1CAT. The resulting plasmid p1.1Cat-B (Fig. 1) was then cut by HindIII and BamHI followed by BspHI digestion. About 60–86 ng/μl DNA of the HindIII–BamHI fragment of the p1.1CAT-B construct with FARE-1 was prepared for generation of the transgenic mice.

Table 1
Primers used for construction of the hybrid gene, genotyping of transgenic mice and RT-PCR

p1.1CAT gene	
1.	Forward, 5′-TTAAGCTTGTATGAAGACGACCCTGA-3′ and Reverse, 5′-CAAGGAGATGTCCACGTTG-3′
Genotyping	
2.	CAT-F4, 5′-CCACGACGATTTCCGGCAGTTT-3′ CAT-R4, 5′-CACTCTATG-CCTGTGTGGAGTAA-3′
3.	TgF6, 5′-TCCGGCCTTTATTCACATTCTTGCCCGCCTG-3′ TgR6, 5′-GGCATTCCACCACTGCTCCCATTCATVCAGTTCC-3′
4.	CAT-F4 (5′-CCACGACGATTTCCGGCAGTTT-3′) CAT-R11 (5′-CCTGCCACTCATCGCAGTACTGTTG-3′)
RT-PCR	
5.	CATF4 (5′-CCACGACGATTTCCGGCAGTTT-3′) and CATR11 (5′-CCTGCCACTCATCGCAGTACTGTTG-3′)
6.	ActinF (5′-TGTGATGGTGGGAATGGGTCAG-3′) and ActinR (5′-TTTGATGTCACGCACGATTTCC-3′)

The hybrid gene, p1.1CAT-B was microinjected into the pronucleus of fertilized mouse embryos by the Transgenic Mice Core Facility at the Oregon Health & Science University, and 40 weaned potential founder transgenic mice carrying the p1.1CAT with FARE-1 were generated. Founder mice carrying the M-CPTI promoter–CAT gene were screened by PCR amplification of tail DNA using the CAT primers shown in Table 1 which were designed to amplify a 380-bp region spanning the junction between the M-CPTI promoter and the CAT reporter gene or nested PCR with an outer primer set that targeted the CAT gene producing a 790 bp PCR product with the tail DNA template, and an inner primer set that amplified a 320 bp PCR product using the 790 bp PCR product of the first reaction as a template. Transgenic founder mice were identified by PCR and Southern blot analysis using tail DNA from 3–4 week old mice [30]. Heterozygous transgenic progenies were obtained by breeding the founders (Fo) to B6D2F1/J wild-type mice. F1 generation mice with the transgene were bred back to B6D2F1/J wild-type mice to produce F2 generation progenies with the transgene, which were again bred to produce the F3 generation. The F3 generation mice with the transgene and the wild-type mice were used for these studies.

Animal treatment

Animals were housed in a temperature controlled room with 12 h-light/12 h-dark cycling and were fed normal chow diet ad libitum. All experiments were performed with age (8–10 weeks old) and sex matched F3 generation mice. Transgenic mice were either fasted for 48 h or fed a normal diet. Diabetes was induced by intraperitoneal injection of streptozotocin (10 mg/100 g body weight) twice per week. Diabetes was confirmed by high blood glucose level (>250 mg/dl). Insulin was administered to diabetic mice at a combined dose of regular insulin (3 U/100 g body weight; Lilly) intraperitoneally and Lente insulin (10 U/100 g body weight; Lilly) subcutaneously [31]. Diabetic mice were studied 96 h following streptozotocin (SZ) treatment. Insulin-treated mice were studied 16 h following insulin treatment. For high fat diet feeding, transgenic mice were fed fish oil (200 g/kg) and corn oil (10 g/kg) based diet (Animal Specialties, Inc., Hubbard, OR) for 6 weeks. All animal procedures were performed with the approval of the Institutional Animal Care and Use Committee at the Oregon Health & Sciences University.

CAT activity assay

The animals were euthanized by 95% CO_2/5% O_2 and sacrificed by cervical dislocation. Tissues were isolated by rapid dissection and frozen in liquid nitrogen. The frozen tissues were pulverized using a ceramic mortar. Three hundred microliters of lysis buffer (250 mM Tris–HCl, pH 8.0) was added to the powdered tissue and the suspension was subjected to three rapid freeze–thaw cycles in dry ice/ethanol bath followed by rapid thawing at 37 °C, vortexed vigorously after each thaw cycle, and finally heated at 60 °C for 10 min to inactivate endogenous deacetylase activity. The suspension was centrifuged at 12,000*g* for 2 min at 4 °C. The protein concentration was determined by the Bio-Rad protein assay using bovine

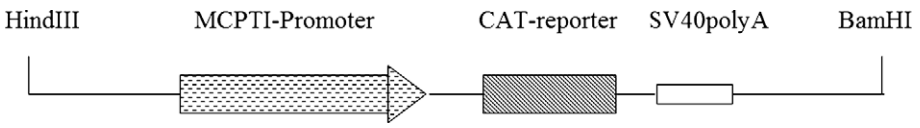

Fig. 1. Schematic representation of the M-CPTI promoter–CAT reporter gene construct used for generation of the transgene.