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THE PHARMACOLOGY OF CARNITINE

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INTRODUCTION

Carnitine (3-hydroxy-4-*N*-trimethylaminobutyric acid; see Figure 1) was isolated from meat in 1905 (1). In 1959 Fritz (2) demonstrated that carnitine has an obligatory role in long-chain fatty acid (LCFA) oxidation. Cederblad & Lindstedt (3) developed a sensitive assay in 1972, which was later modified by McGarry & Foster (4). This assay catalyzed investigation into the biochemical role of L-carnitine. These studies led to the administration of carnitine as a pharmacological agent in various situations. This use of carnitine we view as derivative of its role in intermediary metabolism, generally related to fatty acid metabolism. We refer the reader to the primary literature, reviews, and books describing the physiological and biochemical role of carnitine (5–9).

BIOCHEMICAL ROLE

LCFA oxidation is of some importance in plants, especially in fruits and seeds, but in general carnitine levels are much higher in the fat-oxidizing cells of animals. No dietary requirement is established for carnitine in humans. Biosynthesis of carnitine requires a prior trimethylation of protein-bound lysine. The steps in carnitine biosynthesis are known, but the factors that regulate in vivo rates of biosynthesis are not well characterized (9). Inside the cell, carnitine acts as a cofactor, allowing acyl groups to be shuttled between intra- and extramitochondrial pools of coenzyme A (CoA) (Figure 2). A LCFA such as palmitate is activated by becoming an ester of CoA on the outer aspect of the inner mitochondrial matrix membrane. It is transesterified to

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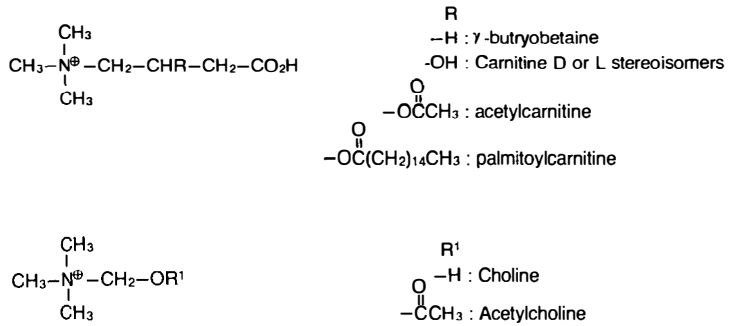

Figure 1 Structures of carnitine, its metabolic precursor gamma-butyrobetaine, and the acetyl and palmitoyl esters of carnitine. The structures of choline and acetyl choline are shown for comparison.

carnitine by the long-chain fatty acylcarnitine transferase enzyme, carnitine palmitoyltransferase I (CPT I). A specific translocase allows the palmitoylcarnitine to enter the mitochondrion and be exchanged for either acetylcarnitine or carnitine. The transesterification by the enzyme CPT II on the inner surface of the matrix membrane presents the enzymes of β-oxidation with the activated substrate palmitoyl-CoA. Short-chain fatty acids (such as propionate in the liver and acetate in all tissues) can enter mitochondria by routes not requiring the carnitine translocator. Medium-chain fatty acids may be activated by the liver peroxisomal medium-chain acyl-CoA synthetase and transesterified to carnitine for entry into mitochondria, or as with short-chain compounds, they may be activated inside of the mitochondrion. The substrate specificity of CPT II and the short-chain acyl transferase (SCAT) ensures that β-oxidation proceeds completely to acetyl-CoA. Medium-chain fatty acids may be able to enter mitochondria of extrahepatic tissue but may not be activated there.

The enzymes of β-oxidation produce acetyl-CoA. If acetyl-CoA is rapidly consumed by the tricarboxylic acid cycle for energy production, free CoA (nonesterified) is regenerated. Depending on the tissue and metabolic state other acetyl-CoA-consuming pathways may be available. Oxaloacetic acid may be used to produce citrate as part of a shuttle mechanism that allows acetate groups to travel to the cytosilic compartment and regenerate free intermitochondrial CoA. In the liver when the rate of acetyl-CoA production is high, ketone bodies are synthesized, again regenerating free CoA. The ratio of acyl-CoA to free CoA increases as the level of LCFA increases in the mitochondria. Without a mechanism to limit the delivery of LCFA as carnitine esters, the capacity of these acetate-consuming reactions would become overwhelmed, and production of LCFA-CoA esters would soon consume the remaining free CoA. The problem with filling the mitochondrial larder with

