Critical Review

Significance of L-Carnitine for Human Health

María M. Adeva-Andany*
Isabel Calvo-Castro
Carlos Fernández-Fernández
Cristóbal Donapetry-García
Ana María Pedre-Piñeiro

Hospital Juan Cardona, Rúa Pardo Bazán, Ferrol, Spain

Abstract

Carnitine acyltransferases catalyze the reversible transfer of acyl groups from acyl-coenzyme A esters to L-carnitine, forming acyl-carnitine esters that may be transported across cell membranes. L-Carnitine is a wáter-soluble compound that humans may obtain both by food ingestion and endogenous synthesis from trimethyl-lysine. Most L-carnitine is intracellular, being present predominantly in liver, skeletal muscle, heart and kidney. The organic cation transporter-2 facilitates L-carnitine uptake inside cells. Congenital dysfunction of this transporter causes primary L-carnitine deficiency. Carnitine acetyltransferase is involved in the export of excess acetyl groups from the mitochondria and in acetylation reactions that regulate gene transcription and enzyme activity. Carnitine octanoyltransferase is a peroxysomal enzyme required for the complete oxidation of very long-chain fatty acids and phytanic acid, a branched-chain fatty acid. Carnitine palmitoyltransferase-1 is a transmembrane protein located on the outer mitochondrial membrane where it catalyzes the conversion of acyl-coenzyme A esters to acyl-carnitine esters. Carnitine acyl-carnitine translocase transports acyl-carnitine esters across the inner mitochondrial membrane in exchange for free L-carnitine that exits the mitochondrial matrix. Carnitine palmitoyltransferase-2 is anchored on the matrix side of the inner mitochondrial membrane, where it converts acyl-carnitine esters back to acyl-coenzyme A esters, which may be used in metabolic pathways, such as mitochondrial β-oxidation. L-Carnitine enhances nonoxidative glucose disposal under euglycemic hyperinsulinemic conditions in both healthy individuals and patients with type 2 diabetes, suggesting that L-carnitine strengthens insulin effect on glycogen storage. The plasma level of acyl-carnitine esters, primarily acetylcarnitine, increases during diabetic ketoacidosis, fasting, and physical activity, particularly high-intensity exercise. Plasma concentration of free L-carnitine decreases simultaneously under these conditions. © 2017 IUBMB Life, 00(0):000–000, 2017

Keywords: *acetyl-coA; organic cation transporter-2; carnitine acetyltransferase; carnitine octanoyltransferase; carnitine palmitoyltransfer*

Abbreviations: *CoA, coenzyme A; CACT, carnitine acylcarnitine translocase; CPT1, carnitine palmitoyltransferase-1; CPT2, carnitine palmitoytransferase-2; CRAT, carnitine acetyltransferase; CROT, carnitine octanoyltransferase; HOMA-IR, homeostasis model assessment-insulin resistance; OCTN2, organic cation transporter-2; PDH, pyruvate dehydrogenase; VO2max, maximal rate of oxygen consumption*

Volume 00, Number 00, Month 2017, Pages 00–00

Address correspondence to: Maria Adeva-Andany, Hospital Juan Cardona, Pardo Bazan s/n, Ferrol 15406, Spain.
E-mail: madevaa@yahoo.com

Received 17 March 2017; Accepted 24 May 2017
DOI 10.1002/iub.1646
Published online 00 Month 2017 in Wiley Online Library (wileyonlinelibrary.com)

Introduction

In humans, L-carnitine is a compound facilitating transfer of acyl groups that operates at the interface between fatty acid metabolism and carbohydrate metabolism promoting fatty acid oxidation and nonoxidative glucose disposal.

Acyl-coenzyme A synthetases are a family of enzymes that catalyze the thioesterification of acyl groups and coenzyme A (CoA) to form acyl-CoA esters. This reaction is required for fatty acids to participate in metabolic pathways (Fig. 1). Carnitine acyltransferases are a family of enzymes that catalyze the reversible transfer of acyl groups between coenzyme A and L-carnitine, converting acyl-CoA esters into acyl-carnitine esters (Fig. 2). Acyl-carnitine esters represent a "dormant" pool of

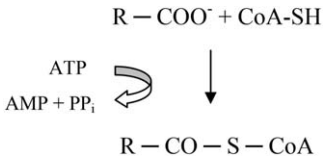

FIG 1 *Basic acyl-coenzyme A synthetases reaction.*

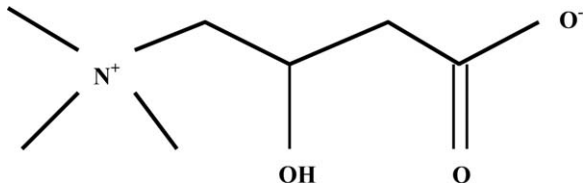

FIG 3 *L-Carnitine.*

acyl groups that may be used in biochemical pathways upon their conversion back into acyl-CoA esters by carnitine acyltransferases. Therefore, L-carnitine and carnitine acyltransferases modulate the intracellular level of "active" acyl groups. For instance, when excess acetyl-CoA is generated inside the mitochondrial matrix, L-carnitine buffers the accumulation of acetyl groups by forming acetyl-carnitine. The formation of acyl-carnitine derivatives allows the transport of acyl groups across cell membranes and their excretion in urine. L-Carnitine and carnitine acyltransferases participate in the oxidation of fatty acids by transporting acyl groups between cell organelles and into the mitochondrial matrix, where β-oxidation occurs. In addition, L-carnitine enhances nonoxidative glucose metabolism and improves slightly glucose tolerance (1).

The IUPAC name for L-carnitine is (3*R*)-3-hydroxy-4-(trimethylazaniumyl)butanoate (Fig. 3). Initially named vitamin B_T, L-carnitine binds acyl groups by establishing ester bonds with carboxylic acids at its 3-hydroxyl position, serving as a carrier for fatty acids.

It has been estimated that the total L-carnitine content in the human body is about 300 mg/kg. Approximately 98% of L-carnitine is intracellular, existing either as free L-carnitine or as several species of acyl-carnitine esters, predominantly in the muscle and liver. The L-carnitine content (mmol) of extracellular fluid, liver, skeletal muscle and kidney is 0.5, 1.3, 127 and 0.2, respectively. The liver contains 500–1,000 nmol total L-carnitine per gram whereas the amount contained in the skeletal muscle is greater, 3,000–5,000 nmol total L-carnitine per gram (2,3).

Endogenous Synthesis and Exogenous Provision of L-Carnitine

L-Carnitine in humans is both endogenously synthesized and obtained through food ingestion. The biochemical pathway to the endogenous synthesis of L-carnitine in humans has not been well-characterized. L-Carnitine is synthesized from the substrate 6-*N*-trimethyl-lysine. Lysine residues in some proteins undergo *N*-methylation using *S*-adenosylmethionine as methyl donor, forming 6-*N*-trimethyl-lysine residues. It is generally assumed that 6-*N*-trimethyl-lysine is generated by degradation of proteins and converted to L-carnitine in four enzymatic steps, namely hydroxylation at carbon 3, aldol cleavage, oxidation of the aldehyde to 4-butyrobetaine and hydroxylation of 4-butyrobetaine at carbon 3 (Fig. 4) (4).

The enzyme trimethyl-lysine dioxygenase (trimethyl-lysine 3-hydroxylase) catalyzes the hydroxylation of trimethyl-lysine at carbon 3 to yield 3-hydroxy-trimethyl-lysine. During this reaction, 2-oxoglutarate (α-ketoglutarate) is converted into succinate, and carbon dioxide is released. The human gene encoding trimethyl-lysine dioxygenase (*TMLHE*) maps to Xp28,

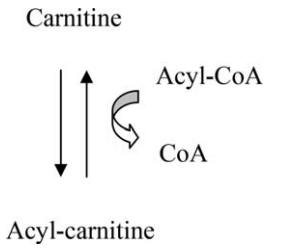

FIG 2 *Basic carnitine acyltransferases reaction.*

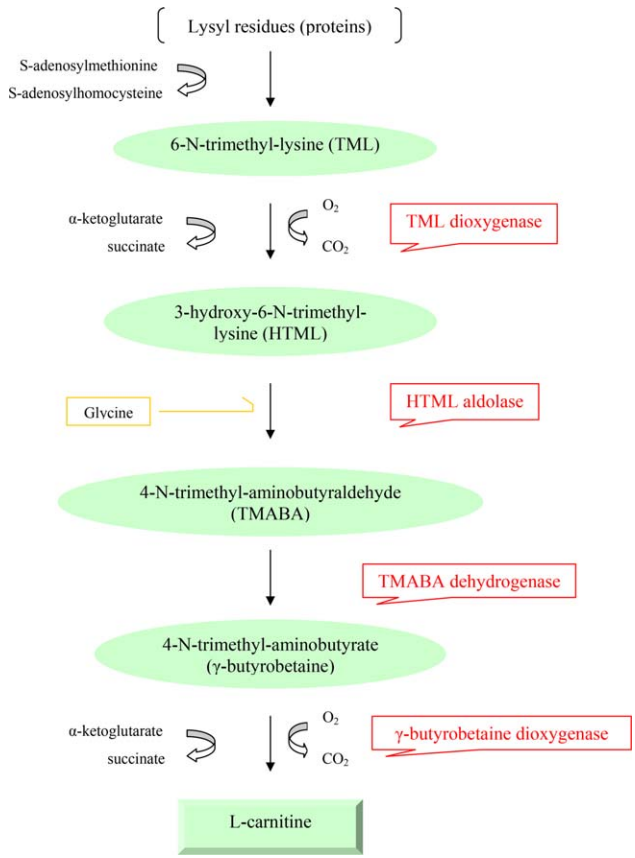

FIG 4 *Pathway of L-carnitine synthesis.*

and mutations in this gene have been found in patients with autism disorders, suggesting that the function of the protein is not well-known (5).

The second enzymatic step in the synthesis of L-carnitine is the aldolytic cleavage of 3-hydroxy-trimethyl-lysine between carbons 2 and 3 to yield glycine and 4-*N*-trimethyl-aminobutyraldehyde. The gene coding the aldolase that catalyzes this reaction has not been identified (4).

The third step in L-carnitine synthesis is assumed to be the dehydrogenation of the aldehyde 4-*N*-trimethyl-aminobutyrate to 4-butyrobetaine (4-*N*-trimethyl-aminobutyrate), but the human gene encoding the enzyme that catalyzes this step has not been identified, and no congenital deficiency has been documented (4).

The last enzymatic step is the hydroxylation of 4-butyrobetaine at carbon 3 by the enzyme 4-butyrobetaine dioxygenase (4-butyrobetaine hydroxylase) to yield L-carnitine. Like the first reaction of the pathway, 2-oxoglutarate (α-ketoglutarate) is converted into succinate, and carbon dioxide is released. The enzyme 4-butyrobetaine dioxygenase adds the hydroxyl group that L-carnitine uses to form ester bonds with acyl moieties. The gene *BBOX1* located at 11p14.2 encodes 4-butyrobetaine dioxygenase (2,3). A homozygous deletion containing the *BBOX1* gene has been reported in a girl with microcephaly, speech delay, growth retardation and facial anomalies (5).

Human tissue distribution and subcellular location of the human enzymes involved in the endogenous synthesis of L-carnitine have been barely investigated; therefore, the sites of L-carnitine synthesis have not been elucidated. In autopsy samples, 4-butyrobetaine dioxygenase activity is found in kidney, liver and brain, being absent from skeletal muscle and heart. The highest activity of 4-butyrobetaine dioxygenase in human tissues is detected in the kidney (4).

Besides endogenous synthesis, humans obtain L-carnitine through dietary food. It has been estimated that the average adult diet provides approximately 75% of daily L-carnitine requirement. The bioavailability of dietary L-carnitine ranges from 54% to 87% while the bioavailability of pharmacological doses of L-carnitine is 5–18%. Therefore, intestinal absorption of supplemental L-carnitine is less efficient than that of dietary L-carnitine (6). Intestinal absorption of L-carnitine occurs via passive and active mechanisms. Studies using human intestinal mucosa have detected passive diffusion of L-carnitine in both the small intestine and the colon and active transport in the duodenum and ileum (7).

