Annu. Rev. Nutr. 1998. 18:179–206

GENETIC DISORDERS OF CARNITINE METABOLISM AND THEIR NUTRITIONAL MANAGEMENT[1]

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KEY WORDS: transporter, translocase, acylcarnitine, carrier, carnitine palmitoyltransferase

ABSTRACT

Carnitine functions as a substrate for a family of enzymes, carnitine acyltransferases, involved in acyl-coenzyme A metabolism and as a carrier for long-chain fatty acids into mitochondria. Carnitine biosynthesis and/or dietary carnitine fulfill the body's requirement for carnitine. To date, a genetic disorder of carnitine biosynthesis has not been described. A genetic defect in the high-affinity plasma membrane carnitine-carrier$_{in}$ leads to renal carnitine wasting and primary carnitine deficiency. Myopathic carnitine deficiency could be due to an increase in efflux moderated by the carnitine-carrier$_{out}$. Defects in the carnitine transport system for fatty acids in mitochondria have been described and are being examined at the molecular and pathophysiological levels. The nutritional management of these disorders includes a high-carbohydrate, low-fat diet and avoidance of those events that promote fatty acid oxidation, such as fasting, prolonged exercise, and cold. Large-dose carnitine treatment is effective in systemic carnitine deficiency.

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INTRODUCTION

Carnitine is ubiquitous in nature and is found in especially high concentration in the muscle tissue of higher organisms, from where in 1905 it was first isolated (60). While its elemental composition was quickly determined, the correct structure of carnitine was not elucidated until 1927 (60). The discovery in the late 1930s of the neurotransmitter acetylcholine, which has a structural similarity to carnitine (carboxymethylcholine), helped to focus the first studies of the function of carnitine (60). Despite these efforts, neither the physiological nor the pharmacological role of carnitine or its biosynthesis or degradation were delineated.

New interest in carnitine was elicited in 1952 when Carter et al (31) established that carnitine was a growth factor for the meal worm *Tenebrio molitor*. Subsequent studies showed that when starved, carnitine-deficient larvae died, with their lipid depot completely intact. This suggested that carnitine had a role in lipid metabolism (60). The role of carnitine in fat metabolism was further substantiated by two observations. In 1955 Fritz (63) showed that a muscle extract stimulated fatty acid oxidation in liver slices and liver homogenates and identified this stimulatory factor as carnitine. At the same time, Friedman & Fraenkel discovered that carnitine can be reversibly acetylated by acetyl-coenzyme A (CoA) (62). These studies not only established the role of carnitine in fatty acid oxidation, they also indicated that the mechanism by which carnitine stimulates fatty acid oxidation might involve a reversible transesterification of carnitine. These observations provided the stimulus for the renewed interest in carnitine research, and subsequent studies established not only the transport role of carnitine in mitochondrial fatty acid oxidation (19, 20, 65, 82, 131, 137, 160, 205), but also its role in shuttling chain-shortened acyl groups from peroxisomes into the mitochondria for further oxidation (10) and in modulating the cellular acyl-CoA/CoASH ratio (134, 179). In conjunction with its acyl-CoA buffering function, carnitine also plays a significant role in the elimination (detoxification) of endogenous (32) and exogenous (xenobiotic) organic acids (136, 186). Therefore, it is not surprising that many metabolic disorders affect carnitine homeostasis, and inadequate carnitine availability may affect a wide variety of metabolic reactions, leading to the diverse clinical manifestations observed in disease states that affect carnitine metabolism.

This review focuses primarily on the known genetic defects of cellular carnitine transport and on the enzymes of the mitochondrial carnitine-dependent fatty acid entry pathway. Several clinical aspects of carnitine metabolism (nutritional, physiological, pharmacological, clinical, and enzymological) have been discussed in previous excellent reviews (4, 9, 15, 21, 23, 47, 49, 60, 64, 145, 161, 198) and books (16, 30, 57, 61, 194). The latest progress and current view of the structural, functional, and regulatory aspects of the mitochondrial carnitine palmitoyltransferases are summarized in a recent review (106).

CARNITINE

Biosynthesis of Carnitine

PATHWAY The ultimate precursors of carnitine are the essential amino acids, lysine and methionine, and the ability to synthesize carnitine seems to be as ubiquitous as carnitine itself. *S*-adenosylmethionine was recognized early as the donor of the three methyl groups (18, 195), but lysine was not recognized as a source of nitrogen and carbon chain until the early 1970s (37, 38, 167). Although the pathway of carnitine biosynthesis has been studied most extensively in rats, the available evidence indicates that the same pathway is operative in humans. In animals, 6-N-trimethyllysine is formed by the methylation of lysine residues in proteins (129). Following proteolysis, 6-N-trimethyllysine is converted to 3-hydroxy-6-N-trimethyllysine (48, 80, 86, 100, 124, 150), which is then cleaved to butyrobetaine aldehyde and glycine by an aldolase that may be identical to serine hydroxymethyltransferase (76, 86). Butyrobetaine aldehyde is then oxidized to γ-butyrobetaine (87) by γ-butyrobetaine aldehyde dehydrogenase that has been purified (87), and in humans, the highest activity is found in liver and kidney (87). The final step in the biosynthetic pathway of carnitine is the conversion of butyrobetaine to L-carnitine by butyrobetaine hydroxylase. While most animal tissues, including brain and skeletal muscle, contain the enzymes necessary to convert trimethyllysine to butyrobetaine (37, 141, 168), the formation of carnitine from butyrobetaine occurs in only a few tissues. The enzyme shows species variation in tissue distribution, but it is present in the liver of all species. In humans, the enzyme is present in the liver, kidney, and brain (52, 141). In both the liver and kidney, the enzyme exists in multiple isoforms (104) and appears to be the dimeric combinations of two subunits that differ in charge only. A genetic disorder of carnitine biosynthesis has yet to be described. Dietary carnitine could provide sufficient carnitine to the body to mask the deficit in synthesis.

REGULATION Carnitine is not considered an essential nutrient for adult humans because it can be synthesized. Although the individual steps of the overall

pathway and the enzymes catalyzing the reactions have been identified, the regulation of carnitine biosynthesis in mammals is less clear. Butyrobetaine hydroxylase is developmentally regulated in the liver but not kidney of rats and humans (128), and in humans the hepatic enzyme activity at birth is about 25% of that in adults. However, subsequent studies in which butyrobetaine was administered to human infants with their diet demonstrated that butyrobetaine hydroxylase is not rate limiting in carnitine biosynthesis (140). Although in rats the rate of carnitine biosynthesis is determined by the availability of 6-N-trimethyllysine (39, 143), whether it is the same in humans has not been conclusively established. Supplementation of a carnitine-free diet with trimethyllysine caused a 1.5-fold increase in carnitine excretion in infants, whereas the same amount of butyrobetaine resulted in a 30-fold increase (128). Likewise, in adults a 4-fold increase was observed with trimethyllysine whereas a 43-fold increase was demonstrated with half the amount of butyrobetaine (140). This does not rule out the possibility that in humans the rate of carnitine biosynthesis is determined by one of the enzymes subserving the conversion of trimethyllysine to butyrobetaine, but the data must be interpreted with the knowledge that trimethyllysine is only poorly taken up by tissues (197) and that trimethyllysine is not reabsorbed in the kidney of humans (102). Consequently, the modest increase in carnitine excretion with trimethyllysine supplementation has been interpreted as meaning that substrate availability rather than the activity of the pathway enzymes is the determinant in carnitine biosynthesis. If this is the case, the only way carnitine biosynthesis could be increased would be through increased methylation of protein-bound lysine and/or increased protein turnover. Thus, the ability of humans to adapt to changes in the need for carnitine would be severely restricted, consequently increasing dependence on exogenous carnitine in conditions of increased demand.

Homeostasis of Body Carnitine

Carnitine is available from dietary sources and from endogenous biosynthesis, as discussed above. The body distribution of carnitine is determined by a series of systems that transport carnitine into cells against a concentration gradient, an independent efflux process, and an exchange mechanism in a tissue-specific fashion. Under physiological conditions, plasma carnitine concentration is maintained within a narrow range. The dominant factor in maintaining plasma carnitine concentrations is renal clearance, which is determined by the kinetic properties of the brush border carnitine transport system (51, 144).

TRANSPORT OF CARNITINE *Liver* Liver plays a unique role in whole-body carnitine homeostasis. Not only is liver the major site of butyrobetaine hydroxylation, but the transport system shows a higher affinity for butyrobetaine

($K_m \cong 0.5$ mM) (34) than for carnitine ($K_m \cong 5.0$ mM) (34, 96), favoring butyrobetaine transport into the liver and the efflux system acylcarnitine over carnitine (77, 153). Thus, the liver contains systems that favor its role of providing synthesized carnitine to the body. Additionally, orally available carnitine is presented to the liver via the portal vein.

The system described above has been studied in isolated hepatocytes (34) and perfused liver (77, 96, 152, 153) and is temperature, energy, and sodium dependent, as well as saturable, consistent with a carrier-mediated process. Additionally, the transporter shows weak stereospecificity. The transporter studied in liver from fasted rats demonstrated a decreased K_m for carnitine (2.6 mM) without a change in $V_{\max}$ (96, 152). It has been suggested that this kinetic response is the mechanism for enhanced carnitine transport into livers from fasted rats. However, because as a substrate the transport of D-carnitine changes similarly, a metabolic reason for enhanced carnitine sequestration in liver is not supported.

A separate system for carnitine efflux has been demonstrated that is saturable, energy independent, and inhibited by mersalyl but not by ouabain (77, 153). It has been suggested that acylcarnitines are transported out of hepatocytes faster than is carnitine. In contrast to other tissues, stimulation of carnitine efflux by extracellular carnitine could not be demonstrated, which suggests an exchange carrier system is not present in the liver (77).

Other tissues Heart (3, 11, 97, 117, 116, 181–183), skeletal muscle (105, 114, 138, 142), kidney (69, 144, 166), and fibroblasts (29, 53, 54, 171, 173–175, 177) all have a temperature- and sodium-dependent saturable uptake system of a high-affinity type (Table 1), which contrasts with the low-affinity type in the liver. The high-affinity uptake of carnitine in the heart is not energy dependent, whereas uptake in skeletal muscle or cultured skin fibroblasts is inhibited by uncouplers or respiratory chain inhibitors. The apparent differences in these studies have not been clarified. Detailed mechanistic studies of the kidney have led to the notion that Na^+-dependent transport of carnitine in brush border membrane vesicles is not directly energy dependent (144). It is noteworthy that fibroblasts from patients with cytochrome oxidase deficiency demonstrated decreased carnitine transport, corresponding to values obtained in heterozygotes of primary deficiency (174).

A different system for efflux of carnitine out of cells has been described in cultured heart cells and skeletal muscle (115, 138). Additionally, an exchange carrier system has been demonstrated in heart tissue and skeletal muscle (138, 154, 155). In maintaining the content of tissue carnitine, this system would be of little significance because net uptake is not affected unless plasma carnitine is exchanged for tissue butyrobetaine.

Table 1 Tissue distribution of carnitine transporter systems[a]

Tissue	High-affinity uptake	Low-affinity uptake	Efflux	Exchange
Liver perfused	—	4.2 mM (96)	0.27 mM (152)	No (77)
Hepatocytes	—	5.6 mM (34)	Yes (96)	—
Heart perfused	24 μM (181–183)	—	—	—
Heart slices	—	—	—	Yes (154, 155)
Cardiomyocytes	$\cong$5.0 μM (11, 117)	—	Yes (115)	Yes (115)
Muscle strips	60 μM (138)	—	Yes (138)	Yes (138)
Myocytes	1.9 μM, 1.5 μM (142, 105)	80–175 μM (142, 105, 114)	Yes (114)	—
Kidney-bbmv[b]	80 μM, 20 μM (144, 166)	3.7 mM (166)	—	—
Fibroblasts	2–8 μM (53, 142, 165, 173, 174, 177)	68.5 (142)	—	—

[a]References in parentheses. Yes/No, transport type present or absent but not investigated in detail; —, not found or not investigated.
[b]Brush border membrane vesicles.