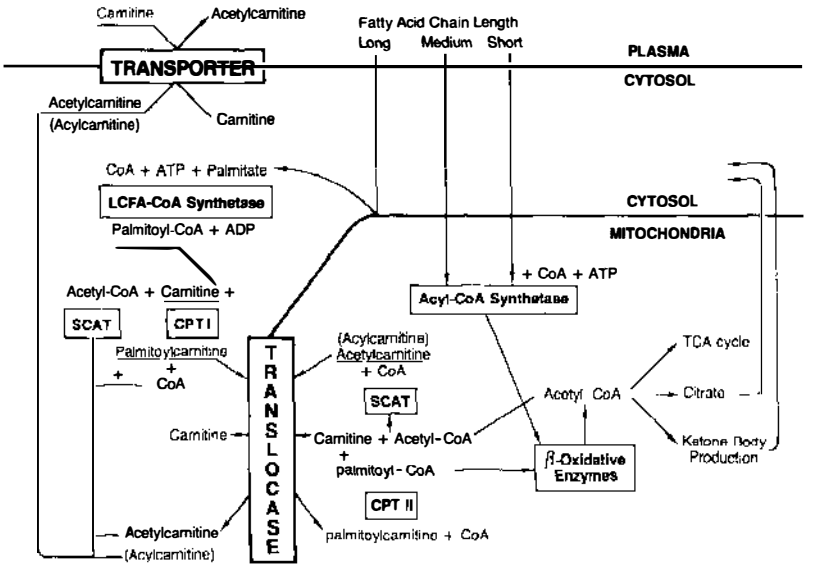

Figure 2 Pathway of intermediary metabolism featuring carnitine's role. Arrowheads indicate general direction of substrate pathway but are readily reversible for all reactions involving carnitine. Abbreviations: CPT, carnitine palmitoyltransferase, SCAT, short-chain acyl transferase.

LCFA groups is that acyl-CoAs inhibit the function of many enzymes. Pyruvate carboxylase and citrate synthetase are enzymes that might participate in pathways that consume acetyl-CoA. These enzymes are inhibited by long-chain acyl-CoAs. The adenine nucleotide translocase, which has similar susceptibility if inhibited by long-chain acyl-CoAs, would stop the translocation of ATP from the mitochondria to the cytoplasm and the intramitochondrial consumption of acetyl-CoA and regeneration of free CoA. As LCFA-CoA esters are good detergents in high concentration, they may damage the structure of the mitochondria. Many substrate pathways are regulated by LCFA-CoA esters or acetyl-CoA inhibiting or activating enzymes. This regulation often depends upon the availability of free CoA, as a high ratio of acyl-CoA to free CoA or high levels of long-chain acyl-CoAs may harm the mitochondria (10, 11).

The majority of the cell's CoA is contained within mitochondria. In the heart 95% and in the liver 60% of the CoA is intramitochondrial (12, 13). Carnitine is more uniformly dispersed in the cell than the CoA. The concentration of CoA and carnitine is similar inside the mitochondria. Transfer of the acetyl group from CoA to carnitine is catalyzed by the SCAT enzyme (see Figure 2, lower center). The equilibrium ratio for this transfer effectively ensures that carnitine and CoA in the mitochondrial pool have the same acyl

CoA to free CoA ratio. The carnitine translocator, while not always achieving a one-for-one exchange (13, 14), generally functions to rapidly exchange free carnitine (nonesterified) from the cytoplasm for acetyl- or other acylcarnitines from the mitochondria. This mechanism would soon exhaust the cytosolic free carnitine except that SCAT on the outer side of the mitochondria reverses the transesterification generating acetyl-CoA. Extramitochondrial LCFA activation decreases as the availability of free CoA becomes limiting to the LCFA-CoA synthetase. This observation provides a regulatory feedback loop for LCFA activation and other cytosolic CoA-dependent reactions that can quickly respond to changes in the mitochondria (10).

In this sequence of events carnitine has been a cofactor in four metabolic events without expending energy: (*a*) delivery of activated LCFA to the intramitochondrial enzymes of β-oxidation; (*b*) removal of intramitochondrial acyl groups, which prevents the mitochondrial acyl to free CoA ratio from rising (regenerating intramitochondrial free CoA); (*c*) regulation of the rate of extramitochondrial LCFA activation; (*d*) removal of acyl groups that are inhibitory and might be catastrophically disruptive (toxic) if left to accumulate in the mitochondrial pool of CoA esters.