The plasma level of total L-carnitine in healthy adults is in the range of 25–50 μM while the plasma level of acetyl-carnitine is 3–6 μM. Circulating L-carnitine filtered at the glomerulus is highly conserved by the kidney tubule, so that approximately 98–99% of the filtered load undergoes tubular reabsorption (6). The threshold concentration for tubular reabsorption is about 40–60 μM, and therefore the increase in the renal clearance of L-carnitine prevents from large elevations in plasma L-carnitine level after exogenous administration (8). The exogenous administration of L-carnitine either orally or intravenously has little effect on skeletal muscle L-carnitine content, but long-term L-carnitine supplementation associated with high carbohydrate ingestion increases slightly the skeletal muscle store of free L-carnitine in healthy subjects (9,10).

The Organic Cation Transporter-2

L-Carnitine enters the cells predominantly across the organic cation transporter-2 (OCTN2), located to the plasma membrane, although other transporters may also carry L-carnitine, including OCTN3, (11) ATB (0,+), (12) and carnitine transporter-2 (13). OCTN3 is expressed in human cultured skin fibroblasts, being likely localized to the peroxisomal membrane (11). ATB (0,+) is a sodium- and chloride-dependent amino acid transporter able to carry carnitine, propionyl-carnitine and acetyl-carnitine (12). Carnitine transporter-2 is specifically located in human testis (13).

OCTN2 is a high affinity, sodium-dependent, carnitine transporter. Human OCTN2 transcript is predominantly present in the intestine, kidney, heart and skeletal muscle, being also detected in the liver, central nervous system, breast and ovary (14). In the human heart, OCTN2 is expressed primarily in endothelial cells. In the human intestine, OCTN2 can be identified both in the brush-border membrane of the small intestine and in colonic epithelial cells. The expression of OCTN2 is increased in biopsy specimens of patients affected with inflammatory bowel disease (15). In vitro studies using human HepG2 cells show that the expression of the *OCTN2* gene is regulated by the transcription factor peroxisome proliferator-activated receptor-α (16). In addition to OCTN2, hepatocytes have a different low-affinity, sodium-independent carnitine transporter (17).

Human OCTN2 has a number of substrates, including free L-carnitine, acetyl-carnitine and some organic cations. The uptake of L-carnitine and acetyl-carnitine by OCTN2 is sodium-dependent with a stoichiometry 1:1. In contrast, OCTN2 transports organic cations without involving sodium. Acetyl-carnitine inhibits OCTN2-mediated L-carnitine transport (14).

Cationic drugs such as verapamil and valproate also inhibit OCTN2-mediated L-carnitine uptake in Caco-2 cells and human fibroblasts (15). Etoposide administration to cancer patients is associated with an excessive loss of L-carnitine in urine, suggesting that this drug may inhibit OCTN2 in the kidney tubule (18). In the human intestine, uptake of L-carnitine is primarily mediated by OCTN2 being inhibited by valproate and levofloxacin (15). Common variants of OCTN2 found in healthy populations may contribute to variation in the handling of L-carnitine and some drugs. OCTN2 haplotypes containing −207G have been associated with a gain of function of the transporter, but healthy subjects homozygous for −207G show no appreciable effect of this single nucleotide polymorphism on L-carnitine metabolism (19).

Primary L-Carnitine Deficiency

The gene encoding OCTN2 (*SLC22A5*) maps to 5q31.2–32. Primary L-carnitine deficiency is an autosomal recessive disorder

due to loss of function mutations in the *SLC22A5* gene (20,21) (Table 1). Reduced levels of mature OCTN2 on the plasma membrane induce defective uptake of L-carnitine into the cells and decreased intracellular L-carnitine concentration in the affected tissues, including liver, skeletal muscle and heart. Defective reabsorption of filtered L-carnitine in the kidney tubule leads to urinary L-carnitine wasting and low serum L-carnitine levels in patients with primary L-carnitine deficiency. Plasma concentration of acyl-carnitine esters is also low in patients with the disease (22). Primary L-carnitine deficiency usually presents early in life with hypoketotic hypoglycemic episodes or later in life with cardiomyopathy or skeletal myopathy. Episodes of hypoketotic hypoglycemia occur particularly during fasting and have been attributed to impaired fatty acid oxidation due to reduced intracellular L-carnitine concentration in the hepatocyte. Defective fatty acid oxidation in the liver may attenuate the capacity to glucose synthesis via the gluconeogenesis pathway (23). Skeletal myopathy and cardiomyopathy are more frequent in older patients. In skeletal muscle, impaired fatty acid oxidation induces intolerance to exercise with occasional episodes of rhabdomyolysis and myoglobinuria. Plasma level of creatine kinase at rest may be mildly elevated. Dilated cardiomyopathy is frequently observed in patients with primary L-carnitine deficiency, but hypertrophic cardiomyopathy has also been documented. The frequency of mutations in the *SLC22A5* gene is similar in patients with unselected cardiomyopathy and the general population. Therefore, heterozygosity for primary L-carnitine deficiency is an unlikely cause of unselected cardiomyopathy (24). There is lipid accumulation in the affected tissues including liver, heart and skeletal muscle. Hyperammonemia may develop in patients with primary L-carnitine deficiency (25). In some patients, the clinical onset of the disease occurs during adulthood. Primary L-carnitine deficiency has been diagnosed in women whose unaffected infants were identified with low free L-carnitine levels in plasma by newborn screening. Affected mothers had minimal or no symptoms at time of diagnosis (26). Although primary L-carnitine deficiency is considered an autosomal recessive disorder, heterozygotes may display clinical features similar to those occurring in the probands, suggesting that they may suffer a partial dysfunction of the OCTN2 transporter. They experience reduced L-carnitine reabsorption in the kidney resulting in urinary L-carnitine wasting and slightly diminished plasma L-carnitine level compared to control subjects. Heterozygotes for the *SLC22A5* gene may show heart involvement in adult life, including dilated cardiomyopathy and hypertrophic cardiomyopathy (21). L-Carnitine uptake in heterozygous fibroblasts shows intermediate values between normal subjects and patients with the disorder (27). Diagnosis of primary L-carnitine deficiency is confirmed by the measurement of diminished OCTN2 transporter activity in skin fibroblasts and mutational analysis of the *SCL22A5* gene. Patients with primary L-carnitine deficiency respond to L-carnitine supplementation, but their response in the affected tissues is blunted (7). After L-carnitine supplementation, the skeletal muscle remains carnitine-depleted whereas the hepatic carnitine store is nearly restored, due to the presence of different carnitine transporters (28).

In addition to primary L-carnitine deficiency, low plasma and tissue concentration of free L-carnitine may occur in other conditions (secondary L-carnitine deficiency). Congenital deficiency of enzymes involved in fatty acid oxidation causes accumulation of unoxidized acyl-CoA esters, which are converted into the respective acyl-carnitine esters, depleting the free L-carnitine pool and resulting in secondary free L-carnitine deficiency. Some congenital disorders of the metabolism of branched-chain amino acids may produce secondary L-carnitine deficiency due to a similar mechanism. Likewise, a ketogenic diet may induce mild hypocarnitinemia usually without clinical repercussion (3). Severe malnutrition leading to acquired L-carnitine deficiency and hyperammonemia has been reported. The dialytic loss of L-carnitine during chronic hemodialysis may contribute to a secondary deficiency (2,3). Antibiotics containing pivalate decrease blood L-carnitine level due to the formation of pivaloyl-carnitine ester, which is excreted in the urine. Zidovudine may cause L-carnitine deficiency due to unclear reasons (29). Medications that inhibit OCTN2 leading to secondary L-carnitine deficiency include levofloxacin, omeprazole, cefepime and etoposide (18). Valproate is a branched-chain fatty acid that may lower plasma free L-carnitine concentration likely by inducing the formation of acyl-carnitine derivatives (30).

L-Carnitine Acyltransferases

L-Carnitine acyltransferases catalyze the reversible transfer of acyl groups between coenzyme A and L-carnitine, converting acyl-CoA esters into acyl-carnitine esters. Human L-carnitine acyltransferases include carnitine acetyltransferase (CRAT), carnitine octanoyltransferase (CROT), carnitine palmitoyltransferase-1 (CPT1) and carnitine palmitoytransferase-2 (CPT2). Each one of them displays typical subcellular location and substrate preference for specific chain-length acyl-CoA esters. CRAT is present in the nucleus, peroxisomes and mitochondrial matrix and prefers short-chain acyl-CoA esters. CROT is a peroxisomal enzyme most active with medium-chain substrates. CPT1 and CPT2 favor longer substrates including medium- and long-chain fatty acids (Table 2). CPT1 is a transmembrane protein sited on the outer mitochondrial membrane while CPT2 is anchored from the matrix side on the inner mitochondrial membrane. During the transfer of acyl groups, the His 343 residue in the active site of carnitine acyltransferases acts as a general base to extract the proton from the 3-hydroxyl group of L-carnitine or the thiol group of CoA, depending on the direction of the reaction. This nucleophilic interaction leads to the formation of a tetrahedral intermediate anion that may be stabilized by the positive charge on the trimethylammonium group of L-carnitine (31).

Carnitine Acetyltransferase. Human CRAT catalyzes the reversible transfer of short-chain acyl groups between coenzyme A and L-carnitine. The X-ray structure of human CRAT has been reported, revealing a monomeric protein of two domains N and C, the active site of the enzyme being located at the interface

TABLE 1 ***Congenital deficiencies of proteins associated with L-carnitine metabolism***

	Organic cation transporter-2 deficiency	*Carnitinepalmitoyl transferase-1A deficiency*	*Carnitinepalmitoyl transferase-2 deficiency*	*Carnitine acylcarnitine translocase deficiency*
Usual age of presentation	Either early or later (skeletal muscle) in life	Infancy	Neonatal (usually lethal), infancy, adult	Neonatal period or early infancy
Metabolic features	Early onset: hypoketotic hypoglycemic episodes	Hypoketotic hypoglycemic episodes	In infancy, hypoketotic hypoglycemic episodes	Hypoketotic hypoglycemic episodes
Skeletal muscle	Late presentation: intolerance to exercise, rhabdomyolysis	Unusual myopathy	In adults, intolerance to exercise, rhabdomyolysis, unusual hypoglycaemia	Skeletal muscle weakness
Heart	Dilated or hypertrophic cardiomyopathy	Unusual cardiomyopathy, sudden death	In infancy, cardiomyopathy, sudden death	Dilated or hypertrophic cardiomyopathy
Plasma carnitine profile	Low free L-carnitine (C0) and acyl carnitines	Elevated ratio C0/C16+C18	Elevated acylcarnitines from C12 to C18	Low free L-carnitine, elevated long-chain acylcarnitines, dicarboxylic aciduria
Diagnosis	Reduced activity of the OCTN2 transporter Mutational analysis	Reduced palmitate oxidation Mutational analysis	Impaired palmitate oxidation during exercise Mutational analysis	Deficient CACT activity Mutational analysis
Therapy	L-Carnitine supplementation	Prevention of fasting, low fat high carbohydrate diet	Prevention of fasting, low fat high carbohydrate diet Bezafibrate?	Prevention of fasting, low fat high carbohydrate diet Consider L-carnitine supplementation

TABLE 2 *Carnitine acyltransferases and carnitine acylcarnitine translocase*

	Carnitine acetyl transferase	*Carnitine octanoyl transferase*	*Carnitine palmitoyl transferase-1 (CPT1)*	*Carnitine palmitoyl transferase 2*	*Carnitine acylcarnitine translocase*
Substrate profile	Short-chain and medium-chain acyl-coA esters	Medium-chain acyl-coA esters	Medium-chain and long-chain acyl-coA esters	Medium-chain and long-chain acyl-coA esters	Exchange of acyl-carnitine esters and L-carnitine across the inner mitochondrial membrane
Maximal activity	Propionyl-coA (C3-coA)	C4-coA to C10-coA esters	C12-coA ester	C10-coA to C14-coA esters	
Isoenzymes			CPT1A (liver) CPT1B (muscle) CPT1C (brain)		
Cellular location	Mitochondrion Peroxisome Nucleus	Peroxisome	Outer mitochondrial membrane Endoplasmic reticulum (CPT1C)	Inner mitochondrial membrane	Inner mitochondrial membrane
Main human tissue distribution	Liver Skeletal muscle Brain	Liver Brain Kidney	CPT1A: heart, liver and pancreas CPT1B: heart and skeletal muscle CPT1C: brain	Heart, liver, skeletal muscle and kidney	Undefined
Function	Export of acetyl groups from mitochondria Acetylation reactions Oxidation of phytanic acid	Oxidation of very long-chain fatty acids and phytanic acid	Oxidation of long-chain fatty acids, deoxyribonucleotides synthesis	Import of acyl-coA esters into the mitochondria Export of acyl-carnitine esters from mitochondria	Fatty acid axidation Export of medium-chain acyl-carnitine esters from mitochondria
Locus	9q34.1	7q21.1	CPT1A: 11q13.1-q13.5 CPT1B: 22q13.3-qter CPT1C: 19q13.33	1p32	3p21.31
Congenital deficiency	One case?	Not reported	CPT1A: autosomal recessive CPT1C: one family	Autosomal recessive	Autosomal recessive

between the two (32). CRAT has been purified from human liver. Valproate induces an eight-fold increase in CRAT activity (33). The relative amount of L-carnitine and acetyl-carnitine may be measured by ^{1}H-magnetic resonance spectroscopy (34).