DEFECTS IN CARNITINE TRANSPORT We have elected to categorize what has been called primary carnitine deficiency according to the carnitine carrier involved. As outlined in the previous section and Table 1, tissue carnitine homeostasis is maintained by a series of transport systems, e.g. carnitine-carrier$_{in}$ (uptake), carnitine-carried$_{out}$ (efflux), and carnitine-carrier$_{exchange}$ (stoichiometric exchange). The exchange system is not discussed in this review because a genetic defect has not been described. Defects in either of the two former systems will result in decreased carnitine concentration in the affected tissue. A defect in the carnitine-carrier$_{out}$ leads to an increased loss of tissue carnitine and may form the underlying mechanism of the myotonic form of primary carnitine deficiencies (50, 114), whereas a defect in carnitine-carrier$_{in}$ results in systemic carnitine deficiency.

Carnitine deficiencies Carnitine deficiency represents a heterogeneous group of diseases with widely varying clinical symptoms. Given that carnitine not only is required for fatty acid oxidation but also plays a crucial role in cellular homeostasis of free and acyl-CoA, the wide variety of clinical manifestation of carnitine deficiency is not unexpected. This is further complicated by the fact that the carnitine requirement depends on several other factors, such as age, diet, metabolic conditions, and tissue dependence on fatty acid oxidation (139). Carnitine deficiency is defined as decreased tissue carnitine concentration that is below the requirement for normal cellular metabolism. Since the initial description of human muscle carnitine deficiency syndrome (50), more than

100 cases with different forms of carnitine deficiency have been reported. It is now clear that many of the reported carnitine deficiency syndromes are due to inherited and acquired diseases and that carnitine deficiency (or insufficiency) is the consequence rather than the cause of the disease. Because of the different etiologies of carnitine deficiencies, they are classified as primary and secondary carnitine deficiencies and discussed briefly below. (For comprehensive reviews on this topic, see References 2, 23, 45, 49, 135, 161.)

Primary carnitine deficiency Two different entities of primary carnitine deficiency syndromes have been described: muscle carnitine deficiency and systemic carnitine deficiency.

The myopathic form (possible carnitine-carrier$_{out}$ deficiency) is more restricted and is characterized by progressive muscle weakness and lipid storage myopathy, primarily in type I fibers. The carnitine content in skeletal muscle is severely reduced but is normal in plasma and the liver, with no sign of renal carnitine leak (49). Plasma acylcarnitine concentrations were normal, and no organic aciduria was observed. Oral L-carnitine treatment has been of benefit in some patients but did not replenish the muscle carnitine stores (45, 135). Although some patients with the myopathic form have been shown to have fatty acid oxidation defects, for others no definitive biochemical defect has been discovered. Direct studies in cultured myoblasts from a patient affected with muscle carnitine deficiency demonstrated normal carnitine uptake (114). However, increased carnitine efflux was found in these myoblasts, resulting in reduced intracellular carnitine content. Intermediate levels of carnitine in skeletal muscle of some parents suggests autosomal recessive inheritance (1).

Primary systemic carnitine deficiency (carnitine-carrier$_{in}$ deficiency) was first described by Karpati et al in 1975 (94). Since this first report, over 20 cases with systemic carnitine deficiency have been described (135). The onset of the clinical symptoms ranges from 1 month to 7 years, with different types of presentation: progressive cardiomyopathy, myopathy, and hypoketotic hypoglycemic encephalopathy. In some cases, all forms of presentation may exist (67, 162, 174). Progressive cardiomyopathy is the most common and usually occurs in older people. Myopathy, manifesting in hypotonia or slowly progressive proximal weakness, is commonly associated with cardiomyopathy or encephalopathy. Acute encephalopathy associated with hypoketotic hypoglycemia is more commonly seen in infants (135). In contrast to the myotonic form of primary carnitine deficiency, systemic carnitine deficiency is characterized by low plasma carnitine concentrations (54, 165, 173, 177). Dicarboxylic aciduria is usually absent, indicating no primary β-oxidation enzyme defect (173). Carnitine concentration is severely reduced in all affected tissues (heart, skeletal muscle, liver) (6, 54, 147, 165, 173, 177). Patients with systemic

carnitine deficiency also demonstrate a severe renal carnitine leak because of a defect in the renal carnitine transporter (148, 165, 173, 177, 188). The diagnosis of systemic carnitine deficiency is made by determination of carnitine uptake in fibroblasts (6, 35, 53, 54, 147, 165, 173, 174, 177), or in cultured lymphoblasts (176), when a compatible clinical picture and laboratory evidence of carnitine deficiency exist. Based on similar kinetic parameters for carnitine uptake in cultured muscle cells (142), heart (11), and fibroblasts (173, 177), it is suggested that these tissues share a common transporter. The concept that primary systemic carnitine deficiency is due to a defect in the specific high-affinity carnitine transporter that is expressed by fibroblasts, muscle, the heart, the kidney, and leukocytes, but not by the liver, is supported by clinical and laboratory findings from patients with symptoms of systemic carnitine deficiency (53, 162, 173, 177). Carnitine-uptake determinations of cultured fibroblasts from parents with affected children demonstrate an autosomal recessive inheritance pattern (6, 53, 165, 173, 174). Given the frequent history of previously unexplained sibling death in families with affected children (135) and the lethal nature of the disease in some early onset cases (6, 54, 147), early identification of the syndrome and institution of oral carnitine therapy may decrease immediate and long-term morbidity and mortality.

Treating primary systemic carnitine deficiency syndromes with carnitine reversed or attenuated the clinical symptoms in most patients without restoring tissue carnitine stores. To reconcile this apparent discrepancy, it was suggested that during carnitine supplementation, a transient increase in plasma carnitine concentration occurs that is sufficient to allow carnitine uptake by the low-affinity transporter to restore function (2).

Secondary carnitine deficiencies Secondary carnitine deficiency, or carnitine insufficiency, is associated with a wide variety of genetic and acquired diseases and is manifested by decreased plasma or tissue carnitine concentrations. The most characteristic causes of secondary carnitine deficiencies are metabolic disorders associated with impaired oxidation of acyl-CoA intermediates in the mitochondria; these are discussed in several previous reviews (2, 23, 45, 161). Below are described two other genetic diseases associated with decreased plasma and/or tissue carnitine concentrations.

1. Defects in the mitochondrial respiratory chain. A few patients with respiratory chain defects have been reported to have low carnitine concentrations in plasma and/or muscle. Marked lipid excess with reduced carnitine levels has been noted in a minority of patients with complex I deficiency (28, 36) and in a 2-year-old female patient with succinate cytochrome *c* reductase activity (157). Mild-to-moderate decreases in total carnitine and increased acylcarnitine have been noted in a few patients also with cytochrome *c* oxidase

defects (5, 118, 146, 158). The significance of these observations is unclear, as is the underlying mechanism of the reduced tissue carnitine concentration. As was recently shown, fibroblasts from patients with cytochrome oxidase deficiency have carnitine uptake rates approximately 50% that of normal fibroblasts (174). A shortage of ATP needed to establish and maintain the Na^+ gradient could explain the moderately reduced skeletal muscle carnitine concentrations.

2. Excessive renal loss of carnitine. The renal tubular Fanconi syndrome is a generalized transport defect affecting tubular reabsorption of small molecules, including carnitine. It is manifested by low plasma and muscle carnitine concentrations and moderate-to-massive lipid accumulation in muscle (8, 66, 121, 126). Patients with renal Fanconi syndrome have an increased fractional excretion of both free carnitine and acylcarnitine, 33% and 26%, respectively, compared with 3% and 5% in normal individuals (8). Plasma-free carnitine concentrations in affected children were low (11.7 μM and 13.2 μM) compared with control values (42 μM), but the esterified fraction was unchanged (8, 66). Skeletal muscle–free carnitine in 12 patients was found to be 37% that of control values (66). Though most of the Fanconi syndrome patients were deficient in plasma-free carnitine to the same extent as reported in systemic carnitine deficiency (51), hepatic fatty acid oxidation was not affected (8). Oral L-carnitine treatment normalized the plasma-free and total carnitine concentrations within 2 days and reduced the initially high plasma-free fatty acid concentration after long-term (7–20 mo) treatment (66). Muscle carnitine content remained low in all patients, even though cystinotic muscle cells in culture took up L-carnitine normally, but muscle lipid accumulation, which varied directly with duration of carnitine deficiency, improved significantly with L-carnitine therapy (66).

NUTRITIONAL ASPECTS OF CARNITINE Dietary carnitine provides more than half of available carnitine in adult humans (139). Although absorption of carnitine in humans has not been studied directly, in vitro experiments established an active transport process across the human proximal small intestinal mucosa (73). The transport of carnitine across this membrane was Na^+ dependent, with an apparent K_m of 974 μM and V_{max} of 27.4 nmol/ml of intracellular water/min. A passive, diffusional process was also identified, which may be important for absorption of large doses of carnitine. Studies from the same laboratory demonstrated an active transport for carnitine on biopsy specimens in the duodenum and the ileum but not in the colon (74). The uptake against a concentration gradient was abolished by anoxia and by replacement of sodium with potassium. The kinetics of carnitine transport was consistent with a two-component system: a saturable system with a K_m of 558 μM, and a linear component probably

representing passive diffusion. The transport of L-carnitine was diminished by D-carnitine and acetyl-L-carnitine, which suggests that these substrates utilize the same transport system. Two-component transport systems have also been demonstrated in different animal models of intestinal carnitine transport. Using everted rat intestinal rings and sacs, Shaw et al (159) demonstrated a saturable carnitine uptake in the duodenum and the jejunum but not in the ileum. The saturable uptake with a K_m between 206 and 316 μM was inhibited by anoxia, 2,4-DNP, cyanide, and removal of Na^+, and D-carnitine and acetyl-L-carnitine were competitive inhibitors of the carnitine uptake. The linear component was presumed to represent diffusion. Active intestinal uptake of carnitine has also been described in more complex in vivo models using a combination of segmental perfusion techniques and bolus intraluminal injection and intestinal vascular perfusion (69, 71, 72). In contrast to the studies above, only passive diffusion could be demonstrated using rat jejunal brush border membrane vesicles (103) and in enterocytes isolated from adult guinea pigs (70). Recently, the mechanism of L-carnitine uptake was studied using a human-derived intestinal epithelial cell line (Caco-2) (113). When grown to confluency, Caco-2 cells undergo enterocyte-like differentiation and develop well-defined intracellular junctions that separate the apical and basolateral membranes into distinct compartments. They also attain many of the absorptive characteristics of villus absorptive cells. These cells demonstrated a temperature- and energy-dependent saturable uptake of carnitine with an apparent K_m of 45.5 μM and $V_{\max}$ of approximately 17 nmol/min/mg of protein. The uptake process was Na^+ dependent and inhibited by structural analogs such as butyrobetaine and D-carnitine but not by structurally unrelated compounds.

While the Na^+-dependent, saturable component of the carnitine uptake system very likely represents the physiological process by which dietary carnitine is taken up from the intestine, the significance of the diffusional component is uncertain. At physiological pH, carnitine forms a zwitterion with no net charge, potentially permitting passive diffusion. However, the significance of diffusion of carnitine as a mechanism of uptake is presumably small under physiological conditions but may assume importance during therapeutic intervention with high oral doses of L-carnitine.