The enzymes that utilize carnitine are all specific for the L-isomer. However, the transporter protein that maintains high intracellular levels of carnitine relative to the plasma does not discriminate between D and L isomers. Choline (see Figure 1) in nonphysiological concentrations can inhibit carnitine transport (15), but it is unclear how these two biologically important molecules affect the transport of each other in vivo or if they always share the same transport system. Injecting choline into a rat can result in the rapid efflux of carnitine from peripheral tissues (16). As this carnitine does not arise from biosynthesis it is likely the result of choline-carnitine exchange. Discrimination of acyl esters from free carnitine is achieved by the transporter, but is not absolute. Liver cells show a preference for the metabolic precursors of carnitine (e.g. γ-butyrobetaine over carnitine) for transport (17). Each tissue, and perhaps each separate cell type in a tissue, has individual kinetic descriptions for carnitine transport (15, 17, 18). The liver has a large capacity for transport and can double its pool size within a short period as plasma carnitine levels rise (16, 19). Skeletal muscle has high levels of carnitine but does not show rapid exchange of radioactive label or change in concentration. The carnitine present in high levels in the heart exchanges with plasma carnitine rapidly. If the level of carnitine in the heart is below the set point, as determined by the kinetic parameters, active carrier-mediated transport results in net accrual (15). Once the pool is filled, carrier-mediated exchange is observed. At this point it is difficult to increase the amount of carnitine above a certain level (20). Generally, rapid turnover indicates an extended period of elevated lipid use, and high levels (less subject to short-term fluctuations) indicate that fatty acid oxidation occurs in proportion to tissue levels of

carnitine. Free carnitine is preferred over acyl carnitine in the kidney tubule's reabsorption of carnitine. Carnitine at elevated concentrations down-regulates its own transport across renal brush border membranes in vitro, although the physiological necessity for this mechanism is not clear (21). The preferential reuptake of free carnitine by the kidneys results in clearance of acyl compounds that might otherwise lower the rate of fatty acid oxidation and other CoA-dependent reactions. Each tissue, and the body as a whole, keeps a low acyl CoA to free CoA ratio by excreting acyl carnitines. Plasma levels typically reported for total carnitine are 50 ± 15 μM; males have higher levels than females (22, 23).

The function of the tissue and the availability of substrate determine the balance of carbohydrates and lipids used for energy. However, the level of carbohydrate and lipid oxidation is tightly regulated in each cell (24, 25). The overall rate of metabolic activity is regulated by ADP, which allows ATP translocation from the mitochondria. Carnitine, its esters, and the corresponding cytosolic and mitochondrial CoA esters partly ensure that fatty acid metabolism occurs to maintain homeostasis. Carnitine supplementation may show pharmacological activity, brought about by increasing pool size or increasing the rate of free carnitine and acyl carnitine exchange. We elaborate on this basic description as appropriate for each of the following sections.

CARNITINE DEFICIENCY SYNDROMES

Two reviews of the literature describing carnitine deficiency syndromes cover the period from the first reported human deficiency in 1973 until 1983 (26, 27). In one case a 3½-year old had systemic carnitine deficiency (SCD), which presented as Reye's Syndrome. The nonfatal 36-hour fast of this patient, with less than 5% of the normal liver and skeletal muscle carnitine levels shows that fatty acids can be delivered to mitochondria even with low levels of carnitine. When treated with carnitine, liver carnitine levels returned to normal and skeletal muscle carnitine was increased to 40% of normal. Neurological disturbances and hepatomegaly disappeared after two weeks of therapy, and the cardiomyopathy was largely resolved by three months (28). A recent review by Stumpf et al (29) defined carnitine deficiency with a focus on possibly the most important clinical role of carnitine yet established. These authors state that "Carnitine deficiency exists when there is insufficient carnitine to buffer toxic acyl-CoA compounds" (29). In eliminating such acyl compounds, a tissue may have a net efflux of carnitine and a syndrome of carnitine deficiency may result.