CRAT is active with a number of substrates, including some fatty acids, ketone bodies, benzoate, salicylate and some intermediates of branched-chain amino acid catabolism whereas this enzyme has no activity with short-chain dicarboxylic acyl-CoA esters, including malonyl-CoA, methylmalonyl-CoA, succinyl-CoA and glutaryl-CoA.

Among fatty acids, CRAT is active toward short- (C2-C6) and medium-chain (C8-C10) acyl-CoA esters. The maximum activity is obtained with propionyl-CoA (C3-CoA). CRAT shows some activity with C12- and C14-CoAs, but no activity is observed with long-chain species such as C16-CoA. CRAT is necessary for the complete oxidation of pristanic acid, as peroxisomal β-oxidation of pristanic acid produces acetyl-CoA (C2-CoA) and propionyl-CoA (C3-CoA) that have to be converted to their respective L-carnitine esters (acetyl-carnitine and propionyl-carnitine) to be transported into the mitochondrial matrix for further oxidation. Acyl-CoA esters with the same chain length but with a *trans* double bond at the C2 position are poor substrates for CRAT showing a catalytic efficiency approximately 100-fold lower than the corresponding straight chain acyl-CoA (33).

The degradation pathways of branched-chain amino acids produce various branched-chain acyl-CoA intermediates. The branched-chain acyl-CoA esters have a much lower affinity towards CRAT than the straight-chain equivalents. A decrease in CRAT activity is observed with acyl-CoA esters in which a methyl group is located at the second position of the acyl unit, suggesting that a methyl group located at this position may interfere with the catalytic mechanism of CRAT, similarly to the double bond at the same position. Among intermediates of valine catabolism, CRAT is active toward isobutyryl-CoA and 3-hydroxy-isobutyryl-CoA, generating the corresponding acylcarnitines (isobutyryl-carnitine and 3-hydroxy-isobutyryl-carnitine). In the isoleucine degradation pathway, the highest CRAT activity is observed with 2-methyl-butyryl-CoA. Tiglyl-CoA and 2-methyl-3-hydroxy-butyryl-CoA are poor substrates for CRAT, but some conversion to the respective acylcarnitines may occur. In the leucine catabolism pathway, isovaleryl-CoA is a good substrate for CRAT. The enzyme shows intermediate activity towards 3-methyl-crotonyl-CoA but none towards 3-hydroxy-3-methylglutaryl-CoA. Among ketone bodies, CRAT is active with acetoacetyl-CoA and 3-hydroxybutyryl-CoA (35). CRAT is active with salicylyl-CoA and benzoyl-CoA, derived from salicylate and benzoate, respectively (36).

In human liver, CRAT activity is detected both in mitochondria and in peroxisomes. Both mitochondrial and peroxisomal CRAT display identical substrate specificity profiles (33,37). CRAT activity has been identified in the nuclei from HEK293 cells (38).

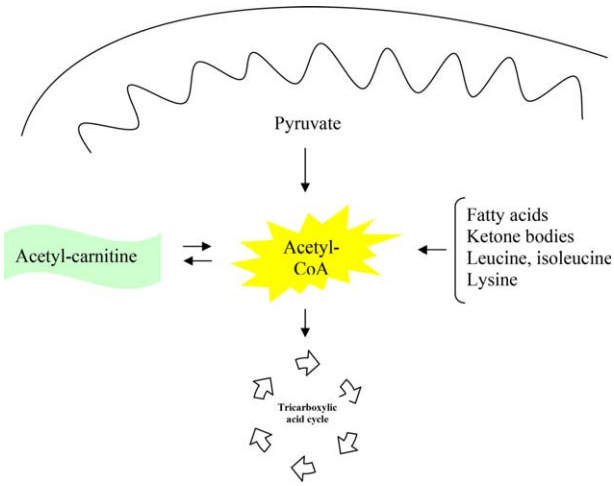

FIG 5 *Summary of acetyl-CoA metabolism.*

CRAT has been found in human liver, skeletal muscle, brain and fibroblasts (33,39). Skeletal muscle from trained athletes show more abundant CRAT protein compared to untrained subjects (40). In patients with type 2 diabetes, there is a higher transcriptional level of CRAT. Higher expression of CRAT may explain the elevated levels of acetyl-carnitine (C2-carnitine) observed in these patients (41).

The reversible conversion between acetyl-CoA esters and acetyl-carnitine esters by CRAT serves to several biological processes, including buffering of acetyl groups and acetylation reactions.

Acetyl-CoA is produced in the mitochondrial matrix primarily from fatty acid oxidation and pyruvate decarboxylation by the pyruvate dehydrogenase (PDH) complex. Ketone bodies and some amino acids (lysine, leucine and isoleucine) also generate acetyl-CoA (Fig. 5).

Conditions that increase acetyl-CoA production include enhanced fatty acid oxidation (fasting and diabetic ketoacidosis) and PDH activation (high-intensity exercise and high-carbohydrate diets). Acetyl-CoA from any source enters the tricarboxylic acid cycle to generate energy. When accelerated production of acetyl-CoA exceeds the capability of the tricarboxylic acid cycle to use it, CRAT transfers acetyl groups from acetyl-CoA to L-carnitine, releasing free coenzyme A (CoA-SH) and forming acetyl-carnitine. Free coenzyme A is required to maintain operative the PDH reaction and the functioning of the tricarboxylic acid cycle (9,38,39,42). In vitro studies with primary human skeletal myotubes show that CRAT can use acetyl-CoA originated from either glucose or fatty acids as substrate. Induction of CRAT activity in these myotubes leads to enhanced acetyl-carnitine efflux from the cells and increased PDH activity. Addition of fatty acids to the culture medium causes a pronounced shift in the carbon source of acetyl-carnitine in favor of fatty acids (43). Incubation of cultured primary human myocytes with increasing doses of long-chain fatty acids (oleate and palmitate) reduces intracellular free

carnitine concentration and results in a dose-dependent decrease in acetyl-carnitine formation. The exposure to excess long-chain fatty acids leads to accumulation of long-chain acyl-carnitines, depleting free carnitine level and thus inhibiting CRAT activity (44).

CRAT is necessary for esterification of acetyl groups. The formation of acetyl-carnitine allows the export of acetyl groups from the mitochondria into the nucleus, where they may be involved in histone acetylation reactions and epigenetic regulation.

Nuclear histone acetyltransferases catalyze the transfer of acetyl groups to lysine residues on histones using acetyl-CoA as substrate whereas histone deacetylases catalyze the removal of acetyl residues from histones. In the mitochondria, CRAT catalyzes the formation of acetyl-carnitine from acetyl-CoA. Acetyl-carnitine exits the mitochondria and enters the nucleus, where is reconverted to acetyl-CoA. Nuclear acetyl-CoA provides acetyl groups for acetylation reactions. In vitro studies using HEK293 cells and primary skin fibroblasts show that L-carnitine inclusion in the incubation increases histone acetylation, which is abolished when the transport of either L-carnitine or pyruvate across the mitochondrial membrane is inhibited. Thus, pyruvate-derived mitochondrial acetyl-CoA leaves mitochondria in the form of acetyl-carnitine and enters nucleus where it is converted to acetyl-CoA and becomes a substrate for histone acetyltransferase. Acetylation may also affect to non-histone proteins such as transcription factors and modulate their function (38).

Mitochondrial acetyl-CoA may be an indicator of nutrient excess signaling the storage of fat via lysine acetylation of some proteins. Excess mitochondrial acetyl-CoA is produced when an oversupply of fuel (predominantly glucose or fatty acids) enters the cell and mitochondrial oxidative capacity is saturated. The elevated concentration of acetyl-CoA may facilitate acetylation of lysine residues on mitochondrial enzymes, modulating their activity. Among the mitochondrial enzymes that might be controlled by lysine acetylation are acetyl-CoA synthetase 2, long-chain acyl-CoA dehydrogenase, 3-hydroxy-3-methylglutaryl-CoA synthetase-2 and ornithine transcarbamylase. Enzyme acetylation may inhibit fuel utilization when energy is in excess, favoring lipogenesis (45).

The gene encoding CRAT, *CRAT*, has been mapped to chromosome 9q34.11. Only one case of CRAT deficiency has been reported, being unclear whether the reduced CRAT activity is a primary genetic defect or a secondary acquired defect. The patient was a 3-year-old girl who presented with liver dysfunction and a progressive encephalopathy with episodes of ataxia associated with oculomotor palsy, hypotonia, mental confusion and disturbances of consciousness. The activity of CRAT was markedly decreased in cultured fibroblasts, liver, kidney and particularly in the brain (39).

Carnitine Octanoyltransferase. Human CROT is a peroxisomal enzyme that catalyzes the reversible transfer of medium-chain acyl groups between coenzyme A and L-carnitine. This enzyme has higher affinity with fatty acyl groups from C4 to C10.

Human peroxisomes are involved in the initial β-oxidation of very long-chain fatty acids and in the α-oxidation of phytanic acid, a branched-chain fatty acid present in the diet. Peroxisomal CROT is required to oxidize these fatty acids. Very long-chain fatty acids undergo initial β-oxidation in the peroxisomes to shorten their chain. The peroxisomal β-oxidation of very long-chain fatty acids leads to the formation of medium-chain acyl-CoA esters such as octanoyl-CoA (C8-CoA), which are exported from the peroxisomes and then imported into the mitochondrial matrix for further β-oxidation. The exit from the peroxisomes of these shortened fatty acids requires their conversion into their respective acyl-carnitines, which is accomplished by CROT. In vitro studies using HepG2 cells, a model of human hepatic cells, show that decreased CROT activity increases the amount of medium-chain saturated fatty acids while no major change is observed in C18 and C20 fatty acids. Conversely, an increase in CROT activity reduces medium-chain and very long-chain fatty acid levels in the cell (46). Phytanic acid is a branched-chain fatty acid that possesses a methyl group in the β position that blocks β-oxidation. Instead, phytanic acid undergoes α-oxidation in peroxisomes yielding pristanic acid. Peroxisomal β-oxidation of pristanic acid produces C11-CoA (4,8-dimethyl-nonanoyl-CoA), which is converted into C11-carnitine by CROT to exit the peroxisomes (37).

The gene encoding CROT (*CROT*) is located to 7q21.12. Human CROT mRNA is found in the brain, liver and kidney. Hepatic and kidney CROT transcripts represent 24% and 59% of the amount recovered in the brain, respectively (46).

Carnitine Palmitoyltransferase-1. Human CPT1 catalyzes the reversible transfer of acyl groups from specific chain-length acyl-CoA esters to L-carnitine to form acyl-carnitine esters in the outer mitochondrial membrane. Then, carnitine acyl-carnitine translocase transports acyl-carnitines through the inner membrane in exchange for free carnitine (Fig. 6).

The highest activity of human CPT1 has been observed with C12-CoA while there is virtually no activity toward acyl-CoA esters of chain length C20 or more (47). Decanoic acid (C10:0) may use CPT1 to access the mitochondrial matrix, although this fatty acid is also able to enter the mitochondrion by diffusion (48).