ACYLCARNITINES

In mammals, all known functions of carnitine involve the reversible acylation of the 3-hydroxyl group of carnitine, with subsequent translocation of these acylcarnitines from one cellular compartment to another. The enzymes responsible for the formation of acylcarnitines are the carnitine acyltransferases with wide and overlapping chain-length specificity, different cellular localization,

and metabolic function (e.g. mitochondrial and peroxisomal fatty acid oxiddation, cellular CoASH homeostasis) (9, 21). Inherited defects in any of the enzymes will affect not only the metabolic pathway the enzyme subserves, but indirectly the cellular metabolism in general through the accumulation of potentially toxic acyl-CoA and acylcarnitine intermediates and by lowering the free CoASH and tissue carnitine concentration. The known genetic defects of the mitochondrial carnitine palmitoyltransferase system, their metabolic consequences, and potential therapeutic intervention are described briefly below.

Mitochondrial β-Oxidation of Fatty Acids—The Role of Mitochondrial Carnitine System

The mitochondrial β-oxidation of fatty acids occurs within the mitochondrial matrix and is catalyzed by the sequential action of acyl-CoA dehydrogenase, enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase, and 3-ketoacyl-CoA thiolase enzyme families with substrate specificities of short-, medium-, long-, and very long-chain acyl-CoAs (22, 99). The successive β-oxidative chain-shortening event in the matrix is preceded by the activation of long-chain fatty acids via the catalytic action of long-chain acyl-CoA synthetase on the mitochondrial outer membrane and by the carnitine-dependent transport of the activated fatty acids into the mitochondrial matrix. This process requires the sequential action of carnitine palmitoyltransferase (CPT)-I, the acylcarnitine-carnitine translocase, and CPT-II (9, 21, 78, 79, 112, 151).

The recognition that L-carnitine stimulates the mitochondrial oxidation of long-chain fatty acids was soon followed by the proposed mechanism of the carnitine-dependent entry of fatty acids into the mitochondria (19, 20, 63–65). This hypothesis took into account the known impermeability of the inner mitochondrial membrane toward acyl-CoAs and two facts: that activated fatty acids are transesterified by CPT-I from CoA to carnitine at the outer face of the mitochondrial inner membrane, and that the acylcarnitines formed traverse the inner membrane into the matrix where the acyl groups are transferred back to CoA by CPT-II before entering the β-oxidation spiral (64). The question of how acylcarnitines enter the mitochondrial matrix and how free carnitine exits from mitochondria was answered with the discovery of acylcarnitine:carnitine translocase, a protein present in the mitochondrial inner membrane (131, 137). Although this conception of the mechanism of fatty acid transport has prevailed some 30 years, understanding about several important aspects has been refined: (*a*) malonyl-CoA is a potent inhibitor of CPT-I but not CPT-II (107, 108, 111); (*b*) CPT-I has been reassigned to the mitochondrial outer membrane (120); (*c*) the catalytic activities of CPT-I and CPT-II reside in structurally and immunologically different polypeptides (40, 98, 191, 193); (*d*) CPT-II is likely to be expressed as the same protein within the same organism (191); (*e*) CPT-I is

expressed in at least two tissue-specific isoforms (the liver and skeletal muscle form) (189, 191, 193): and (*f*) the acylcarnitine:carnitine exchange activity seems to reside in a single polypeptide (90, 92).

CPT-I: ISOENZYMES AND TISSUE SPECIFIC DISTRIBUTION CPT-I catalyzes the formation of long-chain acylcarnitines from long-chain acyl-CoAs and free carnitine. However, a catalytically active form of CPT-I has not been isolated. The existence of a liver-specific (L-CPT-I) and muscle-specific (M-CPT-I) isoform was suggested by differing IC_{50} values for malonyl-CoA and K_m values for carnitine (109) and, following covalent labeling with specific active-site–directed CPT-I inhibitors, by the differing subunit size of the labeled polypeptides for rat liver and skeletal muscle mitochondria (189, 191, 193). Formal proof of the distinction came with the isolation and expression of the liver (44, 55) and skeletal muscle cDNAs (56, 202). The predicted primary structures show 62.6% homology with calculated molecular masses of 88,150 (773 amino acids) and 88,227 (772 amino acids) for the liver and skeletal muscle isoform, respectively. The expressed proteins showed catalytic activity with kinetic parameters similar to those obtained with the respective intact mitochondria. In like manner, the human counterparts were isolated, characterized, and found to be similar to the rat isoforms (25, 203). The genes encoding human liver and skeletal muscle CPT-I have been assigned to chromosome 11q13 (24, 25) and 22q13.3 (24, 180), respectively, and their structural features have been elucidated (25, 180, 204).

The tissue-specific expression of L-CPT-I and M-CPT-I has been investigated in several organs in the rat (56, 106, 202) and somewhat more selectively in humans. Using specific cDNA probes, the human M-CPT-I form is expressed in skeletal muscle and the heart but not in the kidney and liver, whereas the liver form demonstrates high expression in the kidney but is absent in skeletal muscle (203). The heart predominantly expresses M-CPT-I but also has a relative significant expression of the L-CPT-I isoform (203). From a diagnostic standpoint, human fibroblasts express the liver-type enzyme (25, 106).

CPT-I deficiency The metabolic consequences of a selective CPT-I deficiency would be a decrease in long-chain acylcarnitine content in tissue and an increase in cytoplasmic long-chain acyl-CoA concentration. Long-chain acyl-CoA esters are potent inhibitors of the adenine nucleotide translocase and could inhibit oxidative phosphorylation, leading to a generalized energy deficit. A metabolic alternative for long-chain acyl-CoA esters is incorporation into triglycerides, leading to lipid deposition in the affected tissue. Since CPT-I is expressed as tissue-specific isoforms (106, 203), the phenotypic expression of CPT-I deficiency will depend on whether the muscle or liver isoform is affected. The diagnosis is made by measurement of CPT activity under conditions that allow discrimination between CPT-II (malonyl-CoA insensitive) and CPT-I

(malonyl-CoA sensitive) activity either on biopsy material (tissue homogenate or isolated mitochondria) or in fibroblasts. In humans, fibroblasts express only the liver isoform of CPT-I (25); consequently, activity measurement in these cells is useful in hepatic CPT-I deficiency.

CPT-I deficiency appears to be rare, or potentially lethal and underdiagnosed; only a few cases have been reported (7, 12, 14, 17, 41, 43, 46, 81, 83, 149, 156, 164, 172, 185). With a few exceptions (46, 81, 83, 149), in the small number of cases reported to date the affected enzyme is the liver isoform.

CPT-I deficiency with symptoms of hepatic involvement has been described in seven patients (7, 12, 14, 17, 41, 43, 156, 164, 172, 185). The diagnosis was made by CPT activity determination of cultured fibroblasts and was found to be decreased by approximately 85–90% as compared with control fibroblasts, whereas CPT-II activity was within the range of control values. The prominent clinical feature is hypoketotic hypoglycemia, typically precipitated by fasting in infancy, but usually without myopathy or cardiomyopathy, and if not treated is fatal. The absence of myopathic or cardiomyopathic involvement is reflected biochemically by the presence of an enzyme defect only in the liver and in fibroblasts, not in muscle (14, 43, 172). The low capacity for hepatic fatty acid oxidation also explains the concomitant hypoketonemia. The defect affects long-chain fatty acid oxidation only because the ketogenic response to medium-chain triglycerides was normal (7, 43). The biochemical findings included triglyceride accumulation in the liver (7, 43, 156), with normal liver carnitine (43) but elevated plasma-carnitine content (156, 164, 185). Despite the increased total plasma-carnitine concentration, the acylcarnitine fraction remained low. The organic acid profile is normal, and dicarboxylic aciduria is not associated with CPT-I deficiency.

CPT-I deficiency involving the skeletal muscle isoform of the enzyme has been reported in three patients (46, 81, 83, 149). The clinical manifestation in all cases was recurrent rhabdomyolysis. Consistent with selective muscle involvement there was a normal ketogenic response to fasting (83), lipid deposition in skeletal muscle (83, 149), and partially reduced CPT activity in skeletal muscle (46, 81, 83, 149). The selective CPT-I defect was confirmed by the lack of carnitine-dependent palmitate (46) or palmitoyl-CoA oxidation (81, 149) in skeletal muscle homogenate or isolated mitochondria. The oxidation of palmitoyl-L-carnitine, which is not dependent on CPT-I, was normal in all three cases. The symptoms were precipitated by fasting or exercise (46, 81, 83) and, in one patient, by ibuprofen therapy (149). In the latter case there was also a marked decrease in muscle-carnitine content, which following recovery returned to normal, though muscle CPT activity remained abnormally low. It was concluded that the transiently low muscle-carnitine content combined with the partial deficiency of CPT led to the development of the clinical symptoms.

The molecular basis of both forms of CPT-I has yet to be elucidated. From the limited information available, CPT-I deficiency seems to follow autosomal recessive inheritance (84). With the recent emergence of the relevant cDNA structures (25, 203) and characterization of the genes (25, 180, 204), such information is expected to be forthcoming.

Nutritional intervention Because of the small number of patients affected, a systematic clinical trial is not available. However, there is strong testimonial evidence that frequent feedings, night-time snacks, and avoidance of fasting eliminate or ameliorate the development of the clinical symptoms. Medium-chain triglyceride supplementation in the treatment has been proposed, but its role is unclear. For the less clearly defined muscle form, in one patient avoidance of fasting, a high-carbohydrate/low-fat diet, and modification of exercise pattern were reported to almost eliminate clinical deterioration, to prevent attacks, and to markedly reduce episodes of rhabdomyolysis.

MITOCHONDRIAL ACYLCARNITINE-CARNITINE TRANSLOCASE The mitochondrial acylcarnitine-carnitine translocase was the last member of the mitochondrial carnitine-dependent fatty acid transport pathway to be discovered (131, 137). The properties of the transporter have been extensively investigated in intact mitochondria (88, 133, 179, 196). In addition to the unidirectional transport of carnitine, it catalyzes a 1:1 exchange between acylcarnitines of different chain lengths and carnitine. The transporter protein has been isolated and purified to apparent homogeneity from rat liver mitochondria, and its physical, kinetic, and inhibitory properties determined (90–92). It has an apparent molecular mass of 32.5, and when functionally reconstituted the transporter demonstrates the same properties as in intact mitochondria. The mechanism of transport appears to be of the ping-pong type, which makes the carnitine carrier unique among all the mitochondrial metabolite transporters characterized (91, 130). The amino acid sequence of the rat liver acylcarnitine-carnitine translocase has recently been deduced from sequences of overlapping cDNA clones (89). The full-length translation product consists of 301 amino acid residues with a calculated molecular mass of 33.133, which is in good agreement with the value of 32.5 estimated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and consists of three homologous tandem repeats, a feature common to other mitochondrial transporters. Post-translational modification of the transporter is indicated by the fact that the mature transporter is amino-terminally modified. The proposed secondary structure suggests an asymmetric orientation of the membrane-embedded carnitine carrier, a feature previously suggested on the basis of functional studies, indicating different substrate-binding sites on the inner and outer faces both of intact mitochondria (88) and reconstituted liposomes (91). Southern blot analysis of rat and human genomic DNA suggests that both rat and human genomes contain a single gene for the transporter (89).