The term *primary carnitine deficiency,* as pointed out by Irias (30), should probably be restricted to carnitine deficiencies of idiopathic origin. Secondary deficiencies arise from defects in intermediary metabolism, from disease or pathophysiological states, and from normal physiological states that reflect

metabolic stress such as starvation or pregnancy. These authors suggest that the term *primary* be omitted in cases relating to carnitine supply (biosynthesis, diet, absorption), transport (uptake, release, reabsorption), or degradation. This would eliminate the need to reclassify a reported deficiency in biosynthesis that is later shown to be due to a methionine or iron deficiency.

Any condition that causes liver carnitine to be lost from the body may eventually result in depletion of muscle carnitine. Periodic increases of carnitine levels may follow dietary supplementation or periods of intense lipid use by muscle (31). Skeletal muscle levels can fall without a decrease in liver or plasma levels. Loss of carnitine in muscles can result from chronic overuse of lipids by the mitochondria. This overuse may occur during extensive physical activity or may result from a defect in the intermediary metabolism of the muscle. The heart, with its high turnover of carnitine, is susceptible to tissue-specific depletion of L-carnitine by the D isomer (32). The depletion of carnitine caused by net efflux of carnitine as an acyl ester is distinct from the depletion that occurs because of transporter defects or renal loss. In interpreting plasma levels of carnitine, the researcher must recognize that values represent only one moment in time. Although the plasma level may reflect the carnitine level of liver and other tissues, the skeletal muscle pool cannot be estimated by the plasma level. Also, the investigator should know and report both free carnitine and acyl carnitine values. Without knowing the amount of acyl carnitine that accompanies free carnitine, it is impossible to know whether a hypercarnitine- or hypocarnitine-related metabolic state is present.

A redistribution of carnitine may result in increased plasma and liver levels, but also in decreased levels in the tissue supplying the carnitine. The increased level of plasma carnitine might result in a smaller fraction of carnitine being reabsorbed by the kidney, and the levels of carnitine would decrease more rapidly.

In SCD a renal leak occurs in some patients (33). Replacement therapy normalizes plasma and liver carnitine levels only as long as carnitine supplementation is continued. This renal leak is postulated to result from a genetic defect in the renal reuptake system that handles carnitine (34). Glucocorticoids [prednisone (35), dexamethasone (36)] and cholesterol (37) can result in increased tissue (nonliver) and plasma levels of carnitine. The effect of glucocorticoids and cholesterol on renal handling has not been investigated. Therefore, to conclude that all reabsorption defects are genetic may be premature.

Organic Acidemias

Organic acidemias (a fall in blood pH caused by high levels of specific organic acids) provide an example of a group of metabolic diseases that can be successfully treated by the pharmacological use of carnitine (26–29). Acyl CoA metabolites are generated by the oxidation of certain fats or organic acids

that cannot be readily metabolized. This metabolic inability may be due to the inherent structure of the substrate or to a genetic defect. The acyl group becomes a carnitine ester and thus does not remain to retard the function of mitochondrial CoA-dependent metabolic pathways. The continued removal of acyl groups may lead to a depletion of carnitine stores. Patterns of specific tissue protection or depletion may arise because the liver has the enzymes necessary for activating medium-chain fatty acids, but muscle does not (29).

Propionate is only poorly converted to a CoA ester in muscle (38). It is, however, activated in liver, where it inhibits oxidative phosphorylation. Stumpf et al showed that carnitine could reverse the inhibition of oxidative phosphorylation in isolated hepatic mitochondria and suggested carnitine as a potentially effective treatment (39). Propionic acidemia shows clinically significant response to carnitine treatment (40, 41). Other acidemias that result from inborn errors of metabolism also respond to carnitine as does the ingestion of excessive quantities of nonphysiological fatty acids or organic acids that result in acyl CoA accumulation (29). Treatment for carnitine deficiency syndrome is 100 mg/kg/day of L-carnitine, administered orally in 3 or 4 doses (29).