There are three isoforms of human CPT1, CPT1A (the liver isoform), CPT1B (the muscle isoform) and CPT1C (the brain isoform) (49). The brain isoform expressed in yeast does not display catalytic activity with common acyl-CoA esters that are substrates for both liver and muscle CPT1. The structure of the regulatory domain of CPT1C has been determined by nuclear magnetic resonance spectroscopy (50). In vitro studies using human HeLa cell lines show that the C-terminus of the muscle isoform of human CPT1 (CPT1B) is located on the cytosolic face of the outer mitochondrial membrane (51). The brain isoenzyme of CPT1 (CPT1C) localizes to the endoplasmic reticulum (52).

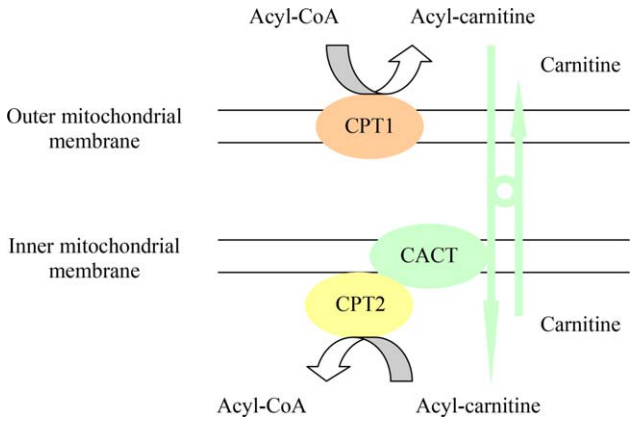

FIG 6 *Transfer of acyl groups by the carnitine system. Acyl groups are transferred from coenzyme A to carnitine by CPT1 on the outer mitochondrial membrane. CACT exchanges acyl-carnitine and carnitine across the inner mitochondrial membrane. In the mitochondrial matrix, acyl groups are transferred back to coenzyme A by CPT2.*

Northern blot analyses of mRNA from different human tissues reveal that the muscle isoform of CPT1 (CPT1B) shows a restricted distribution, being confined to skeletal muscle and heart. By contrast, the liver isoform (CPT1A) displays a wider distribution in the human body, being predominantly detected in liver, heart and pancreas, although other tissues such as endothelium, lung, skeletal muscle and kidney also contain significant amounts of CPT1A transcript. The level of CPT1A transcript is especially high in human pancreas (49). The brain isoform (CPT1C) is present in brain tissue with lower levels in testis and ovary (53). CPT1C is mainly expressed in motor neurons, which are thought to have a limited capacity for lipid β-oxidation in humans. This isoform may play a role in some human brain functions, such as feeding behavior (52).

Endurance training increases the activity of CPT1 in skeletal muscle of healthy individuals and obese patients, and this is associated with an increased rate of mitochondrial fatty acid oxidation (54,55). Proteomic analyses of mitochondria from skeletal muscle show that the protein abundance of the muscle isoform of CPT1 (CPT1B) is lower among obese insulin-resistant patients compared to lean insulin-sensitive individuals (55). In vitro studies using HepG2 cell lines show that daidzein and genistein, the most abundant isoflavones in soy, enhance the activity of the liver isoform of CPT1 (CPT1A). These isoflavones increase the mRNA expression of CPT1A, suggesting that they can upregulate CPT1A activity through upregulation of CPT1A transcription. L-carnitine also enhances CPT1A activity in HepG2 cells (56).

In vitro studies suggest that CPT1 may be inhibited by malonyl-CoA, which is produced in the cytosol from carboxylation of acetyl-CoA. The muscle isoform (CPT1B) is 30–100-fold more sensitive to malonyl-CoA inhibition than the liver isoform (CPT1A) (57). It has been speculated that variations in the amount of malonyl-CoA produced in skeletal muscle during exercise might influence CPT1 activity and fat oxidation. However, no substantial change in the concentration of malonyl-CoA during exercise of any duration at any power output has been demonstrated in human skeletal muscle and therefore malonyl-CoA cannot regulate CPT1 activity during exercise (42,58).

In vitro studies suggest that CPT1 activity may indirectly participate in cell proliferation. Oxidation of fatty acids produces acetyl-CoA that enters the tricarboxylic acid cycle, supplying carbons that are incorporated into aspartate, which is a nucleotide precursor required to synthesize DNA. The blockade of fatty acid oxidation via silencing of CPT1A reduces aspartate levels and compromises de novo DNA synthesis in human umbilical venous endothelial cells. Endothelial loss of CPT1A impairs proliferation of endothelial cells (59).

Three separate loci encode human isoenzymes of CPT1. The *CPT1B* gene encodes the muscle isoform of CPT1 (CPT1B) and maps to 22q13.33 (57). The gene *CPT1A* maps to 11q13.3 and encodes the liver isoenzyme of CPT1 (CPT1A) (60). The gene *CPT1C* encodes the brain isoform of CPT1 (CPT1C), being found on 19q13.33 in the human genome (53).

Epigenetic regulation may modulate the transcriptional activation of *CPT1A* and *CPT1B* genes, affecting the activity of CPT1. There is an association between DNA methylation in the *CPT1A* gene with very low density lipoprotein (VLDL) and low density lipoprotein (LDL) phenotypes. Increased methylation in the *CPT1A* gene is associated with decreased concentration of VLDL and small LDL particles. As smaller LDL particles are considered atherogenic, greater methylation of the *CPT1A* gene might be associated with a favorable lipid profile. DNA methylation marks in the *CPT1A* gene may be detected in peripheral blood, suggesting that such marks could serve as surrogate for methylation at other cells such as hepatocytes (61,62).

In a large healthy population of Caucasians adults, there is an association between variable methylation in *CPT1A* and multiple cardiovascular disease-related traits, including fasting concentration of triacylglycerols, VLDL-cholesterol, adiponectin, insulin and homeostasis model assessment-insulin resistance (HOMA-IR) (63). In primary skeletal muscle cultures from severely obese women, the expression of the *CPT1B* gene in response to lipid is blunted compared with their lean counterparts. This attenuated response is associated with changes in DNA methylation and histone acetylation. These epigenetic modifications might cause reduced expression of *CPT1B* in response to lipid contributing to the pathogenesis of severe obesity (64).

Congenital deficiency of CPT1. Congenital deficiency of the liver isoenzyme of CPT1 (CPT1A) is a rare disease identified in a few families. A mutation in the *CPT1C* gene causing a neurological disorder has been detected in one family. Disease-causing molecular defects in the *CPT1B* gene encoding the muscle isoform of CPT1 have not been reported.

Congenital deficiency of the liver isoenzyme of CPT1 (CPT 1A). Congenital deficiency of CPT1A is an autosomal recessive disorder of hepatic fatty acid oxidation that usually presents in infancy with episodes of hypoketotic hypoglycemia often triggered by fasting that may induce seizures and coma. Other clinical features include hepatomegaly and hyperammonemia. Elevated creatine kinase, cardiomegaly and arrhythmia indicate that muscle is also affected. Renal tubular acidosis suggesting kidney involvement may be present (47). During pregnancy with unaffected fetuses, women with CPT1A deficiency may experience liver disease associated with hemolysis and low platelets (65). CPT1A deficiency confers risk for sudden unexpected death in infancy. Alaska Native newborns show a high prevalence of the sequence variant c.1436C→T in the *CPT1A* gene that may contribute to explain the high infant mortality rate in this population. Children harboring this variant suffer an abnormal metabolic response to fasting. The increase in plasma free fatty acids in response to fasting is similar to that produced in healthy children, but the generation of ketone bodies is blunted (66). CPT1A deficiency is detectable through newborn screening, but not all infants are identified using the standard cut-off values (65). Analysis of free L-carnitine and acyl-carnitines profile in plasma and dried blood spots may be useful to diagnose CPT1A deficiency. The characteristic profile is elevated ratio of free L-carnitine (C0) to the sum of the C16 and C18 acyl-carnitines (C0/C16 + C18). Plasma concentration of free L-carnitine may be normal, and the level of long-chain acyl-carnitines is usually low. Baseline levels of total and free L-carnitine are normal in children homozygous for the c.1436C→T sequence variant in the *CPT1A* gene, but the ratio C0/C16 + C18 is elevated, reflecting CPT1A deficiency (66,67). Whole body palmitate oxidation is markedly reduced (less than 10% of normal) in patients affected with CPT1A deficiency. The diagnosis may be confirmed by sequence analysis of the *CPT1A* gene. Treatment for CPT1A deficiency is based on avoidance of fasting to prevent hypoglycemia and a high-carbohydrate, low-fat diet. Supplementation with medium-chain triglycerides is controversial, as CPT1A is active with medium-chain length substrates (47).

Congenital deficiency of the brain isoenzyme of CPT1 (CPT 1C). A disease-causing mutation in the *CPT1C* gene has been found in a family affected with a novel form of pure autosomal dominant hereditary spastic paraplegia. On overexpression in cells, the mutation reduces the average number and size of lipid droplets (52).

Carnitine Palmitoyltransferase-2. Human CPT2 catalyzes the reversible transfer of acyl groups from specific chain length acyl-CoA esters to L-carnitine to produce the respective acyl-carnitine esters on the matrix side of the inner mitochondrial membrane. CPT2 is a homotetrameric protein (68).

Like CPT1, CPT2 is active with medium-chain (C8–C12) and long-chain (C14–C20) acyl-CoA esters whereas no activity is found with short- and very long-chain acyl-CoA esters. Preferred substrates for CPT2 range from C10- to C14-CoA esters (69). Acyl-CoA esters with the same chain length but with a *trans* double bond at the C2 position (*trans*-2 enoyl-CoA intermediates) are poor substrates for CPT2. These intermediates are also poor substrates for CRAT, suggesting that the *trans*-2 double bond may interfere with the catalytic mechanism of carnitine acyltransferases (37). CPT2 is not active with branched-chain amino acids oxidation intermediates (69).

Human tissue distribution of CPT2 has been barely investigated (49). A case of neonatal CPT2 deficiency showed deficiency of the enzyme activity in heart, liver, skeletal muscle and kidney (70). In vitro studies using fibroblast cell lines show that L-aminocarnitine, an L-carnitine analog, inhibits CPT2 but not CPT1 (71).

Congenital deficiency of CPT2. Human *CPT2* gene has been assigned to human chromosome 1p32.3. Congenital deficiency of CPT2 is more frequent than CPT1A deficiency, being a relatively common disorder of fatty acid oxidation (60). Although it is an autosomal recessive disease, cases of symptomatic carriers have been reported, suggesting that single CPT2 gene mutations may exert a dominant-negative effect on the CPT2 protein (68). Congenital deficiency of CPT2 may cause three clinical phenotypes according to the age of presentation. The neonatal form of the disease is rare, being usually lethal during the first month of life. Patients suffer dysmorphic features, cardiomyopathy, microcephaly and cystic displasia of the brain and kidney. At autopsy, diffuse lipid accumulation is found (70). Clinical presentation of CPT2 deficiency in infants is severe and includes cardiomyopathy and episodes of hypoketotic hypoglycemia. Sudden death has been reported. It has been suggested that the F352C variant of *CPT2* could be a genetic risk factor for sudden death in infancy (72). The adult form of CPT2 deficiency is called the myopathic form, being characterized by recurrent episodes of muscle pain, rhabdomyolysis and myoglobinuria triggered by prolonged exercise. The adult form is more frequent than the other two being a common cause of rhabdomyolysis and myoglobinuria in adults. Skeletal muscle biopsy reveals lipid accumulation and reduced CPT2 activity (68). The adult type of CPT2 deficiency may rarely present with severe hypoglycemia (73). In patients with congenital deficiency of CPT2, oxidation of palmitate is normal at rest but severely impaired during prolonged low-intensity exercise. Carriers who display symptoms of CPT2 deficiency have a fatty acid oxidation defect comparable with the patients (68). The capacity to oxidize medium-chain fatty acids in the mitochondrial network is partially preserved in patients with CPT2 deficiency, likely due to CPT2-independent uptake. After medium-chain triglyceride loading, patients show dicarboxylic aciduria, suggesting that extramitochondrial pathways of fatty acid oxidation are used when the limited capacity of the CPT2-independent uptake is exceeded, leading to the production of dicarboxylic acids (47). In patients with CPT2 deficiency, the enzyme activity in fibroblasts is approximately 19% of

normal whereas the activity of CPT2 is 58% of normal in carriers with single CPT2 gene mutations. Fibroblasts from patients with severe manifestations of CPT2 deficiency generally show very low levels of residual enzyme activity whereas appreciable activity is detected in fibroblasts from patients with mild manifestations. Molecular analysis detects mutations in the CPT2 gene (68). Patients with CPT2 deficiency should follow a carbohydrate-rich diet avoiding prolonged fasting. Medium-chain triglycerides supplementation should not be recommended, as medium-chain fatty acids are substrates for the enzyme (47). Bezafibrate normalizes palmitate and myristate oxidation rates in cultured skin fibroblasts from patients with mild CPT2 deficiency and significant residual enzyme activity, but the drug does not correct fatty acid oxidation in fibroblasts from patients with severe phenotype. In a pilot trial conducted in six patients with the myopathic form of CPT2 deficiency, bezafibrate improves the oxidation rates of palmitate, being associated with a decline in muscular pain and an increase in physical activity (74).