Deficiency The acylcarnitine-carnitine translocase deficiency results in defective intramitochondrial transport of acylcarnitines formed by CPT-I and is one of the most severe disorders of fatty acid oxidation. Although the occurrence of translocase deficiency as a clinical entity has been speculated earlier based on indirect evidence (178), a direct diagnosis was made possible only recently, with the development of an assay for translocase activity (119). Only five patients with acylcarnitine-carnitine translocase deficiencies have been reported in the literature (26, 33, 122, 125, 132, 163). The disease shows autosomal recessive inheritance with very early onset and lethal outcome in the perinatal and early infantile period of life. Most of the patients show signs of multiorgan involvement, e.g. liver, heart, and skeletal muscle. The symptoms are triggered by fasting or by ingestion of fat and include hyperammonemia, hypoketotic hypoglycemia, and hypocarnitinemia. A characteristic feature of the disease is a persistent elevation of plasma long-chain acylcarnitines with very low free carnitine. In one patient, elevated plasma short- and medium-chain acylcarnitines were demonstrated (163). This finding would be consistent with one transporter (92). As a consequence, acylcarnitine-carnitine translocase deficiency affects not only mitochondrial fatty acid oxidation, it also severely impairs mitochondrial metabolism in general as a result of its acyl-CoA buffer function. This may explain the severity and the lethal nature of the disease. Clinically and biochemically, acylcarnitine-carnitine translocase deficiency appears similar in presentation and severity to multiorgan (generalized) CPT-II deficiency (42, 85). All reported so far have been diagnosed by measurement of the acylcarnitine-carnitine translocase in the patients' fibroblasts. Direct measurement of CPT-II activity demonstrated that CPT-II is not affected in these cases.

Because of the early and lethal nature of the disorder and the lack of a pathophysiological mechanism, nutritional or other interventions have not been studied. A recent report describes a nonfatal translocase deficiency (6% activity remaining) (127) that was successfully treated with intravenous glucose and carnitine; follow-up treatment was a high-carbohydrate, low-fat diet. The use of carnitine in these patients must be reconsidered because the accumulation of long-chain acylcarnitines are thought to be toxic.

CPT-II CPT-II catalyzes the last step of the carnitine-dependent entry of activated long-chain fatty acids into mitochondria for β-oxidation, i.e. the reconversion of long-chain acylcarnitines to the respective CoA esters. The enzyme is localized on the matrix side of the mitochondrial inner membrane (9, 79, 82) and, in disrupted mitochondria, can be functionally distinguished from CPT-I by its insensitivity toward malonyl-CoA and etomoxiryl/tetradecylglycidyl-CoA inhibition. The enzyme is easily solubilized in the presence of detergents. It has been isolated in catalytically active form from a variety of tissues of different species, and its physical and kinetic properties determined (reviewed in

9, 79). All preparations have an apparent subunit size of 66–68 kDa, were not inhibited by malonyl-CoA, and displayed immunological crossreactivity, which indicates a great degree of homology (191). The full-length cDNA of rat liver mitochondrial CPT-II predicted a protein of 658 amino acid residues (74,119 Da) and contained a 25-amino-acid amino-terminal leader sequence that is cleaved upon mitochondrial import, yielding a mature protein of 71 kDa (27, 192). Expression of the full-length cDNA in a variety of systems (COS cells, insect cells, and yeast lacking endogenous CPT) resulted in the overproduction of malonyl-CoA–insensitive CPT, which had the same properties as the original CPT-protein purified from rat liver mitochondria (44, 93, 192). Since CPT-II mRNA was found to be identical in size (approximately 2.5 kb) and the product was immunologically indistinguishable, it was suggested that the CPT-II protein is the product of a single gene (110, 191). Simultaneously with the isolation of rat liver mitochondrial CPT-II cDNA, the human counterpart was isolated by Finocchiaro et al (59) based on amino acid sequence information obtained from isolated human liver CPT-II (58). The human cDNA also predicted a nascent product of 658 amino acids, and the two proteins showed strong similarity (85% and 82% identity at the nucleotide and amino acid level, respectively). The gene encoding the human liver CPT-II has been localized to chromosome 1p32 (59, 68), and the structure of the gene analyzed in detail (184). The availability of the human CPT-II cDNA and the known structural features of the gene provided the necessary tools and information for the analysis of the molecular basis of CPT-II deficiencies in humans.

CPT-II deficiencies In contrast to the few patients with documented CPT-I deficiency, CPT-II deficiency is more frequent. Although it is generally accepted that CPT-II is expressed as a single protein, the deficiency manifests itself in several clinical phenotypes. Although the typical adult muscle form is by far the most frequent (198), during the past few years a number of new complex phenotypes have been ascribed to CPT-II deficiency. Lethal neonatal CPT-II deficiency presenting with myopathy has been described by Land et al (101); cases of CPT-II deficiency with hypoketotic hypoglycemia, cardiomegaly, dysorganogenesis, and early death have occurred in four neonates and one child (85, 123, 190, 200); and CPT-II deficiency with hepatic involvement in a female and male infant has been reported by Yamamoto et al (201). The phenotypic heterogeneity of CPT-II deficiency is not understood. It has been suggested that the clinical and metabolic consequences of CPT-II deficiency might depend on the residual enzyme activity (42). However, this suggestion does not explain the clinical heterogeneity. For example, residual skeletal muscle CPT-II activity was 7% in a patient with neonatal CPT-II deficiency with an Arg631Cys substitution (170), as well as in one with typical adult

muscular form with a Ser113Leu substitution (169). Furthermore, residual activity of a similar percentage was also found in both patients' lymphoblasts and fibroblasts. A more complex pathogenic mechanism for the different phenotypic expression has been proposed by Taroni et al (169), who considered an altered interaction of the mutated CPT-II with other enzymes of the fatty acid oxidation pathway, or a yet undefined tissue-specific metabolic role of CPT-II. Further research directed toward the identification of other mutations combined with in vitro expressions of the different mutations will be needed to reveal the molecular basis underlying the clinical heterogeneity of the disease.

Muscle form of CPT-II deficiency CPT-II deficiency is the most common disorder of lipid metabolism affecting skeletal muscle, and more than 60 patients have been reported with this disorder (see 198, and references therein, for an extensive review). Classically, the disease presents in young adulthood with recurrent episodes of exercise-induced myoglobinuria. In some, massive myoglobinuria may occur, leading to acute renal failure. Fasting, exposure to cold, or high-fat intake can precipitate the symptoms even without exercise or can contribute to the effects of exercise. Although the enzyme defect is evident in tissues other than skeletal muscle (198), the disease usually presents only with muscular symptomatology.

For the typical adult phenotype, four missense mutations have been reported (169, 184). Three amino acid substitutions (Arg631Cys, Pro50His, and Asp553Asn) appear to be rare, whereas the Ser113Leu mutation appears to be prevalent (169). Of 25 patients studied, 8 were homozygous and 13 heterozygous for the Ser113Leu mutation (169). Two subsequent studies of 22 and 20 patients, respectively, confirmed the high frequency of the Ser113Leu mutation (95, 199). The effect of the Ser113Leu mutation on CPT-II activity was investigated by introducing the substitution into the human CPT-II cDNA and expressing the mutated CPT in COS-1 cells (169). The transfection experiments revealed that the catalytic activity of the mutant enzyme was drastically reduced. The mutation resulted in normal synthesis but a markedly reduced steady-state level of the protein, indicating decreased stability of the mutant CPT-II. Similar transfection experiments with the three rare mutations (Pro50His, Asp553Asn, and Arg631Cys) also demonstrated that these mutations cause a decreased stability of CPT-II, resulting in a markedly reduced steady-state level of enzyme protein and, hence, enzyme activity (170, 184). Kinetic analysis of CPT-II activity in the patients' lymphoblasts carrying the Arg631Cys mutation (170) or in fibroblasts with the Pro50His mutation (184) revealed an unaltered K_m for all substrates in both the forward and reverse direction of the reaction. However, in some patients who present with the typical adult-type muscular form of CPT-II deficiency, the molecular basis is not so

clear. Three other CPT-II–deficient patients with typical adult-type muscular form have been described recently (156, 187). Two patients studied were heterozygous for the common Ser113Leu mutation, and a third was heterozygous for the rare Pro50His mutation. In addition, in a consanguineous family with four members affected with CPT-II deficiency and the homozygous Ser113Leu mutation, the clinical presentation varied from asymptomatic to fatal (75). One member had the typical adult muscular presentation. The combined molecular and functional approaches to the defect are essential in understanding the underlying genetic and pathophysiological basis for CPT-II deficiency.

Another patient was described with an early onset lethal myopathic CPT-II deficiency (101). Total CPT activity in isolated skeletal muscle mitochondria was decreased 75% compared with controls, and the residual activity was inhibited by malonyl-CoA. Thus, the CPT-II enzyme activity was essentially absent with retention of CPT-I. In contrast, total activity in liver mitochondria was inhibited about 52% by malonyl-CoA. Quantitative immunoblotting of CPT-II protein showed that the protein is expressed in skeletal muscle mitochondria. In this patient, the CPT-II deficiency was restricted to skeletal muscle, where a lethal functional loss of activity in a structurally conserved region existed. The molecular defect has not been described.

Because in patients with CPT-II deficiency the enzyme activity is reduced in fibroblasts and lymphoblasts (42, 169, 170, 184) as well as in muscle, the diagnosis can be made by measuring CPT-II activity (malonyl-CoA insensitive) on any of the above tissues.

Early onset hepatic and multiorgan forms Neonatal presentations of CPT-II deficiency in the first few days of life has been described (85, 123, 190, 200). A common feature is the increased long-chain acylcarnitine content of plasma and tissues with marked lipid accumulation. The activity of CPT-II is markedly, but variably, decreased with normal CPT-I activity. Cystic dysplasia has been described in three of these patients (123, 190, 200).

The original description of a fatal infantile multiorgan CPT-II deficiency documented impaired long-chain fatty acid oxidation in lymphoblasts and fibroblasts (42). The activity of CPT-II was 10% of control, with normal CPT-I activity. The CPT-II protein was barely detectable by immunoprecipitation, and biosynthesis of the CPT-II protein in fibroblasts was reduced. Recently, a Tyr628Ser substitution was identified (13). The expressed protein with this defect has only 10% of activity consistent with the activity determinations in the patient's material.

The molecular basis of the early onset form of CPT-II deficiency has been studied by Taroni et al (170) in a 5-year-old patient presenting with hypoketotic hypoglycemia and cardiomyopathy. Oxidation of palmitate was decreased (less then 25% of normal), but oxidation of octanoate and butyrate was normal.

Although there was no impairment of CPT-I activity, CPT-II activity was reduced to 16%, 9%, and 7% of normal controls in fibroblasts, lymphoblasts, and skeletal muscle, respectively. CPT-II activity was also reduced in the parent's fibroblasts by 40% and 35%, which suggests an autosomal recessive inheritance. Kinetic analysis of CPT-II activity in lymphoblast homogenate from the patient revealed a significant reduction of the estimated V_{max} but no changes in the apparent K_m values, determined both in the forward and backward direction. Metabolic labeling studies showed normal biosynthesis of CPT-II protein; however, the stability of the mutant enzymes was greatly reduced. Genomic and cDNA analysis demonstrated that the patient was homozygous for a mutant CPT-II allele (ICV allele) carrying three missense mutations, resulting in the following substitutions: Val368Ile, Arg631Cys, and Met647Val. Although the mutations Val368Ile and Met647Val represent sequence polymorphism, the Arg631Cys was not detected in 22 healthy individuals nor in 12 of 14 patients with the adult muscular form of CPT-II deficiency. To correlate these mutations with the CPT-II activity, the three mutations were individually expressed in COS cells, and the enzyme activity determined. The Arg631Cys substitution resulted in drastic reduction of CPT-II activity, whereas neither of the two other mutations affected the CPT-II activity. However, they exacerbated the effects of the Arg631Cys if all three were coexpressed.