Lipid deposits are found in muscle biopsy specimens of patients with carnitine deficiencies, and often nonketotic hypoglycemia occurs with fasting. After carnitine supplementation clinical improvement occurs (26, 27). Carnitine depletion can cause lower rates of LCFA oxidation, suggesting that carnitine has become limiting for CPT I (42). In addition, CPT I (24) and SCAT (43) are inhibited by malonyl CoA, the first step in fatty acid synthesis or chain elongation. Long et al demonstrated that malonyl-CoA, produced when carbohydrate is used as the main source of mitochondrial substrate, inhibits fatty acid oxidation and increases synthesis of fatty acids. When acetyl CoA is provided by the citrate shuttle for elongating a fatty acid chain, SCAT inhibition prevents the acetyl CoA from being consumed while generating acetyl carnitine. Carnitine-deficient cells become more dependent on carbohydrate, as depleted carnitine levels result in lower rates of fatty acid oxidation (42). With a greater dependence on carbohydrate and decreased fatty acid oxidation, the increase in triglyceride (TG) may represent synthesis of fatty acids in excess of oxidation and phospholipid synthesis. Carnitine supplementation increases plasma and usually liver stores at least to control levels but is less effective in replenishing skeletal muscle carnitine (44). Carnitine replacement results in a more efficient metabolism of both carbohydrates and lipids, with patients showing weight gain as tissue lipid stores decrease.

Cardiomyopathies

A number of reports indicate that cardiomyopathic patients with low levels of serum carnitine may benefit from L-carnitine supplementation (28, 45–47).

Following chronic carnitine supplementation, thickness of the left ventricle returns to normal as does cardiac performance, with EKG abnormalities disappearing. In one study of cardiomyopathic patients an unexpected inverse correlation between survival and plasma carnitine levels was observed (47). However, a recent abstract suggests that the elevated level of serum carnitines in cardiomyopathic patients results from decreased renal clearance (48). Not all of the patients with elevated levels of plasma carnitine had high amounts of plasma acyl carnitines, but it may be that acyl carnitines increased in the sequence of events that led to death.

Diphtheria results in low levels of cardiac carnitine. These levels have been increased by treatment with D,L-carnitine (49).

Reye's and Reye's-Like Syndromes

Several cases of Reye's-like syndromes (RLS) with associated carnitine deficiency have been reported (28, 33, 44, 50). The clinical description—hypoglycemia, hypoketonemia, and coma—that appears episodically may represent a failure of the carnitine and CoA acyl regulatory mechanism that normally functions to prevent accumulation of acyl CoA esters.

One group of RLS patients has a defect in their medium-chain acyl-CoA dehydrogenase (44, 50). For heuristic purposes let us assume that the defect is absolute and the LCFA is metabolized to the eight-carbon acid octanoate. For every palmitic acid (C16:O) taken into the mitochondria only four acetyl CoA and one octanoyl CoA would be generated instead of the normal eight acetyl CoAs. To provide the equivalent "acetate pressure" produced by five palmitates, eight would have to be used. The altered metabolic economy from using only half of the LCFA would suggest that ten palmitates be used rather than five. Thus, for any level of LCFA metabolic activity, the percentage of the acyl pool that would be nonacetyl CoA would increase. Chronically, carnitine could become depleted as the octanoyl CoA was continuously removed. SCAT and CPT II would remove the octanoyl CoA only as its concentration became elevated.

Decreased stores of carnitine lower the buffering capacity and increase the accumulation of nonacetyl medium- and long-chain acyl CoA's during intense lipid use. Episodic symptoms of RLS might occur even during fasting for a short duration. The hypoglycemia reflects the lack of acetyl CoA as a positive regulator of pyruvate carboxylase activity inhibiting gluconeogenesis. The lack of ketone body formation reflects the lack of acetyl CoA as substrate for ketone body production. The defect in medium-chain acyl CoA dehydrogenase observed clinically may not be absolute. Plasma levels of carnitine are approximately one half of normal, and a single dose of supplemental carnitine results in a large efflux of acyl carnitines, 63% of which are octanoyl carnitines (44). The management of this RLS includes a diet low in fat,

avoidance of fasting, as well as administering L-carnitine supplementation (44, 50).