Carnitine-Acylcarnitine Translocase

Carnitine acyl-carnitine translocase (CACT) or carnitine acyl-carnitine carrier is located to the inner mitochondrial membrane and transports acyl-carnitine esters across the membrane in exchange for free L-carnitine that exits from the mitochondrial matrix (75). Its primary structure has been reported (76). CACT is needed for oxidation of medium- and long-chain saturated fatty acids. Fatty acid oxidation studies in a patient with CACT deficiency show deficient oxidation of saturated fatty acids at all chain lengths from C10:0 to C24:0 with partially reduced oxidation of C26:0 fatty acid, suggesting that CACT is required for the mitochondrial transport of these medium- and long-chain fatty acyl moieties. The oxidation of short-chain and medium-chain fatty acids with less than 10 carbons is normal, indicating that these fatty acids do not require CACT to be imported into the mitochondria (77).

In human skin fibroblasts from patients with a defect in CACT, oxidation of pristanic acid is reduced to about 30% of control levels, suggesting that complete oxidation of pristanate requires the participation of CACT (78). In vitro studies using human fibroblast cell lines reveal that CACT is required for the mitochondrial export of medium-chain acyl-carnitines (C8-carnitine) (48). Supply of acetyl groups for nuclear histone acetylation requires the activity of CACT, as histone acetylation is reduced in skin fibroblasts from a patient with CACT deficiency compared to normal subjects (38).

Congenital CACT Deficiency. The CACT gene (*SLC25A20*) has been assigned to human chromosome 3p21.31, and its exon–intron structure has been described. Congenital CACT deficiency is an autosomal recessive disease with high mortality rate that usually presents in the neonatal period or early infancy. Typical clinical features include severe fasting-induced hypoketotic hypoglycemia, myopathy with muscle weakness and high plasma creatine kinase, cardiac involvement with hypertrophic or dilated cardiomyopathy, auriculo-ventricular block, arrhythmia and hyperammonemia. Liver dysfunction and hepatomegaly may occur. Increased serum lactate levels have been reported. Some patients present with sudden cardiac arrest and death in the neonatal or infantile period. Pre-eclampsia may occur in the mother of affected patients (77,79,80). Post-mortem examination of patients with CACT deficiency reveals fatty infiltration of the liver, kidney, heart and skeletal muscle. Histological examination confirms extensive lipid infiltration of these tissues. Stainable glycogen could not be demonstrated in liver, heart or skeletal muscles in one patient with CACT deficiency (77,80). Patients with CACT deficiency show low plasma level of free L-carnitine, marked elevation of the long-chain acyl-carnitines and dicarboxylic aciduria. Approximately 92% of the total carnitine in plasma is acyl-carnitine. The species of acyl-carnitine elevated in plasma are C16:0, C18:1 and C18:2. Dicarboxylic aciduria affects C6-C10 fatty acids (79). CACT activity is deficient in cultured fibroblasts from patients with CACT deficiency. An elevated level of palmitoyl-carnitine is present in these cells (80). Definitive diagnosis of CACT deficiency by molecular analysis of the *SLC25A20* gene should be performed. CACT deficiency has a broad mutation spectrum, molecular changes being distributed along the entire gene without a hot spot. Therapy includes avoidance of fasting with frequent carbohydrate-rich meals, restriction of long-chain fatty acids in the diet, and likely supplementation with L-carnitine and medium-chain triglycerides. Bezafibrate has been used in one patient with no success (79).

L-Carnitine and Glucose Metabolism

L-Carnitine administration improves insulin-stimulated glucose disposal, suggesting that this metabolite functions at the interface between fatty acid metabolism and glucose metabolism. Conditions associated with insulin deficiency or insulin resistance show abnormal levels of plasma L-carnitine.

Plasma L-Carnitine Concentration in Diabetes and Fasting. The plasma level of free L-carnitine and acyl-carnitine esters is altered in situations associated with accelerated fatty acid oxidation, including diabetes mellitus and fasting.

In patients with diabetic ketoacidosis, there is a striking change in the distribution of plasma free L-carnitine and acyl-carnitine esters. The plasma level of free L-carnitine is markedly reduced while the plasma concentration of acyl-carnitine esters, particularly short-chain acyl-carnitine species, is elevated compared to healthy controls. This abnormal distribution is promptly returned to normal by insulin therapy (81,82). Acetyl-carnitine (C2-carnitine) accounts for a major fraction of the acyl-carnitine species generated during diabetic ketoacidosis (83). Insulin deficiency promotes adipose lypolysis and fatty acid oxidation, increasing the formation of acyl-carnitine esters and depleting the pool of free L-carnitine.

In patients with type 2 diabetes, fasting plasma concentration of total L-carnitine (free L-carnitine + acyl-carnitine esters) is similar to healthy controls. However, the ratio of acyl-carnitine esters to free L-carnitine is increased (0.6 vs. 0.3), suggesting that the plasma level of free L-carnitine is lower

while plasma concentration of acyl-carnitine esters is higher in diabetic patients compared to healthy controls (84). Consistently, fasting plasma concentration of acyl-carnitine esters has been found elevated in patients with type 2 diabetes (85,86). Acetyl-carnitine (C2-carnitine), C6-carnitine, C8-carnitine and C10-carnitine represent the most abundant acyl-carnitine species in African American women with type 2 diabetes compared with nondiabetic controls (85). Similarly, fasting plasma concentration of C4-carnitine, C6-carnitine and C8-carnitine is elevated in another population of patients with type 2 diabetes relative to healthy controls. Fasting levels of plasma long-chain acyl-carnitine species (C14:1-carnitine, C16-carnitine and C18-carnitine) are also increased (86). Fasting plasma concentration of propionyl-carnitine (C3-carnitine) has been found lower (85) and higher (86) in type 2 diabetes patients relative to healthy controls.

Insulin infusion reduces plasma concentration of acyl-carnitine esters in both healthy subjects and type 2 diabetic patients, but this reduction is blunted in type 2 diabetes so that the decrease in plasma level of acyl-carnitine esters is less pronounced than that observed in healthy controls (86).

In a cross-sectional analysis, patients with impaired glucose tolerance show elevated fasting serum acetyl-carnitine (C2-carnitine) concentration as compared to those with normal glucose tolerance. However, baseline plasma level of C2-carnitine does not predict the risk of developing type 2 diabetes in prospective analysis (41). In a case–control study, plasma level of total and free L-carnitine is lower in neonates who later develop type 1 diabetes compared to control neonates (87).

Similarly to diabetic ketoacidosis, fasting is associated with a marked reduction in the plasma level of free L-carnitine while the plasma concentration of acyl-carnitine esters increases, particularly acetyl-carnitine (88,89). Serum long-chain acyl-carnitine esters also increase during fasting but to a lesser extent than the short-chain acyl-carnitine species (83,89). Refeeding corrects the abnormal pattern of L-carnitine and acyl-carnitine esters associated with fasting (89). The elevation of acetyl-carnitine (C2-carnitine) triggered by fasting is blunted in obese individuals, so that the magnitude of the increase is greater in normal-weight subjects compared to obese during the same period of fasting (83). In lean humans, plasma long-chain acyl-carnitine esters level is higher after 62 h of fasting compared with 14 h of fasting. However, skeletal muscle content of long-chain acyl-carnitine esters is similar, suggesting that the liver is a likely source of plasma long-chain acyl-carnitine species (90).

A few studies have analyzed plasma concentration of L-carnitine and acyl-carnitine esters in obesity with inconsistent results. In obese individuals, fasting levels of plasma long-chain acyl-carnitine species are increased relative to lean subjects (86). Plasma concentration of short-chain acyl-carnitine esters (C2-carnitine, C4-carnitine and C6-carnitine) is similar in obese and lean subjects (86,89). Plasma level of free L-carnitine is similar in obese and normal-weight subjects (89). In obese subjects, the plasma level of acyl-carnitine esters increase after weight loss achieved with calorie restriction compared to baseline values (91,92). However, this elevation is not observed when weight loss is achieved with calorie restriction plus exercise (92). Plasma total L-carnitine level is lower in lean and obese women with polycystic ovary syndrome, a condition frequently associated with insulin resistance, compared to healthy controls (93,94).

Effect of L-Carnitine on Glucose Metabolism in Healthy Subjects and Type 2 Diabetes. L-Carnitine administration is effective at improving insulin-mediated glucose disposal and storage both in healthy subjects and type 2 diabetic patients. L-Carnitine infusion to healthy subjects attenuates the increase in plasma glucose level induced by a glucose solution (95,96). L-Carnitine administration stimulates nonoxidative glucose disposal in healthy volunteers. During a euglycemic hyperinsulinemic clamp, L-carnitine increases from 8% to 17% the whole-body glucose disposal rate owing to an increase in nonoxidative metabolism (9,97). Chronic administration of L-carnitine prevents the increase in body fat associated with high ingestion of carbohydrates. Healthy subjects supplemented with carbohydrate without L-carnitine endure a 1.9 kg increase in body mass accounted for by an increase of 1.8 kg in body fat mass. Simultaneous administration of L-carnitine with carbohydrate prevents the increase in body fat (10).

There is a correlation between L-carnitine concentration and glycogen content in skeletal muscle from healthy subjects. A 15% increase in L-carnitine content is associated with a 30% increase in glycogen content, suggesting that an increase in muscle L-carnitine content may divert glucose uptake toward glycogen storage in healthy subjects (98). Chronic administration (6 months) of oral acetyl-carnitine improves glucose tolerance among nondiabetic individuals with baseline glucose disposal rate ≤ 7.9 mg/kg/min. However, this treatment has no appreciable effect on subjects with higher baseline glucose disposal rate (>7.9). The mechanisms mediating the differential effect of acetyl-carnitine according to the degree of glucose disposal are unclear (99,100). A meta-analysis of randomized controlled trials analyzing the effect of L-carnitine on weight loss might suggest that L-carnitine supplementation results in weight loss in adults (101,102).

In patients with type 2 diabetes, the improvement on glucose disposal induced by L-carnitine is similar to that observed in healthy individuals. L-Carnitine infusion enhances whole body glucose utilization compared to saline infusion. The improvement of total glucose metabolism is accounted for by an increase in nonoxidative glucose disposal and glycogen store in skeletal muscle increases during L-carnitine infusion (97). Short-term (10 days) and prolonged (6 months) oral administration of L-carnitine to insulin-resistant patients reduces plasma insulin level and HOMA-IR, suggesting that oral L-carnitine supplementation improves insulin resistance (43,103). In patients with type 2 diabetes, infusion of acetyl-carnitine increases whole-body glucose storage compared to placebo (104).

Similarly, in patients with mildly impaired glucose tolerance (fasting blood glucose 100–125 mg/dl), administration of acetyl-carnitine results in slight improvement in HOMA-IR (105).

In patients with nonalcoholic hepatic steatosis, oral L-carnitine supplementation for 6 months reduces plasma insulin concentration and improves HOMA-IR, compared to control subjects. The association of L-carnitine to metformin in patients with impaired glucose metabolism and nonalcoholic fatty liver improves serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) compared with metformin alone (106).