Additional examples of nonfatal early onset CPT-II deficiency with hepatic presentation were described in two siblings by Yamamoto at al (201). Both patients were hospitalized by an illness triggered by fever and had hepatomegaly with hypoglycemia and hypoketonemia. In one patient, a liver biopsy revealed macrovesicular steatosis. The CPT-I activity in the lymphoblasts of the female patient was normal (for the male patient the activity was not reported), whereas CPT-II activity was 3% of the control value for both patients. Both recovered after intravenous treatment with glucose and fluid. cDNA sequence analysis revealed four single-base substitutions, with three (Phe352Cys, Val368Ile, and Phe383Tyr) on one allele and the fourth (Glu174Lys) on the other. Two of these mutations (Val368Ile and Phe352Cys) represent sequence polymorphism (169); the other two mutations (Glu174Lys and Phe383Tyr) are probably related to the pathogenesis of the disease. Based on prediction analysis, the authors suggested that a change in the secondary structure of CPT-II due to extension of the length of the α-helix (Glu174Lys) and change in the direction of turn (Phe383Tyr) could cause the hepatic form of CPT-II deficiency.

NUTRITIONAL INTERVENTION

There are no systematic controlled therapeutic trials in CPT-I, CPT-II, or translocase deficiency. Avoidance of the triggering events is generally recommended. Glucose and fluids are the mainstay in the emergent hypoglycemia of these

disorders or the presentation of rhabdomyolysis. Most patients with hepatic CPT-I, presumed muscular CPT-I, and muscular CPT-II deficiency benefit from high-carbohydrate and low-fat diets with moderation of life style, including avoidance of fasting. Recent report of a nonfatal translocase deficiency (6% activity remaining) (127) also utilized this approach but added carnitine. It seems that treatment with carnitine of the translocase and CPT-II deficiency is contraindicated because long-chain acylcarnitines are already increased and further increases in these potential toxic compounds should be avoided.

SUMMARY AND FUTURE DIRECTIONS

The molecular basis for most genetic disorders of carnitine metabolism is not known, because the carnitine carrier proteins and their genes have yet to be isolated and characterized. The recent descriptions and expression of the cDNAs for the liver and muscle isoforms of CPT-I, even though a catalytically active protein is still not available, and additionally for the carnitine-acylcarnitine translocase forecast a rapid characterization of the defects in the carnitine-dependent transport of long-chain fatty acids into mitochondria. Advances in the molecular analysis of CPT-II defects are occurring rapidly and highlight our incomplete understanding. The integration of the mitochondrial carnitine-dependent transport system should be elucidated from these exciting studies.

ACKNOWLEDGMENTS

We thank Drs. Edward Lesnefsky and John Mieyal for reviewing this manuscript. This research was supported by the Medical Research Service of the Department of Veterans Affairs and NIH grant DK-36069.

Literature Cited

1. Angelini C, Trevisan G, Isaya G, Pegolo G, Vergani L. 1987. Clinical varieties of carnitine and carnitine palmitoyltransferase deficiency. *Clin. Biochem.* 20:1–7
2. Angelini C, Vergani L, Martinuzzi A. 1992. Clinical and biochemical aspects of carnitine deficiency and insufficiency: transport defects and inborn errors of β-oxidation. *Crit. Rev. Clin. Lab. Sci.* 29:217–42
3. Bahl J, Navin T, Manian AA, Bressler R. 1981. Carnitine transport in isolated adult rat heart myocytes and the effect of 7,8-diOH chlorpromazine. *Circ. Res.* 48: 378–85
4. Bahl JJ, Bressler R. 1987. The pharmacology of carnitine. *Annu. Rev. Pharmacol. Toxicol.* 27:257–77
5. Barth PG, Scholte HR, Berden JA, et al. 1983. An X-linked mitochondrial disease affecting cardiac muscle, skeletal muscle and leucocytes. *J. Neurol. Sci.* 62:327
6. Bennett M, Hale DE, Pollitt RJ, Stanley CA, Variend S. 1996. Endocardial fibroelastosis and primary carnitine deficiency due to a defect in plasma membrane carnitine transporter. *Clin. Cardiol.* 19:243–46

7. Bergman AJ, Donckerwolke RA, Duran A, Smeitink JA, Mousson B, et al. 1994. Rate-dependent distal renal tubular acidosis and carnitine palmitoyltransferase I deficiency. *Pediatr. Res.* 36:582–88
8. Bernardini I, Rizzo WB, Dalakas M, Bernar J, Gahl WA. 1985. Plasma and muscle free carnitine deficiency due to renal Fanconi syndrome. *J. Clin. Invest.* 75:1124–30
9. Bieber LL. 1988. Carnitine. *Annu. Rev. Biochem.* 57:261–83
10. Bieber LL, Emaus R, Valkner K, Farrell S. 1982. Possible functions of short-chain and medium-chain carnitine acyltransferases. *Fed. Proc.* 41:2858–62
11. Bohmer T, Eiklid K, Jonsen J. 1977. Carnitine uptake into human heart cells in culture. *Biochim. Biophys. Acta* 465:627–33
12. Bonnefont J-P, Haas R, Wolff J, Thuy LP, Buchta R, et al. 1989. Deficiency of carnitine palmitoyltransferase I. *J. Child. Neurol.* 4:198–203
13. Bonnefont J-P, Taroni F, Cavadini P, Cepanec C, Brivet M, et al. 1996. Molecular analysis of carnitine palmitoyltransferase II deficiency with hepatocardiomuscular expression. *Am. J. Hum. Genet.* 58:971–78
14. Bonnefont J-P, Tein I, Saudubray J-M, Demaugre F. 1990. Hepatic and muscular forms of palmitoyl carnitine transferase deficiency. In *Fatty Acid Oxidation: Clinical, Biochemical, Molecular Aspects*, ed. K Tanaka, PM Coates, pp. 451–56. New York: Liss
15. Borum P. 1983. Carnitine. *Annu. Rev. Nutr.* 3:233–59
16. Borum PR, ed. 1986. *Clinical Aspects of Human Carnitine Deficiency*. New York: Pergamon
17. Bougneres PF, Saudubray JM, Marsac C, Bernard O, Odievre M, Girard J. 1981. Fasting hypoglycemia resulting from hepatic carnitine palmitoyltransferase deficiency. *J. Pediatr.* 98:742–46
18. Bremer J. 1961. Biosynthesis of carnitine in vivo. *Biochim. Biophys. Acta* 48:622–24
19. Bremer J. 1962. Carnitine in intermediary metabolism. The metabolism of fatty acid esters of carnitine by mitochondria. *J. Biol. Chem.* 237:3628–32
20. Bremer J. 1963. Carnitine in intermediary metabolism. The biosynthesis of palmitoylcarnitine by cell subfractions. *J. Biol. Chem.* 238:2774–79
21. Bremer J. 1983. Carnitine—metabolism and function. *Physiol. Rev.* 63:1420–80
22. Bremer J, Osmundsen H. 1984. Fatty acid oxidation and its regulation. In *Fatty Acid Metabolism and Its Regulation*, ed. S Numa, pp. 113–54. New York: Elsevier
23. Breningstall GN. 1990. Carnitine deficiency syndromes. *Pediat. Neurol.* 6:75–81
24. Britton CH, Mackey DW, Esser V, Foster DW, Burns DK, et al. 1997. Fine chromosome mapping of the genes for human liver and muscle carnitine palmitoyltransferase I (CPT1A and CPT1B). *Genomics* 40:209–11
25. Britton CH, Schultz RA, Zhang B, Esser V, Foster DW, McGarry JD. 1995. Human liver mitochondrial carnitine palmitoyltransferase I: characterization of its cDNA and chromosomal localization and partial analysis of the gene. *Proc. Natl. Acad. Sci. USA* 92:1984–88
26. Brivet M, Slama A, Ogier H, Bourton A, Demaugre F, et al. 1994. Diagnosis of carnitine acylcarnitine translocase deficiency by complemetation analysis. *J. Inherit. Metab. Dis.* 17:271–74
27. Brown NF, Esser V, Gonzalez AD, Evans CT, Slaughter CA, et al. 1991. Mitochondrial import and processing of rat liver carnitine palmitoyltransferase II defines the amino terminus of the mature protein: possibility of differential modification of the rat and human isoforms. *J. Biol. Chem.* 266:15446–49
28. Busch HFM, Scholte HR, Arts WF, Luyt-Houwen IEM. 1981. A mitochondrial myopathy with a respiratory chain defect and carnitine deficiency. In *Mitochondria and Muscle Diseases*, ed. HFM Busch, FGI Jennekens, HR Scholte, pp. 207–11. Beetsterzwag, The Netherlands: Mefar
29. Carnicero HH, Englard S, Seifter S. 1982. Carnitine uptake and fatty acid utilization by diploid cells aging in culture. *Arch. Biochem. Biophys.* 215:78–88
30. Carter AL, ed. 1992. *Current Concepts in Carnitine Research*. Boca Raton, FL: CRC
31. Carter HE, Bhattacharyya PK, Weidman KR, Fraenkel G. 1952. Chemical studies on vitamin BT isolation and characterization as carnitine. *Arch. Biochem. Biophys.* 38:405–16
32. Chalmers RA, Roe CR, Stacey TE, Hoppel CL. 1984. Urinary excretion of L-carnitine and acylcarnitines by patients with disorders of organic acid metabolism: evidence for secondary insufficiency of L-carnitine. *Pediatr. Res.* 18:1325–28
33. Chalmers RA, Stanley CA, English N, Wiggleworth JS. 1997. Mitochondrial