The anticonvulsant valproic acid induces RLS in a small percentage of patients. Fatty acid oxidation rates are depressed by valproic acid, and neither carnitine nor glycine causes rapid exit of valproyl CoA from mitochondria (51). After a single injection of valproic acid into infant mice acetyl CoA fell 71%, medium-chain acyl carnitine rose 650%, and long-chain esters increased 68% (52). These observations suggest that valproyl CoA inhibits the mitochondrial β-oxidative pathway in a similar manner to a deficiency in medium-chain acyl dehydrogenase. Hypoglycin, the active component of the unripe Ackee fruit that causes the vomiting sickness of Jamacia (53), also inhibits the mitochondrial β-oxidative pathway. Adult mice showed a similar response, only with higher doses of valproate, when starved (54). Increased acyl group flux (and accumulation) may be necessary in the adult animals that start with larger pools of tissue carnitine and may activate valproate at a different fractional rate. Becker & Harris reported that valproate does not accumulate in the brain (51). This finding suggests that valproate's action to deplete carnitine may be more closely related to its mechanism of action than previously appreciated. Carnitine deficiency might be linked in three ways: (*a*) by decreasing the rate of choline biosynthesis (55) (see "Carnitine in the Neonate"); (*b*) by effecting a redistribution of choline to the periphery, as carnitine no longer competes for peripheral transport into liver and muscle and/or increased phosphatidyl choline synthesis does not compete for choline; and (*c*) by altering the metabolism of neurotransmitter production secondary to the carnitine deficiency state, thereby causing a greater utilization of glucose by all tissues. Plasma levels of carnitine correlate inversely with the dose of valproic acid in patients (56). In that relatively short-term study D,L-carnitine did not alter the electroencephalogram or frequency of seizures; however, it did normalize the associated hyperammonemia and plasma L-carnitine concentration.

Thurston et al (54) suggest testing patients for susceptibility to valproate-induced Reye's Syndrome by measuring the capacity for normal ketogenesis during a fast to uncover unrecognized metabolic deficiencies. Roe et al (57) recently warned that fasting in patients who may have low plasma carnitine levels can result in induction of coma and death. Alternatively, administration of a bolus of L-carnitine with identification of abnormal distribution, type, or quantity of acyl esters appearing in the urine is suggested (52).

Patients with true Reye's Syndrome do not respond to carnitine therapy (58). Aspirin use in juveniles is associated with Reye's Syndrome. It would be analogous with the previous example if salicylic acid or acetyl-salicylic acid (the parent compound) were found to form a CoA ester that decreased the rate of LCFA oxidation. Induction of acyl transferases may contribute to a

higher concentration of thc intramitochondrial CoA ester than usually occurs. However, any defect in the enzymes of β-oxidation, or inhibition of their activity by any exogenous or endogenous compound with valproatelike activity, would set the stage for Reye's Syndrome. The uncoupling of oxidative phosphorylation by salicylate would create high flux rates that would amplify any problem in LCFA oxidation. Decreasing liver levels of carnitine following the metabolic stress associated with illness may also contribute to susceptibility to Reye's Syndrome. During recovery from an attack, carnitine could be restored, and tissue levels would appear normal (59). Until the acyl CoA responsible for altering fatty acid oxidation is cleared from the mitochondria, episodic attacks would be expected. Other diseases or conditions where long-chain esters of carnitine or CoA are increased might respond to carnitine supplementation. In limb girdle dystrophy and Duchenne muscular dystrophy, long-chain acyl CoA levels are elevated (60). Also, in Duchenne dystrophy, muscle and plasma levels of carnitine are decreased (61).

Carnitine in the Neonate

Carnitine levels are lower in newborns than in adults (62, 63). During fetal development glucose is the predominant energy source for most tissues. Around the time of birth many tissues begin to utilize fatty acids for energy and therefore are dependent on carnitine for this function. The brain is an important exception to this generalization; it never develops a reliance on LCFA use for energy although it does use medium-chain fatty acids (64). The brain uses sugars as the main energy source only at some time after birth (65, 66). Ketone bodies, initially from the mother and subsequently from other tissues that begin to switch to LCFA utilization, may be crucial for normal development (67, 68). Carnitine in milk is a major source of the carnitine that fills the neonate's body pool (69).

Orzali et al (70) reported that D,L-carnitine administration [and later L-carnitine (71)] did not significantly alter existing fatty acid metabolism; lipid loading occurred in neonates receiving total parental nutrition (TPN). These authors concluded that enough tissue carnitine was present to maintain good lipid utilization in the neonate receiving TPN. Schmidt-Sommerfeld et al (72) pointed out that the lipid bolus contained glucose, which might tend to mask potential differences in lipid use. They also indicatcd that the 6-hr i.v. administration of carnitine following a bolus loading dose may not have been long enough for the carnitine to enter a pool where it could be useful (72). Schmidt-Sommerfeld et al studied TPN-fed neonates with chronic carnitine supplementation. Only premature, and not full-term, neonates showed an augmented ability to use lipid with supplemental carnitine (73). Skeletal muscle carnitine lcvels correlate with gestational age (62, 63), although those

of the liver or heart do not (63). Carnitine supplementation in premature neonates does increase carnitine tissue levels (62).