L-Carnitine and Exercise

Human skeletal muscle oxidizes glucose and fatty acids to generate ATP during physical exercise. These metabolites are derived from plasma and from intramyocellular stores of glycogen and triacylglycerol. The workload of the working muscles determines in part the fuel consumed. During short-lived exercise at high-intensity (above 75% of peak oxygen consumption), carbohydrates are the preferred fuel while fatty acids are favored as source of energy during prolonged low-intensity (below 50% of maximal oxygen consumption) physical activity. At moderate-intensity workload (between 50% and 75% of maximal rate of oxygen consumption), both glucose and fatty acids may be used, being a gradual increase in glucose over fat consumption with incremental exercise intensity. Accordingly, the respiratory exchange ratio rises in healthy subjects with increasing power output, being 0.84, 0.92 and >1 at 35%, 65% and 90% maximal rate of oxygen consumption (VO_{2max}), respectively, indicating a gradual change of energy source from fatty acids to glucose as the workload becomes more intense. Exercise at 35% and 65% VO_{2max} is associated with a larger energy contribution from fat oxidation (58). The rate of fat oxidation increases from rest to exercise at 60% and 85% VO_{2max} while decreasing at 100% VO_{2max}. By contrast, carbohydrate oxidation increases progressively from rest to exercise at 60%, 85% and 100% VO_{2max} (107).

Glucose and fatty acids availability may modify the fuel consumed by skeletal muscle at rest and during exercise. During exercise at 65% of peak oxygen consumption, glucose oxidation increases as the amount of dietary carbohydrate increases. Reciprocally, fat oxidation is lower on high-carbohydrate diet compared to low-carbohydrate diet. When less glucose is available (low-carbohydrate diet), glucose use decreases whereas fat consumption increases (42). Likewise, augmenting the provision of fatty acids to skeletal muscle with a lipid infusion leads to glycogen sparing during low-intensity (40%), moderate-intensity (65%) and high-intensity (85% maximal oxygen uptake) exercise in healthy subjects, indicating greater consumption of the available fatty acids over glucose (108).

Training increases the capability to store glucose (glycogen) and fat (triacylglycerols) inside the skeletal muscle. Fuel store in skeletal muscle (glycogen and triacylglycerols) represents a reserve of energy source to be used predominantly during high-intensity exercise. In trained subjects, adipose lipolysis is high whereas little intramuscular lipolysis occurs during low-intensity exercise compared with moderate- and high-intensity exercise. Lipolysis of intramuscular triacylglycerol store is stimulated only at higher intensities (109).

At rest, approximately 77% of L-carnitine present in human skeletal muscle is free L-carnitine. Physical activity, particularly high-intensity exercise, increases the production of acyl-carnitine esters, predominantly acetyl-carnitine, in healthy subjects. Short-chain acyl-carnitine concentration in skeletal muscle increases from approximately 8% at rest to 39% after 30 min of high-intensity exercise. Free L-carnitine concentration is reduced. Plasma concentration of free L-carnitine and acyl-carnitine esters shows similar profile than that observed in skeletal muscle. With 30 min of high-intensity exercise, plasma short-chain and long-chain acyl-carnitine level increases by 46% and 23%, respectively (110). The increase in acetyl-carnitine (and free L-carnitine reduction) during exercise at 65% peak oxygen consumption is greater in subjects receiving a high-carbohydrate diet compared to individuals on low-carbohydrate diet. When the availability of glucose is higher (high-carbohydrate diet), more acetyl-CoA and acetyl-carnitine are produced in skeletal muscle during moderate-intensity exercise. Correspondingly, the decline in free carnitine concentration is greater among subjects on high-carbohydrate diet than in those on low-carbohydrate diet at the same workload (42).

The increase in acetyl-carnitine production during high-intensity exercise is not consistently modified by L-carnitine supplementation prior to exercise. Among healthy volunteers, L-carnitine supplementation versus placebo has no effect on fuel selection either at high-intensity or low-intensity workloads. Short-term L-carnitine supplementation neither enhances lipid oxidation during exercise at 70% VO_{2max} nor spares skeletal muscle glycogen during exercise of any intensity (42). However, long-term (24 weeks) L-carnitine supplementation in combination with carbohydrate reduces skeletal muscle glycogen utilization during low-intensity exercise (50% of maximal oxygen consumption), suggesting an increase in lipid utilization (10).

The concentration of acetyl-carnitine (C2-carnitine) in skeletal muscle determined by proton magnetic resonance spectroscopy correlates with insulin sensitivity in endurance-trained athletes, lean and obese sedentary subjects and type 2 diabetic patients (111). In patients with type 1 diabetes mellitus postexercise levels of skeletal muscle acetyl-carnitine measured by proton magnetic resonance spectroscopy are higher than pre-exercise values. In addition, the increase in intramyocellular acetyl-carnitine post-exercise is greater in euglycemia as compared to hyperglycemia whereas pre-exercise concentration of skeletal muscle acetyl-carnitine does not differ according to the glycemic level (112).

L-Carnitine and Blood Ammonium Concentration

Hyperammonemia has been documented in primary L-carnitine deficiency and some conditions associated with secondary

L-carnitine deficiency such as malnutrition and valproate administration. Hyperammonemia also occurs among patients with congenital deficiency of CPT1A and CACT. The occurrence of hyperammonemia in these conditions suggest a link between metabolism of fatty acids, glucose and amino acids, but the mechanism underlying the increase in plasma ammonium concentration associated with these conditions is unclear. In the mitochondrial matrix of hepatocytes, acetyl-CoA is required to synthesize urea. The enzyme *N*-acetylglutamate synthase generates *N*-acetylglutamate from acetyl-CoA and glutamate. *N*-Acetylglutamate activates the enzyme carbamoylphosphate synthetase-1 that combines bicarbonate and ammonia to produce carbamoylphosphate initiating the urea cycle. Impaired mitochondrial fatty acid oxidation due to deficiency of L-carnitine, CPTA1 or CACT might reduce acetyl-CoA production, inhibiting N-acetylglutamate synthesis. Shortage of *N*-acetylglutamate may induce hyperammonemia by inhibiting the urea cycle (25).

Summary

L-Carnitine receives fatty acids (acyl groups) from acyl-CoA esters to create a reservoir of acyl-carnitine esters that can be used in metabolic reactions by being converted back into acyl-CoA esters. In addition, L-carnitine facilitates the transport of acyl groups across cell membranes. Humans may synthesize L-carnitine from trimethyl-lysine and obtain it via dietary food. Most L-carnitine is intracellular, being present predominantly in skeletal muscle, heart, liver and kidney. In muscle, intestine and kidney, L-carnitine enters the cells predominantly across the organic cation transporter-2. In hepatocytes, a sodium-independent L-carnitine transport is also operative. Carnitine acyltransferases catalyze the reversible transfer of acyl groups between coenzyme A and L-carnitine. Among them, carnitine acetyltransferase is active toward short- and medium-chain acyl-CoA esters, being located to the mitochondrial matrix, nucleus and peroxixomes. Carnitine octanoyltransferase is a peroxisomal enzyme active with medium-chain length substrates. Carnitine palmitoyltransferase-1 is a transmembrane protein located to the outer mitochondrial membrane while carnitine palmitoytransferase-2 lies on the matrix side of the inner mitochondrial membrane. Both favor longer substrates including medium- and long-chain fatty acids and allow the transport of these fatty acids across the mitochondrial membrane to be oxidized. Carnitine acyl-carnitine translocase transports acyl-carnitine esters across the inner mitochondrial membrane in exchange for free L-carnitine that exits the mitochondrial matrix, being required for oxidation of medium- and long-chain saturated fatty acids. L-Carnitine improves whole-body glucose disposal by enhancing nonoxidative glucose metabolism in both healthy subjects and patients with type 2 diabetes. In addition, chronic administration of L-carnitine prevents the increase in body fat associated with high ingestion of carbohydrates. The production of acyl-carnitine esters, predominantly acetyl-carnitine, is markedly increased during diabetic ketoacidosis, fasting and physical activity, particularly high-intensity exercise.

Acknowledgement

We are very grateful to Ms. Gema Souto for her help writing this manuscript.

References

[1] Lee, B. J., Lin, J. S., Lin, Y. C., and Lin, P. T. (2016) Effects of l-carnitine supplementation on lipid profiles in patients with coronary artery disease. Lipids Health Dis. 15, 107.

[2] Brass, E. P. (1995) Pharmacokinetic considerations for the therapeutic use of carnitine in hemodialysis patients. Clin. Ther. 17, 176–185. discussion 5.

[3] Scaglia, F., and Longo, N. (1999) Primary and secondary alterations of neonatal carnitine metabolism. Semin. Perinatol. 23, 152–161.

[4] Rebouche, C. J., and Engel, A. G. (1980) Tissue distribution of carnitine biosynthetic enzymes in man. Biochim. Biophys. Acta 630, 22–29.

[5] El-Hattab, A. W., and Scaglia, F. (2015) Disorders of carnitine biosynthesis and transport. Mol. Genet. Metab. 116, 107–112.

[6] Evans, A. M., and Fornasini, G. (2003) Pharmacokinetics of L-carnitine. Clin. Pharmacokinet. 42, 941–967.

[7] Hamilton, J. W., Li, B. U., Shug, A. L., and Olsen, W. A. (1986) Carnitine transport in human intestinal biopsy specimens. Demonstration of an active transport system. Gastroenterology 91, 10–16.

[8] Bain, M. A., Milne, R. W., and Evans, A. M. (2006) Disposition and metabolite kinetics of oral l-carnitine in humans. J. Clin. Pharmacol. 46, 1163–1170.

[9] Ferrannini, E., Buzzigoli, G., Bevilacqua, S., Boni, C., Del Chiaro, D., et al. (1988) Interaction of carnitine with insulin-stimulated glucose metabolism in humans. Am. J. Physiol. 255, E946–E952.

[10] Wall, B. T., Stephens, F. B., Constantin-Teodosiu, D., Marimuthu, K., Macdonald, I. A., et al. (2011) Chronic oral ingestion of L-carnitine and carbohydrate increases muscle carnitine content and alters muscle fuel metabolism during exercise in humans. J. Physiol. 589, 963–973.

[11] Lamhonwah, A. M., Ackerley, C. A., Tilups, A., Edwards, V. D., Wanders, R. J., et al. (2005) OCTN3 is a mammalian peroxisomal membrane carnitine transporter. Biochem. Biophys. Res. Commun. 338, 1966–1972.

[12] Nakanishi, T., Hatanaka, T., Huang, W., Prasad, P. D., Leibach, F. H., et al. (2001) Na+- and Cl–coupled active transport of carnitine by the amino acid transporter ATB(0,+) from mouse colon expressed in HRPE cells and Xenopus oocytes. J. Physiol. 532, 297–304.

[13] Enomoto, A., Wempe, M. F., Tsuchida, H., Shin, H. J., Cha, S. H., et al. (2002) Molecular identification of a novel carnitine transporter specific to human testis. Insights into the mechanism of carnitine recognition. J. Biol. Chem. 277, 36262–36271.

[14] Tamai, I., Ohashi, R., Nezu, J., Yabuuchi, H., Oku, A., Shimane, M., et al. (1998) Molecular and functional identification of sodium ion-dependent, high affinity human carnitine transporter OCTN2. J. Biol. Chem. 273, 20378–20382.

[15] Elimrani, I., Lahjouji, K., Seidman, E., Roy, M. J., Mitchell, G. A., et al. (2003) Expression and localization of organic cation/carnitine transporter OCTN2 in Caco-2 cells. Am. J. Physiol. Gastrointest. Liver Physiol. 284, G863–G871.

[16] Luo, H., Zhang, Y., Guo, H., Zhang, L., Li, X., Ringseis, R., et al. (2014) Transcriptional regulation of the human, porcine and bovine OCTN2 gene by PPARalpha via a conserved PPRE located in intron 1. BMC Genet. 15, 90.

[17] Wang, Y., Ye, J., Ganapathy, V., and Longo, N. (1999) Mutations in the organic cation/carnitine transporter OCTN2 in primary carnitine deficiency. Proc. Natl. Acad. Sci. USA 96, 2356–2360.

[18] Hu, C., Lancaster, C. S., Zuo, Z., Hu, S., Chen, Z., et al. (2012) Inhibition of OCTN2-mediated transport of carnitine by etoposide. Mol. Cancer Ther. 11, 921–929.

[19] Tahara, H., Yee, S. W., Urban, T. J., Hesselson, S., Castro, R. A., et al. (2009) Functional genetic variation in the basal promoter of the organic cation/carnitine transporters OCTN1 (SLC22A4) and OCTN2 (SLC22A5). J. Pharmacol. Exp. Ther. 329, 262–271.