carnitine-acylcarnitine translocase deficiency presenting as sudden neonatal death. *J. Pediatr.* 131:220–25
34. Christiansen RZ, Bremer J. 1976. Active transport of butyrobetaine and carnitine into isolated liver cells. *Biochim. Biophys. Acta* 448:562–77
35. Christodoulou J, Teo SH, Hammond J, Sim KG, Hsu BYL, et al. 1996. First prenatal diagnosis of the carnitine transporter defect. *Am. J. Med. Genet.* 66:21–24
36. Clark JB, Hayes DJ, Morgan-Hughes JA, Byrne E. 1984. Mitochondrial myopathies: disorders of the respiratory chain and oxidative phosphorylation. *J. Inherit. Metab. Dis.* 7(Suppl. 1):62–68
37. Cox RA, Hoppel CL. 1974. Carnitine and trimethylaminobutyrate synthesis in the rat tissues. *Biochem. J.* 142:699–701
38. Cox RA, Hoppel CL. 1974. Carnitine biosynthesis in rat liver slices. *Biochim. Biophys. Acta* 362:403–13
39. Davies AT, Hoppel CL. 1986. Effect of starvation on the disposition of free and peptide-linked trimethyllysine in the rat. *J. Nutr.* 116:760–67
40. Declercq PE, Falck JR, Kuwajima M, Tyminski H, Foster DW, McGarry JD. 1987. Characterization of the mitochondrial carnitine palmitoyltransferase enzyme system. I. Use of inhibitors. *J. Biol. Chem.* 262:9812–21
41. Demaugre F, Bonnefont J-P, Cepanec C, Scholte J, Saudubray JN, Leroux JP. 1990. Immunoquantitative analysis of human carnitine palmitoyltransferase I and II defects. *Pediatr. Res.* 27:497–500
42. Demaugre F, Bonnefont J-P, Colonna M, Cepanec C, Leroux J-P, Saudubray J-M. 1991. Infantile form of carnitine palmitoyltransferase II deficiency with hepatomuscular symptoms and sudden death: physiopathological approach to carnitine palmitoyltransferase II deficiencies. *J. Clin. Invest.* 87:859–64
43. Demaugre F, Bonnefont J-P, Mitchell G, NguyenHoang N, Pelet A, et al. 1988. Hepatic and muscular presentations of carnitine palmitoyltransferase deficiency: two distinct entities. *Pediatr. Res.* 24:308–12
44. DeVries Y, Arvidson DN, Waterham HR, Cregg JM, Woldegiorgis G. 1997. Functional characterization of mitochondrial carnitine palmitoyltransferases I and II expressed in yeast *Pichia pastoris*. *Biochemistry* 36:5285–92
45. DiDonato S. 1994. Disorders of lipid metabolism affecting skeletal muscle: carnitine deficiency syndromes, defects in catabolic pathway, Chanarin disease. In *Myology*, ed. AG Engel, C Franzini-Armstrong, pp. 1587–609. New York: McGraw-Hill. 2nd ed.
46. DiMauro S, Melis-DiMauro PM. 1973. Muscle carnitine palmitoyltransferase deficiency and myoglobinuria. *Science* 182: 929–31
47. DiMauro S, Trevisan C. 1982. Carnitine palmitoyltransferase (CPT) deficiency: a review. In *Disorders of the Motor Unit*, ed. DL Schotland, pp. 657–66. New York: Wiley
48. Dunn WA, Aronson NN, Englard S. 1981. Carnitine biosynthesis by the perfused rat liver from exogenous protein-bound trimethyllysine. Metabolism of methylated lysine derivatives arising from degradation 6-N-[methyl-^{3}H]lysine-labeled glycoprotein. *J. Biol. Chem.* 256:12437–44
49. Engel AG. 1986. Carnitine deficiency syndromes and lipid storage myopathies. In *Myology*, ed. AG Engel, BQ Benker, pp. 1663–96. New York: McGraw-Hill
50. Engel AG, Angelini C. 1973. Carnitine deficiency of human skeletal muscle with associated lipid storage myopathy: a new syndrome. *Science* 179:899–902
51. Engel AG, Rebouche CJ, Wilson DM, Glasgow AM, Romshe CA, Cruse RP. 1981. Primary systemic carnitine deficiency. II. Renal handling of carnitine. *Neurology* 31:819–25
52. Englard S. 1979. Hydroxylation of γ-butyrobetaine to carnitine in human and monkey tissues. *FEBS Lett.* 102:297–300
53. Erikson BO, Lindstedt S, Nordin I. 1988. Hereditary defect in carnitine membrane transport is expressed in skin fibroblasts. *Eur. J. Pediatr.* 147:662–68
54. Esser V, Britton CH, Weis BC, Foster DW, McGarry JD. 1994. Cloning, sequencing, expression of a cDNA encoding rat liver carnitine palmitoyltransferase I: direct evidence that a single polypeptide is involved in inhibitor interaction and catalytic function. *J. Biol. Chem.* 268:5817–22
55. Eriksson BO, Gustafson B, Lindstedt S, Nordin I. 1989. Transport of carnitine into cells in hereditary carnitine deficiency. *J. Inherit. Metab. Dis.* 12:108–11
56. Esser V, Brown NF, Cowan AT, Foster DW, McGarry JD. 1996. Expression of a cDNA isolated from rat brown adipose tissue and heart identifies the product as the muscle isoform of carnitine palmitoyltransferase I (M-CPT I): M-CPT I is the predominant CPT I isoform expressed in both white (epididymal) and brown

adipocytes. *J. Biol. Chem.* 271:6972–77
57. Ferrari R, DiMauro S, Sherwood G, eds. 1992. L-*Carnitine and Its Role in Medicine: From Function to Therapy*. New York: Academic
58. Finocchiaro G, Colombi I, DiDonato S. 1990. Purification, characterization and partial amino acid sequences of carnitine palmitoyl-transferase from human liver. *FEBS Lett.* 274:163–66
59. Finocchiaro G, Taroni F, Rocchi M, Martin AL, Colombo I, et al. 1991. cDNA cloning, sequence analysis, chromosomal localization of the gene for human carnitine palmitoyltransferase. *Proc. Natl. Acad. Sci. USA* 88:661–65
60. Fraenkel G, Friedman S. 1957. Carnitine. In *Vitamins and Hormones*, ed. RS Harris, GF Marian, KV Thiman, 15:73–118. New York: Academic
61. Frenkel RA, McGarry JD, eds. 1980. *Carnitine Biosynthesis, Metabolism, Functions*. New York: Academic
62. Friedman S, Fraenkel G. 1955. Reversible enzymatic acetylation of carnitine. *Arch. Biochem. Biophys.* 59:491–501
63. Fritz IB. 1955. The effect of muscle extracts on the oxidation of palmitic acid by liver slices and homogenates. *Acta Physiol. Scand.* 34:367–85
64. Fritz IB. 1963. Carnitine and its role in fatty acid metabolism. *Adv. Lipid Res.* 1:285–334
65. Fritz IB, Yu KTN. 1963. Long-chain carnitine acyltransferase and the role of acylcarnitine derivatives in the catalytic increase of fatty acid oxidation induced by carnitine. *J. Lipid Res.* 4:279–88
66. Gahl WA, Bernardini I, Dalakas M, Rizzo WB, Harper GS, et al. 1988. Oral carnitine therapy in children with cystinosis and renal Fanconi syndrome. *J. Clin. Invest.* 81:549–60
67. Garavaglia B, Uziel G, Dworzak F, Carrara F, DiDonato S. 1991. Primary carnitine deficiency: heterozygote and intrafamilial variation. *Neurology* 41:1691–93
68. Gellera C, Verderio E, Floridia G, Finocchiro G, Montermini L, et al. 1994. Assignment of the human carnitine palmitoyltransferase II gene (CPT I) to chromosome 1p32. *Genomics* 24:195–97
69. Gross CJ, Henderson LM. 1984. Absorption of D- and L-carnitine by the intestine and kidney tubule in the rat. *Biochim. Biophys. Acta* 772:209–19
70. Gross CJ, Savaiano DA. 1993. Effect of development and nutritional state on the uptake, metabolism and release of free and acetyl-L-carnitine by the rodent small intestine. *Biochim. Biophys. Acta* 1170:265–74
71. Gudjonsson H, Li BUK, Shug A, Olsen WA. 1985. In vivo studies of intestinal carnitine absorption in rats. *Gastroenterology* 88:1880–87
72. Gudjonsson H, Li BUK, Shug A, Olsen WA. 1985. Studies of carnitine metabolism in relation to intestinal absorption. *Am. J. Physiol.* 248:G313–19
73. Hamilton JW, Li BUK, Shug A, Olsen WA. 1983. Studies of L-carnitine absorption in man. *Gastroenterology* 84(2):1180
74. Hamilton JW, Li BUK, Shug A, Olsen WA. 1986. Carnitine transport in human intestinal biopsy specimens. *Gastroenterology* 91:10–16
75. Handig I, Dams E, Taroni F, Van Laere S, de Barsy T, Willems PJ. 1996. Inheritance of the S113L mutation within an inbred family with carnitine palmitoyltransferase enzyme deficiency. *Hum. Genet.* 97:291–93
76. Hochalter JB, Henderson LM. 1976. Carnitine biosynthesis: the formation of glycine from carbons 1 and 2 of 6-N-trimethyl-L-lysine. *Biochim. Biophys. Res. Commun.* 70:364–66
77. Hokland BM. 1988. Uptake, metabolism and release of carnitine and acylcarnitines in the perfused liver. *Biochim. Biophys. Acta* 961:234–41
78. Hoppel CL. 1976. Carnitine palmitoyltransferase and transport of fatty acids. In *The Enzymes of Biological Membranes*, ed. A Martonosi, 2:119–43. New York: Plenum
79. Hoppel CL, Brady L. 1985. Carnitine palmitoyltransferase and transport of fatty acids. In *The Enzymes of Biological Membranes*, ed. AN Martonosi, 2:139–75. New York: Plenum
80. Hoppel CL, Cox RA, Novak RF. 1980. N^6-trimethyl-lysine metabolism. 3-Hydroxy-N^6-trimethyl-lysine and carnitine biosynthesis. *Biochem. J.* 188:509–19
81. Hoppel CL, Genuth S, Brass E, Fuller R, Hostetler K. 1980. Carnitine and carnitine palmitoyltransferase in metabolic studies. See Ref. 61, pp. 287–305
82. Hoppel CL, Tomec RJ. 1972. Carnitine palmitoyltransferase. Location of two enzymatic activities in rat liver mitochondria. *J. Biol. Chem.* 247:832–41
83. Hostetler KY, Hoppel CL, Romine JS, Sipe JC, Gross SR, Higginbottom PA. 1978. Partial deficiency of muscle carnitine palmitoyltransferase with normal ketone production. *N. Engl. J. Med.* 298: 553–57

84. Hostetler KY, Yazaki PJ. 1980. Deficiency of carnitine palmitoyltransferase in transformed lymphoblasts from a patient having a deficiency of carnitine palmitoyltransferase in skeletal muscle. *Biochem. Biophys. Res. Commun.* 94:270–77
85. Hug G, Bove KE, Soukup S. 1991. Lethal neonatal multiorgan deficiency of carnitine palmitoyltransferase II. *N. Engl. J. Med.* 325:1862–64
86. Hulse JD, Ellis SR, Henderson LM. 1978. Carnitine biosynthesis. β-Hydroxylation of trimethylysine by an α-ketoglutarate-dependent mitochondrial dioxygenase. *J. Biol. Chem.* 253:1654–59
87. Hulse JD, Henderson LM. 1980. Carnitine biosynthesis. Purification of 4-N-trimethylaminobutyraldehyde dehydrogenase from beef liver. *J. Biol. Chem.* 255:1146–51
88. Idell-Wenger JA. 1981. Carnitine:acylcarnitine translocase of rat heart mitochondria. *J. Biol. Chem.* 256:5597–603
89. Indiveri C, Iacobazzi V, Giangregorio N, Palmieri F. 1997. The mitochondrial carnitine carrier protein: cDNA cloning, primary structure and comparison with other mitochondrial transporter proteins. *Biochem. J.* 321:713–19
90. Indiveri C, Tonazzi A, Palmieri F. 1990. Identification and purification of the carnitine carrier from rat liver mitochondria. *Biochim. Biophys. Acta* 1020:81–86
91. Indiveri C, Tonazzi A, Palmieri F. 1994. The reconstituted carnitine carrier from rat liver mitochondria: evidence for a transport mechanism different from that of the other mitochondrial translocators. *Biochim. Biophys. Acta* 1189:65–73
92. Indiveri C, Tonazzi A, Prezioso G, Palmieri F. 1991. Kinetic characterization of the reconstituted carnitine carrier from rat liver mitochondria. *Biochim. Biophys. Acta* 1065:231–38
93. Johnson TM, Mann WR, Dragland CJ, Anderson RC, Nemecek GM, Bell PA. 1995. Over-expression and characterization of active recombinant rat liver carnitine palmitoyltransferase II using baculovirus. *Biochem. J.* 309:689–93
94. Karpati G, Carpenter S, Engel AG, Watters G, Allen J, et al. 1975. The syndrome of systemic carnitine deficiency. *Neurology* 25:16–24
95. Kaufmann P, El-Schahawi M, DiMauro S. 1997. Carnitine palmitoyltransferase II deficiency: diagnosis by molecular analysis of blood. *Mol. Cell. Biochem.* 174:237–39
96. Kispal Gy, Melegh B, Alkonyi I, Sandor A. 1987. Enhanced uptake of carnitine by perfused rat liver following starvation. *Biochim. Biophys. Acta* 896:96–102
97. Kjernes U, Molstad P. 1981. The effect of sulfhydryl blocking agents on the uptake of L-carnitine in an established human cell line (CCL 27). *Scand. J. Clin. Lab. Invest.* 41:209–13
98. Kolodziej MP, Crilly PJ, Corstorphine CG, Zammit VA. 1990. Development and characterization of a polyclonal antibody against rat liver mitochondrial overt carnitine palmitoyltransferase (CPT I). *Biochem. J.* 282:415–21
99. Kunau W-H, Dommes V, Schulz H. 1995. β-Oxidation of fatty acids in mitochondria, peroxisomes, bacteria: a century of continued progress. *Prog. Lipid Res.* 34:267–342
100. Labadie J, Dunn WA, Aronson NN. 1976. Hepatic synthesis of carnitine from protein-bound trimethyllysine. Lysosomal digestion of methyl-lysine-labeled asialo-fetuin. *Biochem. J.* 160:85–95
101. Land JM, Mistry S, Wquier M, Hope P, Ghadiminejad I, et al. 1995. Neonatal carnitine palmitoyltransferase-2 deficiency: a case presenting with myopathy. *Neuromusc. Disord.* 5:129–37
102. Lange HW, Lower R, Hempel K. 1973. Quantitative determination of N^{ε}-methylated lysins in human plasma and urine. *Hoppe-Seyler's Z. Physiol. Chem.* 354:117–20
103. Li BUK, Bummer PM, Hamilton JW, Gudjonsson H, Zografi G, Olsen WA. 1990. Uptake of L-carnitine by rat jejunal brush border microvillous membrane vesicles. *Dig. Dis. Sci.* 35:333–39
104. Lindstedt S, Nordin I. 1984. Multiple isoforms of γ-butyrobetaine hydroxylase (EC 1.14.11.1). *Biochem. J.* 223:119–27
105. Martinuzzi A, Vergani L, Rosa M, Angelini C. 1991. L-Carnitine uptake in differentiating human cultured muscle. *Biochim. Biophys. Acta* 1095:217–22
106. McGarry JD, Brown NF. 1997. The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. *Eur. J. Biochem.* 244:1–14
107. McGarry JD, Foster DW. 1979. In support of the roles of malonyl-CoA and carnitine acyltransferase I in the regulation of hepatic fatty acid oxidation and ketogenesis. *J. Biol. Chem.* 254:8163–68
108. McGarry JD, Leatherman GF, Foster DW. 1978. Carnitine palmitoyltransferase I. The site of inhibition of hepatic fatty acid oxidation by malonyl-CoA. *J. Biol. Chem.* 253:4128–36