[20] Nezu, J., Tamai, I., Oku, A., Ohashi, R., Yabuuchi, H., et al. (1999) Primary systemic carnitine deficiency is caused by mutations in a gene encoding sodium ion-dependent carnitine transporter. Nat. Genet. 21, 91–94.
[21] Koizumi, A., Nozaki, J., Ohura, T., Kayo, T., Wada, Y., et al. (1999) Genetic epidemiology of the carnitine transporter OCTN2 gene in a Japanese population and phenotypic characterization in Japanese pedigrees with primary systemic carnitine deficiency. Hum. Mol. Genet. 8, 2247–2254.
[22] de Boer, L., Kluijtmans, L. A., and Morava, E. (2013) Primary carnitine (OCTN2) deficiency without neonatal carnitine deficiency. JIMD Rep. 10, 39–40.
[23] Magoulas, P. L., and El-Hattab, A. W. (2012) Systemic primary carnitine deficiency: an overview of clinical manifestations, diagnosis, and management. Orphanet. J. Rare Dis. 7, 68.
[24] Amat di San Filippo, C., Taylor, M. R., Mestroni, L., Botto, L. D., and Longo, N. (2008) Cardiomyopathy and carnitine deficiency. Mol. Genet. Metab. 94, 162–166.
[25] Hwu, W. L., Chien, Y. H., Tang, N. L., Law, L. K., Lin, C. Y., et al. (2007) Deficiency of the carnitine transporter (OCTN2) with partial *N*-acetylglutamate synthase (NAGS) deficiency. J. Inherit. Metab. Dis. 30, 816.
[26] Schimmenti, L. A., Crombez, E. A., Schwahn, B. C., Heese, B. A., Wood, T. C., et al. (2007) Expanded newborn screening identifies maternal primary carnitine deficiency. Mol. Genet. Metab. 90, 441–445.
[27] Garavaglia, B., Uziel, G., Dworzak, F., Carrara, F., and DiDonato, S. (1991) Primary carnitine deficiency: heterozygote and intrafamilial phenotypic variation. Neurology 41, 1691–1693.
[28] Scaglia, F., Wang, Y., and Longo, N. (1999) Functional characterization of the carnitine transporter defective in primary carnitine deficiency. Arch. Biochem. Biophys. 364, 99–106.
[29] Ohashi, R., Tamai, I., Yabuuchi, H., Nezu, J. I., Oku, A., et al. (1999) Na(+)-dependent carnitine transport by organic cation transporter (OCTN2): its pharmacological and toxicological relevance. J. Pharmacol. Exp. Ther. 291, 778–784.
[30] Triggs, W. J., Bohan, T. P., Lin, S. N., and Willmore, L. J. (1990) Valproate-induced coma with ketosis and carnitine insufficiency. Arch. Neurol. 47, 1131–1133.
[31] Jogl, G., Hsiao, Y. S., and Tong, L. (2004) Structure and function of carnitine acyltransferases. Ann. N Y Acad. Sci. 1033, 17–29.
[32] Wu, D., Govindasamy, L., Lian, W., Gu, Y., Kukar, T., et al. (2003) Structure of human carnitine acetyltransferase. Molecular basis for fatty acyl transfer. J. Biol. Chem. 278, 13159–13165.
[33] Bloisi, W., Colombo, I., Garavaglia, B., Giardini, R., Finocchiaro, G., et al. (1990) Purification and properties of carnitine acetyltransferase from human liver. Eur. J. Biochem. 189, 539–546.
[34] Ren, J., Lakoski, S., Haller, R. G., Sherry, A. D., and Malloy, C. R. (2013) Dynamic monitoring of carnitine and acetylcarnitine in the trimethylamine signal after exercise in human skeletal muscle by 7T 1H-MRS. Magn. Reson. Med. 69, 7–17.
[35] Violante, S., Ijlst, L., Ruiter, J., Koster, J., van Lenthe, H., et al. (2013) Substrate specificity of human carnitine acetyltransferase: implications for fatty acid and branched-chain amino acid metabolism. Biochim. Biophys. Acta 1832, 773–779.
[36] Vessey, D. A., Chen, W. W., and Ramsay, R. R. (1991) Effect of carboxylic acid xenobiotics and their metabolites on the activity of carnitine acyltransferases. Biochem. J. 279, 895–897.
[37] Violante, S., Ijlst, L., Te Brinke, H., Koster, J., Tavares de Almeida, I., et al. (2013) Peroxisomes contribute to the acylcarnitine production when the carnitine shuttle is deficient. Biochim. Biophys. Acta 1831, 1467–1474.
[38] Madiraju, P., Pande, S. V., Prentki, M., and Madiraju, S. R. (2009) Mitochondrial acetylcarnitine provides acetyl groups for nuclear histone acetylation. Epigenetics 4, 399–403.
[39] DiDonato, S., Rimoldi, M., Moise, A., Bertagnoglio, B., and Uziel, G. (1979) Fatal ataxic encephalopathy and carnitine acetyltransferase deficiency: a functional defect of pyruvate oxidation?. Neurology 29, 1578–1583.
[40] Seiler, S. E., Koves, T. R., Gooding, J. R., Wong, K. E., Stevens, R. D., et al. (2015) Carnitine acetyltransferase mitigates metabolic inertia and muscle fatigue during exercise. Cell. Metab. 22, 65–76.
[41] Wang-Sattler, R., Yu, Z., Herder, C., Messias, A. C., Floegel, A., et al. (2012) Novel biomarkers for pre-diabetes identified by metabolomics. Mol. Syst. Biol. 8, 615.
[42] Roepstorff, C., Halberg, N., Hillig, T., Saha, A. K., Ruderman, N. B., et al. (2005) Malonyl-CoA and carnitine in regulation of fat oxidation in human skeletal muscle during exercise. Am. J. Physiol. Endocrinol. Metab. 288, E133–E142.
[43] Muoio, D. M., Noland, R. C., Kovalik, J. P., Seiler, S. E., Davies, M. N., et al. (2012) Muscle-specific deletion of carnitine acetyltransferase compromises glucose tolerance and metabolic flexibility. Cell. Metab. 15, 764–777.
[44] Seiler, S. E., Martin, O. J., Noland, R. C., Slentz, D. H., DeBalsi, K. L., et al. (2014) Obesity and lipid stress inhibit carnitine acetyltransferase activity. J. Lipid Res. 55, 635–644.
[45] Ghanta, S., Grossmann, R. E., and Brenner, C. (2013) Mitochondrial protein acetylation as a cell-intrinsic, evolutionary driver of fat storage: chemical and metabolic logic of acetyl-lysine modifications. Crit. Rev. Biochem. Mol. Biol. 48, 561–574.
[46] Le Borgne, F., Ben Mohamed, A., Logerot, M., Garnier, E., and Demarquoy, J. (2011) Changes in carnitine octanoyltransferase activity induce alteration in fatty acid metabolism. Biochem. Biophys. Res. Commun. 409, 699–704.
[47] Schaefer, J., Jackson, S., Taroni, F., Swift, P., and Turnbull, D. M. (1997) Characterisation of carnitine palmitoyltransferases in patients with a carnitine palmitoyltransferase deficiency: implications for diagnosis and therapy. J. Neurol. Neurosurg. Psychiatry 62, 169–176.
[48] Violante, S., Ijlst, L., Te Brinke, H., Tavares de Almeida, I., Wanders, R. J., et al. (2013) Carnitine palmitoyltransferase 2 and carnitine/acylcarnitine translocase are involved in the mitochondrial synthesis and export of acylcarnitines. FASEB J. 27, 2039–2044.
[49] Yamazaki, N., Shinohara, Y., Shima, A., Yamanaka, Y., and Terada, H. (1996) Isolation and characterization of cDNA and genomic clones encoding human muscle type carnitine palmitoyltransferase I. Biochim. Biophys. Acta 1307, 157–161.
[50] Samanta, S., Situ, A. J., and Ulmer, T. S. (2014) Structural characterization of the regulatory domain of brain carnitine palmitoyltransferase 1. Biopolymers 101, 398–405.
[51] van der Leij, F. R., Kram, A. M., Bartelds, B., Roelofsen, H., Smid, G. B., et al. (1999) Cytological evidence that the C-terminus of carnitine palmitoyltransferase I is on the cytosolic face of the mitochondrial outer membrane. Biochem. J. 341, 777–784.
[52] Rinaldi, C., Schmidt, T., Situ, A. J., Johnson, J. O., Lee, P. R., et al. (2015) Mutation in CPT1C associated with pure autosomal dominant spastic paraplegia. JAMA Neurol. 72, 561–570.
[53] Price, N., van der Leij, F., Jackson, V., Corstorphine, C., Thomson, R., et al. (2002) A novel brain-expressed protein related to carnitine palmitoyltransferase I. Genomics 80, 433–442.
[54] Berthon, P. M., Howlett, R. A., Heigenhauser, G. J., and Spriet, L. L. (1998) Human skeletal muscle carnitine palmitoyltransferase I activity determined in isolated intact mitochondria. J. Appl. Physiol. (1985) 85(1), 148–53.
[55] Lefort, N., Glancy, B., Bowen, B., Willis, W. T., Bailowitz, Z., et al. (2010) Increased reactive oxygen species production and lower abundance of complex I subunits and carnitine palmitoyltransferase 1B protein despite normal mitochondrial respiration in insulin-resistant human skeletal muscle. Diabetes 59, 2444–2452.
[56] Shin, E. S., Cho, S. Y., Lee, E. H., Lee, S. J., Chang, I. S., et al. (2006) Positive regulation of hepatic carnitine palmitoyl transferase 1A (CPT1A) activities by soy isoflavones and L-carnitine. Eur. J. Nutr. 45, 159–164.
[57] Zhu, H., Shi, J., de Vries, Y., Arvidson, D. N., Cregg, J. M., et al. (1997) Functional studies of yeast-expressed human heart muscle carnitine palmitoyltransferase I. Arch. Biochem. Biophys. 347, 53–61.
[58] Odland, L. M., Howlett, R. A., Heigenhauser, G. J., Hultman, E., and Spriet, L. L. (1998) Skeletal muscle malonyl-CoA content at the onset of exercise at varying power outputs in humans. Am. J. Physiol. 274, E1080–E1085.
[59] Schoors, S., Bruning, U., Missiaen, R., Queiroz, K. C., Borgers, G., et al. (2015) Fatty acid carbon is essential for dNTP synthesis in endothelial cells. Nature 520, 192–197.

[60] Gobin, S., Bonnefont, J. P., Prip-Buus, C., Mugnier, C., Ferrec, M., et al. (2002) Organization of the human liver carnitine palmitoyltransferase 1 gene (CPT1A) and identification of novel mutations in hypoketotic hypoglycaemia. Hum. Genet. 111, 179–189.
[61] Frazier-Wood, A. C., Aslibekyan, S., Absher, D. M., Hopkins, P. N., Sha, J., Tsai, M. Y., et al. (2014) Methylation at CPT1A locus is associated with lipoprotein subfraction profiles. J. Lipid Res. 55, 1324–1330.
[62] Gagnon, F., Aïssi, D., Carrié, A., Morange, P. E., and Trégouët, D. A. (2014) Robust validation of methylation levels association at CPT1A locus with lipid plasma levels. J. Lipid Res. 55, 1189–1191.
[63] Irvin, M. R., Aslibekyan, S., Hidalgo, B., and Arnett, D. (2014) CPT1A: the future of heart disease detection and personalized medicine?. Clin. Lipidol. 9, 9–12.
[64] Maples, J. M., Brault, J. J., Witczak, C. A., Park, S., Hubal, M. J., et al. (2015) Differential epigenetic and transcriptional response of the skeletal muscle carnitine palmitoyltransferase 1B (CPT1B) gene to lipid exposure with obesity. Am. J. Physiol. Endocrinol. Metab. 309, E345–E356.
[65] Collins, S. A., Sinclair, G., McIntosh, S., Bamforth, F., Thompson, R., et al. (2010) Carnitine palmitoyltransferase 1A (CPT1A) P479L prevalence in live newborns in Yukon, Northwest Territories, and Nunavut. Mol. Genet. Metab. 101, 200–204.
[66] Gillingham, M. B., Hirschfeld, M., Lowe, S., Matern, D., Shoemaker, J., et al. (2011) Impaired fasting tolerance among Alaska native children with a common carnitine palmitoyltransferase 1A sequence variant. Mol. Genet. Metab. 104, 261–264.
[67] Heiner-Fokkema, M. R., Vaz, F. M., Maatman, R., Kluijtmans, L. A., van Spronsen, F. J., et al. (2017) Reliable diagnosis of carnitine palmitoyltransferase type IA deficiency by analysis of plasma acylcarnitine profiles. JIMD Rep. 32, 33–39.
[68] Orngreen, M. C., Duno, M., Ejstrup, R., Christensen, E., Schwartz, M., et al. (2005) Fuel utilization in subjects with carnitine palmitoyltransferase 2 gene mutations. Ann. Neurol. 57, 60–66.
[69] Violante, S., Ijlst, L., van Lenthe, H., de Almeida, I. T., Wanders, R. J., et al. (2010) Carnitine palmitoyltransferase 2: new insights on the substrate specificity and implications for acylcarnitine profiling. Biochim. Biophys. Acta 1802, 728–732.
[70] North, K. N., Hoppel, C. L., De Girolami, U., Kozakewich, H. P., and Korson, M. S. (1995) Lethal neonatal deficiency of carnitine palmitoyltransferase II associated with dysgenesis of the brain and kidneys. J. Pediatr. 127, 414–420.
[71] Chegary, M., Te Brinke, H., Doolaard, M., Ijlst, L., Wijburg, F. A., et al. (2008) Characterization of L-aminocarnitine, an inhibitor of fatty acid oxidation. Mol. Genet. Metab. 93, 403–410.
[72] Yamamoto, T., Tanaka, H., Emoto, Y., Umehara, T., Fukahori, Y., et al. (2014) Carnitine palmitoyltransferase 2 gene polymorphism is a genetic risk factor for sudden unexpected death in infancy. Brain Dev. 36, 479–483.
[73] Deutsch, M., Vassilopoulos, D., Sevastos, N., Papadimitriou, A., Vasiliou, K., et al. (2008) Severe rhabdomyolysis with hypoglycemia in an adult patient with carnitine palmitoyltransferase II deficiency. Eur. J. Intern. Med. 19, 289–291.
[74] Bonnefont, J. P., Bastin, J., Laforet, P., Aubey, F., Mogenet, A., et al. (2010) Long-term follow-up of bezafibrate treatment in patients with the myopathic form of carnitine palmitoyltransferase 2 deficiency. Clin. Pharmacol. Ther. 88, 101–108.
[75] Indiveri, C., Iacobazzi, V., Tonazzi, A., Giangregorio, N., Infantino, V., et al. (2011) The mitochondrial carnitine/acylcarnitine carrier: function, structure and physiopathology. Mol. Aspects Med. 32, 223–233.
[76] Indiveri, C., Iacobazzi, V., Giangregorio, N., and Palmieri, F. (1997) The mitochondrial carnitine carrier protein: cDNA cloning, primary structure and comparison with other mitochondrial transport proteins. Biochem. J. 321, 713–719.
[77] Chalmers, R. A., Stanley, C. A., English, N., and Wigglesworth, J. S. (1997) Mitochondrial carnitine-acylcarnitine translocase deficiency presenting as sudden neonatal death. J. Pediatr. 131, 220–225.
[78] Jakobs, B. S., and Wanders, R. J. (1995) Fatty acid beta-oxidation in peroxisomes and mitochondria: the first, unequivocal evidence for the involvement of carnitine in shuttling propionyl-CoA from peroxisomes to mitochondria. Biochem. Biophys. Res. Commun. 213, 1035–1041.
[79] Pande, S. V., Brivet, M., Slama, A., Demaugre, F., Aufrant, C., et al. (1993) Carnitine-acylcarnitine translocase deficiency with severe hypoglycemia and auriculo ventricular block. Translocase assay in permeabilized fibroblasts. J. Clin. Invest. 91, 1247–1252.
[80] Geven, W. B., Niezen-Koning, K. E., Timmer, A., van Loon, A. J., Wanders, R. J., et al. (2007) Pre-eclampsia in a woman whose child suffered from lethal carnitine-acylcarnitine translocase deficiency. BJOG 114, 1028–1030.
[81] Genuth, S. M., and Hoppel, C. L. (1979) Plasma and urine carnitine in diabetic ketosis. Diabetes 28, 1083–1087.
[82] Soltész, G., Melegh, B., and Sándor, A. (1983) The relationship between carnitine and ketone body levels in diabetic children. Acta Paediatr. Scand. 72, 511–515.
[83] Hoppel, C. L., and Genuth, S. M. (1982) Urinary excretion of acetylcarnitine during human diabetic and fasting ketosis. Am. J. Physiol. 243, E168–E172.
[84] Okuda, Y., Kawai, K., Murayama, Y., and Yamashita, K. (1987) Postprandial changes in plasma ketone body and carnitine levels in normal and non-insulin-dependent diabetic subjects. Endocrinol. Jpn. 34, 415–422.
[85] Adams, S. H., Hoppel, C. L., Lok, K. H., Zhao, L., Wong, S. W., et al. (2009) Plasma acylcarnitine profiles suggest incomplete long-chain fatty acid beta-oxidation and altered tricarboxylic acid cycle activity in type 2 diabetic African-American women. J. Nutr. 139, 1073–1081.
[86] Mihalik, S. J., Goodpaster, B. H., Kelley, D. E., Chace, D. H., Vockley, J., et al. (2010) Increased levels of plasma acylcarnitines in obesity and type 2 diabetes and identification of a marker of glucolipotoxicity. Obesity (Silver Spring) 18, 1695–1700.
[87] la Marca, G., Malvagia, S., Toni, S., Piccini, B., Di Ciommo, V., et al. (2013) Children who develop type 1 diabetes early in life show low levels of carnitine and amino acids at birth: does this finding shed light on the etiopathogenesis of the disease?. Nutr. Diabetes 3, e94.
[88] Frohlich, J., Seccombe, D. W., Hahn, P., Dodek, P., and Hynie, I. (1978) Effect of fasting on free and esterified carnitine levels in human serum and urine: correlation with serum levels of free fatty acids and beta-hydroxybutyrate. Metabolism 27, 555–561.
[89] Hoppel, C. L., and Genuth, S. M. (1980) Carnitine metabolism in normal-weight and obese human subjects during fasting. Am. J. Physiol. 238, E409–E415.
[90] Soeters, M. R., Sauerwein, H. P., Duran, M., Wanders, R. J., Ackermans, M. T., et al. (2009) Muscle acylcarnitines during short-term fasting in lean healthy men. Clin. Sci. (Lond) 116, 585–592.
[91] Schooneman, M. G., Napolitano, A., Houten, S. M., Ambler, G. K., Murgatroyd, P. R., et al. (2016) Assessment of plasma acylcarnitines before and after weight loss in obese subjects. Arch. Biochem. Biophys. 606, 73–80.
[92] Redman, L. M., Huffman, K. M., Landerman, L. R., Pieper, C. F., Bain, J. R., et al. (2011) Effect of caloric restriction with and without exercise on metabolic intermediates in nonobese men and women. J. Clin. Endocrinol. Metab. 96, E312–E321.
[93] Fenkci, S. M., Fenkci, V., Oztekin, O., Rota, S., and Karagenc, N. (2008) Serum total L-carnitine levels in non-obese women with polycystic ovary syndrome. Hum. Reprod. 23, 1602–1606.
[94] Celik, F., Kose, M., Yilmazer, M., Köken, G. N., Arioz, D. T., et al. (2017) Plasma L-carnitine levels of obese and non-obese polycystic ovary syndrome patients. J. Obstet. Gynaecol. 37, 1–4.
[95] Angelini, A., Imparato, L., Landi, C., Porfido, F. A., Ciarimboli, M., et al. (1993) Variation in levels of glycaemia and insulin after infusion of glucose solutions with or without added L-carnitine. Drugs Exp. Clin. Res. 19, 219–222.
[96] Negro, P., Gossetti, F., La Pinta, M., Mariani, P., and Carboni, M. (1994) The effect of L-carnitine, administered through intravenous infusion of glucose, on both glucose and insulin levels in healthy subjects. Drugs Exp. Clin. Res. 20, 257–262.
[97] Mingrone, G., Greco, A. V., Capristo, E., Benedetti, G., Giancaterini, A., et al. (1999) L-carnitine improves glucose disposal in type 2 diabetic patients. J. Am. Coll. Nutr. 18, 77–82.

[98] Stephens, F. B., Constantin-Teodosiu, D., Laithwaite, D., Simpson, E. J., and Greenhaff, P. L. (2006) An acute increase in skeletal muscle carnitine content alters fuel metabolism in resting human skeletal muscle. J. Clin. Endocrinol. Metab. 91, 5013–5018.

[99] Ruggenenti, P., Cattaneo, D., Loriga, G., Ledda, F., Motterlini, N., et al. (2009) Ameliorating hypertension and insulin resistance in subjects at increased cardiovascular risk: effects of acetyl-L-carnitine therapy. Hypertension 54, 567–574.

[100] Muniyappa, R. (2010) Oral carnitine therapy and insulin resistance. Hypertension 55, e13.

[101] Pooyandjoo, M., Nouhi, M., Shab-Bidar, S., Djafarian, K., and Olyaeemanesh, A. (2016) The effect of (L-)carnitine on weight loss in adults: a systematic review and meta-analysis of randomized controlled trials. Obes. Rev. 17, 970–976.

[102] Del Vecchio, F. B., Coswig, V. S., and Galliano, L. M. (2017) Comment on 'The effect of (L-)carnitine on weight loss in adults: a systematic review and meta-analysis of randomized controlled trials'. Obes. Rev. 18, 277–278.

[103] Molfino, A., Cascino, A., Conte, C., Ramaccini, C., Rossi Fanelli, F., et al. (2010) Caloric restriction and L-carnitine administration improves insulin sensitivity in patients with impaired glucose metabolism. J. Parenter. Enteral. Nutr. 34, 295–299.

[104] Giancaterini, A., De Gaetano, A., Mingrone, G., Gniuli, D., Liverani, E., et al. (2000) Acetyl-L-carnitine infusion increases glucose disposal in type 2 diabetic patients. Metabolism 49, 704–708.

[105] Bloomer, R. J., Fisher-Wellman, K. H., and Tucker, P. S. (2009) Effect of oral acetyl L-carnitine arginate on resting and postprandial blood biomarkers in pre-diabetics. Nutr. Metab. (Lond) 6, 25.

[106] Malaguarnera, M., Gargante, M. P., Russo, C., Antic, T., Vacante, M., et al. (2010) L-Carnitine supplementation to diet: a new tool in treatment of nonalcoholic steatohepatitis—a randomized and controlled clinical trial. Am. J. Gastroenterol. 105, 1338–1345.

[107] Dean, D., Daugaard, J. R., Young, M. E., Saha, A., Vavvas, D., et al. (2000) Exercise diminishes the activity of acetyl-CoA carboxylase in human muscle. Diabetes 49, 1295–1300.

[108] Odland, L. M., Heigenhauser, G. J., Wong, D., Hollidge-Horvat, M. G., and Spriet, L. L. (1998) Effects of increased fat availability on fat-carbohydrate interaction during prolonged exercise in men. Am. J. Physiol. 274, R894–R902.

[109] Romijn, J. A., Coyle, E. F., Sidossis, L. S., Gastaldelli, A., Horowitz, J. F., et al. (1993) Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. Am. J. Physiol. 265, E380–E391.

[110] Hiatt, W. R., Regensteiner, J. G., Wolfel, E. E., Ruff, L., and Brass, E. P. (1989) Carnitine and acylcarnitine metabolism during exercise in humans. Dependence on skeletal muscle metabolic state. J. Clin. Invest. 84, 1167–1173.

[111] Lindeboom, L., Nabuurs, C. I., Hoeks, J., Brouwers, B., Phielix, E., et al. (2014) Long-echo time MR spectroscopy for skeletal muscle acetylcarnitine detection. J. Clin. Invest. 124, 4915–4925.

[112] Boss, A., Kreis, R., Jenni, S., Ith, M., Nuoffer, J. M., et al. (2011) Noninvasive assessment of exercise-related intramyocellular acetylcarnitine in euglycemia and hyperglycemia in patients with type 1 diabetes using (1)H magnetic resonance spectroscopy: a randomized single-blind crossover study. Diabetes Care 34, 220–222.