109. McGarry JD, Mills SE, Long CS, Foster DW. 1983. Observations on the affinity for carnitine, malonyl-CoA sensitivity, of carnitine palmitoyltransferase I in animal and human tissues. Demonstration of the presence of malonyl-CoA in non-hepatic tissues of the rat. *Biochem. J.* 214:21–28
110. McGarry JD, Sen A, Esser V, Woeltje KF, Weis B, Foster DW. 1991. New insights into the mitochondrial carnitine palmitoyltransferase system. *Biochimie* 73:77–84
111. McGarry JD, Takabayashi Y, Foster DW. 1978. The role of malonyl-CoA in the coordination of fatty acid synthesis and oxidation in isolated hepatocytes. *J. Biol. Chem.* 253:8294–300
112. McGarry JD, Woeltje KF, Kuwajima M, Foster DW. 1989. Regulation of ketogenesis and the renaissance of carnitine palmitoyltransferase. *Diabetes Metab. Rev.* 5:271–84
113. McLoud E, Ma TY, Grant KE, Mathis RK, Said HM. 1996. Uptake of L-carnitine by a human intestinal epithelial cell line, Caco-2. *Gastroenterology* 111:1534–40
114. Mesmer OT, Lo TCY. 1990. Hexose transport properties of myoblasts isolated from a patient with suspected muscle carnitine deficiency. *Biochem. Cell Biol.* 68:1372–79
115. Molstad P. 1980. The efflux of L-carnitine from cells in culture (CCL 27). *Biochim. Biophys. Acta* 597:166–73
116. Molstad P, Bohmer T. 1979. Transport of L-carnitine induced by prednisolone in an established cell line (CCL 27). *Biochim. Biophys. Acta* 585:94–99
117. Molstad P, Bohmer T, Eiklid K. 1977. Specificity and characteristics of the carnitine transport in human heart cells (CCL 27) in culture. *Biochim. Biophys. Acta* 471:296–304
118. Muller-Hocker J, Pongratz D, Deufel Th, Trijbels JMF, Endres W, Hubner G. 1983. Fatal lipid storage myopathy with deficiency of cytochrome-c-oxidase and carnitine: a contribution to the combined cytochemical-fine-structural identification of cytochrome-c-oxidase in long term frozen muscle. *Virchows Arch. Pathol. Anat. Physiol.* 399:11–23
119. Murthy MSR, Kammana VS, Pande SV. 1986. A carnitine/acylcarnitine translocase assay applicable to biopsied specimens without requiring mitochondrial isolation. *Biochem. J.* 236:143–48
120. Murthy MSR, Pande SV. 1987. Malonyl-CoA binding site and overt carnitine palmitoyltransferase activity reside on opposite sides of the mitochondrial membrane. *Proc. Natl. Acad. Sci. USA* 84:378–82
121. Netzloff ML, Kohrman AF, Jones MZ, Emaus RK, Bieber LL, DiMauro S. 1982. Carnitine deficiency and renal Fanconi syndrome. *Pediatr. Res.* 16:262A
122. Nienzen-Koning KE, Van Spronsen FJ, Iljst L, Wanders RJA, Brivet M, et al. 1995. Patient with lethal cardiomyopathy and carnitine-acylcarnitine translocase deficiency. *J. Inherit. Metab. Dis.* 18:230–32
123. North KN, Hoppel CL, De Girolami U, Kozakewich HP, Korson MS. 1995. Lethal neonatal deficiency of carnitine palmitoyltransferase II associated with dysgenesis of the brain and kidneys. *J. Pediatr.* 127:414–20
124. Novak RF, Swift TJ, Hoppel CL. 1980. N^6-trimethyl-lysine metabolism. Structural identification of the metabolite 3-hydroxy-N^6-trimethyllysine. *Biochem. J.* 188:521–27
125. Ogier de Baulny H, Slama A, Touati G, Turnbull DM, Pourfarzam M, Brivet M. 1995. Neonatal hyperammonemia caused by a defect of carnitine-acylcarnitine translocase. *J. Pediatr.* 127:723–28
126. Ohtani Y, Nishiyama S, Matsude I. 1984. Renal handling of free and acylcarnitine in secondary carnitine deficiency. *Neurology* 34:977–79
127. Olpin SE, Bonham JR, Downing M, Manning NJ, Pollitt RJ, et al. 1997. Carnitine-acylcarnitine translocase deficiency—a mild phenotype. *J. Inherit. Metab. Dis.* 20:714–15
128. Olson AL, Rebouche CJ. 1987. γ-Butyrobetaine hydroxylase activity is not rate limiting for carnitine biosynthesis in the human infant. *J. Nutr.* 117:1024–31
129. Paik WK, Kim S. 1975. Protein methylation: chemical, enzymological, biological significance. *Adv. Enzymol. Relat. Areas Mol. Biol.* 42:227–86
130. Palmieri F, Indiveri C, Bisaccia F, Kramer R. 1993. Functional properties of purified and reconstituted mitochondrial metabolite carriers. *J. Bioenerg. Biomembr.* 25:525–35
131. Pande SVA. 1975. A mitochondrial carnitine acylcarnitine translocase system. *Proc. Natl. Acad. Sci. USA* 72:883–87
132. Pande SV, Brivet M, Slama A, Demaugre F, Aufrant C, Saudubray J-M. 1993. Carnitine-acylcarnitine translocase deficiency with severe hypoglycemia and auriculo ventricular block. *J. Clin. Invest.* 91:1247–52
133. Pande SV, Parvin R, 1980. Carnitine-

acylcarnitine translocase-mediated transport of fatty acids into mitochondria: its involvement in the control of fatty acid oxidation in liver. See Ref. 61, pp. 143–57
134. Pearson DJ, Tubbs PK. 1967. Carnitine and derivatives in rat tissues. *Biochem. J.* 105:953–63
135. Pons R, DeVivo DC. 1995. Primary and secondary carnitine deficiency syndromes. *J. Child Neurol.* 10:2S8–24
136. Quistad GB, Staiger LE, Schooley DA. 1986. The role of carnitine in the conjugation of acidic xenobiotics. *Drug Metab. Dispos.* 14:521–26
137. Ramsay RR, Tubbs PK. 1975. The mechanism of fatty acid uptake by heart mitochondria: an acylcarnitine-carnitine exchange. *FEBS Lett.* 54:21–25
138. Rebouche CJ. 1977. Carnitine movement across muscle cell membranes. Studies in isolated rat muscle. *Biochim. Biophys. Acta* 471:145–55
139. Rebouche CJ, 1992. Carnitine function and requirements during life cycle. *FASEB J.* 6:3379–86
140. Rebouche CJ, Bosch EP, Chenard CA, Schabold KJ, Nelson SE. 1989. Utilization of dietary precursors for carnitine biosynthesis in human adults. *J. Nutr.* 119:1907–13
141. Rebouche CJ, Engel AG. 1980. Tissue distribution of carnitine biosynthetic enzymes in man. *Biochim. Biophys. Acta* 630:22–29
142. Rebouche CJ, Engel AG. 1982. Carnitine transport in cultured muscle cells and skin fibroblasts from patients with primary systemic carnitine deficiency. *In Vitro* 18:495–500
143. Rebouche CJ, Lehman LJ, Olson AL. 1986. ε-N-trimethyllysine availability regulates the rate of carnitine biosynthesis in the growing rat. *J. Nutr.* 116:751–59
144. Rebouche CJ, Mack DL. 1984. Sodium gradient-stimulated transport of L-carnitine into renal brush border membrane vesicles: kinetics, specificity, regulation by dietary carnitine. *Arch. Biochem. Biophys.* 235:393–402
145. Rebouche CJ, Paulson DJ. 1986. Carnitine metabolism and function in humans. *Annu. Rev. Nutr.* 6:41–66
146. Rimoldi M, Boittacchi E, Rossi L, Cornelio F, Uziel G, DiDonato S. 1982. Cytochrome c oxidase deficiency in muscles of a floppy infant without mitochondrial myopathy. *J. Neurol.* 227:201
147. Rinaldo P, Stanley CA, Hsu BYL, Sanchez LA, Stern HJ. 1997. Sudden infant death in carnitine transporter deficiency. *J. Pediatr.* 131:304–5
148. Rodriguez-Pereira R, Scholte HR, Luyt-Houven IEM, Vaandrager-Verduin MHM. 1988. Cardiomyopathy associated with carnitine loss in kidneys and small intestine. *Eur. J. Pediatr.* 148:193–97
149. Ross NS, Hoppel CL. 1987. Partial muscle carnitine palmitoyltransferase-A deficiency. Rhabdomyolysis associated with transiently decreased muscle carnitine content after ibuprofen therapy. *JAMA* 257:62–65
150. Sachan DS, Hoppel CL. 1980. Carnitine biosynthesis. Hydroxylation of N^6-trimethyl-lysine to 3-hydroxy-N^6-trimethyl-lysine. *Biochem. J.* 188:529–34
151. Saggerson D, Ghadiminejad I, Awan M. 1992. Regulation of mitochondrial carnitine palmitoyltransferases from liver and extrahepatic tissues. *Adv. Enzyme Regul.* 32:285–306
152. Sandor A, Kispal Gy, Melegh B, Alkonyi I. 1985. Release of carnitine from the perfused rat liver. *Biochim. Biophys. Acta* 835:83–91
153. Sandor A, Kispal Gy, Melegh B, Alkonyi I. 1987. Ester composition of carnitine in the perfusate of liver and in the plasma of donor rats. *Eur. J. Biochem.* 170:443–45
154. Sartorelli L, Ciman M, Mantovani G, Siliprandi N. 1985. Carnitine transport in rat heart slices: II. The carnitine/deoxycarnitine antiport. *Ital. J. Biochem.* 34: 282–87
155. Sartorelli L, Ciman M, Siliprandi N. 1985. Carnitine transport in rat heart slices: I. The action of thiol reagents on the acetylcarnitine/carnitine exchange. *Ital. J. Biochem.* 34:275–81
156. Schaefer J, Jackson S, Taroni F, Swift P, Turnbull DM. 1997. Characterization of carnitine palmitoyltransferases in patients with a carnitine palmitoyltransferase deficiency: implications for diagnosis and therapy. *J. Neurol. Neurosurg. Psych.* 62:169–76
157. Sengers RCA, Fischer JC, Trijbels JFM, Ruitenbeck W, Stadhouders AM, ter Laak HJ. 1983. A mitochondrial myopathy with a defective respiratory chain and carnitine deficiency. *Eur. J. Pediatr.* 140:332–37
158. Sengers RCA, Trijbels JMF, Bakkeren JAJM, Ruitenbeck W, Fischer JC, Janssen AJ. 1984. Deficiency of cytochromes b and aa_3 in muscle from a floppy infant with cytochrome oxidase deficiency. *Eur. J. Pediat.* 141:178
159. Shaw RD, Li BUK, Hamilton JW, Shug

AL, Olsen WA. 1983. Carnitine transport in rat small intestine. *Am. J. Physiol.* 245:G376–81
160. Skrede S, Bremer J. 1970. The compartmentation of CoA and fatty acid activating enzymes in rat liver mitochondria. *Eur. J. Biochem.* 14:465–72
161. Stanley CA. 1995. Carnitine disorders. *Adv. Pediatr.* 42:209–42
162. Stanley CA, De Leeuw S, Coates PM, Vianay LC, Divry P, et al. 1991. Chronic cardiomyopathy and weakness or acute coma in children with a defect in carnitine uptake. *Ann. Neurol.* 30:709–16
163. Stanley CA, Hale DE, Berry GT, Deleuw S, Boxer J, Bonnefont J-P. 1992. Brief report: a deficiency of carnitine-acylcarnitine translocase in the inner mitochondrial membrane. *N. Engl. J. Med.* 327:19–23
164. Stanley CA, Sunaryo F, Hale DE, Bonnefont JP, Demaugre F, Saudubray JM. 1992. Elevated plasma carnitine in the hepatic form of carnitine plamitoyltransferase-1 deficiency. *J. Inherit. Metab. Dis.* 15:785–89
165. Stanley CA, Treem WR, Hale DE, Coates PM. 1990. A genetic defect in carnitine transport causing primary carnitine deficiency. In *Fatty Acid Oxidation: Clinical, Biochemical, Molecular Aspects*, ed. K Tanaka, PM Coates, pp. 457–64. New York: Liss
166. Stieger B, O'Neill B, Krahenbuhl S. 1995. Characterization of L-carnitine transport by rat kidney brush-border-membrane vesicles. *Biochem. J.* 309:643–47
167. Tanphaichitr V, Broquist HP. 1973. Role of lysine and ε-N-trimethyllysine in carnitine biosynthesis. II. Studies in the rat. *J. Biol. Chem.* 248:2176–81
168. Tanphaichitr V, Broquist HP. 1974. Site of carnitine biosynthesis in the rat. *J. Nutr.* 104:1669–73
169. Taroni F, Verderio E, Dworzak F, Willems PJ, Cavadini P, DiDonato S. 1993. Identification of a common mutation in the carnitine palmitoyltransferase II gene in familial recurrent myoglobinuria patients. *Nat. Genet.* 4:314–20
170. Taroni F, Verderio E, Fiorucci S, Cavadini P, Finocchiaro G, et al. 1992. Molecular characterization of inherited carnitine palmitoyltransferase II deficiency. *Proc. Natl. Acad. Sci. USA* 89:8429–33
171. Tein I, Bukovac SW, Xie Z-W. 1996. Characterization of the human plasmalemmal carnitine transporter in cultured skin fibroblasts. *Arch. Biochem. Biophys.* 329:145–55
172. Tein I, Demaugre F, Bonnefont JP, Saudubray JP. 1989. Normal muscle CPT1 and CPT2 activities in hepatic presentation patients with CPT-I deficiency in fibroblasts: tissue-specific isoforms of CPT1? *J. Neurol. Sci.* 92:229–45
173. Tein I, De Vivo DC, Bierman F, Pulver P, De Meirleir LJ, et al. 1990. Impaired skin fibroblast carnitine uptake in primary systemic carnitine deficiency manifested by childhood carnitine-responsive cardiomyopathy. *Pediatr. Res.* 28:247–55
174. Tein I, De Vivo DC, Ranuci D, DiMauro S. 1993. Skin fibroblast carnitine uptake in secondary carnitine deficiency disorders. *J. Inherit. Metab. Dis.* 16:135–46
175. Tein I, DiMauro S, Xie Z-W, De Vivo DC. 1993. Valproic acid impairs carnitine uptake in cultured human skin fibroblasts. An *in vitro* model for the pathogenesis of valproic acid-associated carnitine deficiency. *Pediatr. Res.* 34:281–87
176. Tein I, Xie Z-W. 1996. The human plasmalemmal carnitine transporter defect is expressed in cultured lymphoblasts: a new non-invasive method for diagnosis. *Clin. Chim. Acta* 252:201–4
177. Treem WR, Stanley CA, Finegold DN, Hale DE, Coates PM. 1988. Primary carnitine deficiency due to a failure of carnitine transport in kidney, muscle, fibroblasts. *N. Engl. J. Med.* 319:1331–36
178. Trevisan CP, Reichman H, DeVivo DC, DiMauro S. 1985. Beta-oxidation enzymes in normal human muscle and in muscle from a patient with an unusual form of myopathic carnitine deficiency. *Muscle Nerve* 8:672–75
179. Tubbs PK, Ramsay RR, Edwards MR. 1980. Inhibitors of carnitine transport and metabolism. See Ref. 61, pp. 207–18
180. Van der Leif FR, Takens J, Van der Veen AY, Terpstra P, Kuipers JRG. 1997. Localization and intron usage analysis of the human CPT1B gene for muscle type carnitine palmitoyltransferase I. *Biochim. Biophys. Acta* 1352:123–28
181. Vary TC, Neely JR. 1982. Characterization of carnitine transport in isolated perfused adult rat hearts. *Am. J. Physiol.* 242:H585–92
182. Vary TC, Neely JR. 1982. A mechanism for reduced myocardial carnitine levels in diabetic animals. *Am. J. Physiol.* 243:H154–58
183. Vary TC, Neely JR. 1983. Sodium dependence of carnitine transport in isolated perfused adult rat hearts. *Am. J. Physiol.* 244:H247–52
184. Verderio E, Cavadini P, Montermini L, Wang H, Lamantea E, et al. 1995. Carnitine palmitoyltransferase II deficiency:

structure of the gene and characterization of two novel disease-causing mutations. *Hum. Mol. Gen.* 4:19–29
185. Vianey-Saban C, Mousson B, Bertrand C, Stamm D, Dumoulin R, et al. 1993. Carnitine palmitoyltransferase I deficiency presenting as a Reye-like syndrome without hypoglycemia. *Eur. J. Pediatr.* 152:334–38
186. Vickers S, Duncan CAH, White SD, Ramjit HG, Smith JL, et al. 1985. Carnitine and glucuronic acid conjugates of pivalic acid. *Xenobiotica* 15:453–58
187. Villard J, Fischer A, Mandon G, Collombet JM, Taroni F, Mousson B. 1996. Recurrent myoglobinuria due to carnitine palmitoyltransferase II deficiency: expression of the molecular phenotype in cultured muscle cells. *J. Neurol. Sci.* 136:178–81
188. Waber LJ, Valle D, Neill C, DiMauro S, Shug A. 1982. Carnitine deficiency presenting as a familial cardiomyopathy: a treatable defect in carnitine transport. *J. Pediatr.* 101:700–5
189. Weis BC, Esser V, Foster DW, McGarry JD. 1994. Rat heart expresses two forms of mitochondrial carnitine palmitoyltransferase I. *J. Biol. Chem.* 269: 18712–15
190. Witt DR, Theobald M, Santa-Maria M, Packman S, Townsend S, et al. 1991. Carnitine palmitoyl transferase type 2 deficiency: two new cases and successful prenatal diagnosis. *Am. J. Hum. Genet.* 49:A109
191. Woeltje KF, Esser V, Weis BC, Cox WF, Schroeder JG, et al. 1990. Inter-tissue and inter-species characteristics of the mitochondrial carnitine palmitoyltransferase system. *J. Biol. Chem.* 265:10714–19
192. Woeltje KF, Esser V, Weis BC, Sen A, Cox WF, et al. 1990. Cloning, sequencing, expression of a cDNA encoding rat liver mitochondrial carnitine palmitoyltransferase II. *J. Biol. Chem.* 265:10720–25
193. Woeltje KF, Kuwajima M, Foster DW, McGarry JD. 1987. Characterization of the mitochondrial carnitine palmitoyltransferase enzyme system. Use of detergents and antibodies. *J. Biol. Chem.* 262:9822–27
194. Wolf D, ed. 1965. *Recent Research on Carnitine: Its Relation to Lipid Metabolism*. Cambridge, MA: MIT Press
195. Wolf G, Berger RA. 1961. Studies on the biosynthesis and turnover of carnitine. *Arch. Biochem. Biophys.* 92:360–65
196. Wolkowicz PE, Pauly DF, Van Winkle WB, McMillin JB. 1989. Chymotrypsin activates cardiac mitochondrial carnitine-acylcarnitine translocase. *Biochem. J.* 261:363–70
197. Zaspel BJ, Sheridan KJ, Henderson LM. 1980. Transport and metabolism of carnitine precursors in various organs of the rat. *Biochim. Biophys. Acta* 631:1192–202
198. Zierz S. 1994. Carnitine palmitoyltransferase deficiency. In *Myology*, ed. AG Engel, C Franzini-Armstrong, 2:1577–86. New York: McGraw-Hill. 2nd ed.
199. Zierz S, Engel AG, Olek K. 1994. The Ser113Leu mutation in the carnitine palmitoyltransferase II gene in patients with muscle carnitine palmitoyltransferase deficiency. *Muscle Nerve* 1(Suppl.):S129
200. Zinn AB, Hoppel CL. 1991. An unusual form of carnitine palmitoyltransferase B (CPT B) deficiency associated with neonatal cardiomyopathy and renal dysorganogenesis. *Am. J. Hum. Genet.* 49:A109
201. Yamamoto S, Abe H, Kohgo T, Ogawa T, Ohtake A, Hayashibe H, et al. 1996. Two novel gene mutations (Glu174→Lys, Phe383→Tyr) causing the hepatic form of carnitine palmitoyltransferase II deficiency. *Hum. Genet.* 98:116–18
202. Yamazaki N, Shinohara Y, Shima A, Terada H. 1995. High expression of a novel carnitine palmitoyltransferase I like protein in rat brown adipose tissue and heart: isolation and characterization of its cDNA clone. *FEBS Lett.* 363:41–45
203. Yamazaki N, Shinohara Y, Shima A, Yamanaka Y, Terada H. 1996. Isolation and characterization of cDNA and genomic clones encoding human muscle type carnitine palmitoyltransferase I. *Biochim. Biophys. Acta* 1307:157–61
204. Yamazaki N, Yamanaka Y, Hashimoto Y, Shinohara Y, Shima A, Terade H. 1997. Structural features of the gene encoding human muscle type carnitine palmitoyltransferase I. *FEBS Lett.* 409:401–6
205. Yates DW, Garland PB. 1970. Carnitine palmitoyltransferase activities (EC 2.3.1.-) of rat liver mitochondria. *Biochem. J.* 119:547–52