Exogenous carnitine supplementation in the premature neonate may be especially important in TPN-fed neonates (73, 74). Biosynthetic enzymatic activity may be greatly reduced in all neonates (75), but with TPN-fed neonates the overall biosynthetic rate may become substrate limited. Infusion of nutrient solutions into the superior vena cava for TPN-fed neonates bypasses the intestine and the first-pass effect of the liver. Methionine in peripheral tissue is largely consumed by reactions leading to sulfate rather than by those leading to methyl groups available for transmethylation (76). Limitation of active methyl groups may lead to diminished rates of synthesis of both carnitine and choline in TPN-fed patients (77). Ethanolamine levels doubled in the heart and increased fourfold in the brain of miniature piglets when carnitine was added to the TPN solution (78). This observation suggests that ethanolamine production stimulated by carnitine was probably not converted to choline because of the lack of methyl donors. Because of the competition for methyl donors a lack of dietary choline causes a functional deficit of carnitine in the liver (55).

Maternal human plasma carnitine levels gradually fall during the course of pregnancy, and acute postpartum crisis can result (79). Maternal carnitine levels correspond to levels in full-term neonates delivered by elective cesarean section following uncomplicated pregnancies (80). Carnitine is used to treat respiratory distress syndrome associated with premature births, a syndrome caused by inadequate synthesis of phosphatidyl choline necessary for surfactant production (81, 82). When injected into pregnant female rats, carnitine caused an increase in total phospholipid synthesis in the lungs of fetal pups (83). This finding suggests that the importance of carnitine extends beyond fatty acid oxidation, especially for the fetus and neonate. It also points out that regulation of carnitine and choline biosynthesis may be related.

PHARMACOLOGY OF CARNITINE IN NONDEFICIENCY SYNDROMES

Neuropharmacology of Carnitine

The actions of acetyl carnitine and carnitine on the central nervous system were previously reviewed (84). Similarities in structure between acetyl carnitine and acetylcholine are often cited as circumstantial evidence that these compounds interact at the same receptor, but direct evidence has not been forthcoming. Electrophysiological studies suggest that carnitine and its derivatives may have important interactions with neuronal metabolism; neurotransmitter uptake, reuptake, and/or turnover; and receptor coupling in a variety of neurons. Insightful studies combining multiple areas of neuro-

pharmacology are needed to help clarify and interpret the existing data of this area.

As previously discussed, choline is taken up by peripheral tissues for phosphatidyl choline synthesis and may share a common transporter with carnitine. Choline competes with the carnitine biosynthetic pathway for methyl donors, and the rate of choline biosynthesis may be related to tissue carnitine levels.

Hemodialysis

Patients on hemodialysis have elevated levels of acyl carnitine esters in both plasma and tissue (85, 86). Unlike the functional kidney that excretes acyl carnitine and preferentially reabsorbs free carnitine, hemodialysis results in the preferential loss of free carnitine; therefore, acyl carnitines accumulate (87, 88). In this semiclosed system the absolute level of LCFA acyl esters of CoA and carnitine may be more important than the acyl to free ester ratio. During hemodialysis plasma carnitine levels fall, and concurrently the rate of spontaneous cardiac arrhythmias increases. Administration of L-carnitine prevents these arrhythmias from increasing (89). At the conclusion of hemodialysis plasma carnitine levels rebound (85, 86). However, chronic hemodialysis results in depletion of muscle stores of carnitine. Plasma triglyceride (TG) levels increase, possibly because of both decreased peripheral use of lipid and increased hepatic synthesis of fatty acids. In six separate studies in hemodialysis patients (90–95), two studies in noninsulin-dependent diabetics (96, 97), and two studies in rats chronically administered alcohol (98, 99), D,L-carnitine administration resulted in a decrease in the level of TG. In another study in uremic rats L-carnitine prevented the rise of TG that accompanied the establishment of uremia but did not decrease the TG level once the level was elevated (100). There was, however, no effect with L-carnitine on TG level in at least four studies of hemodialysis patients (89, 101–103), and a paradoxical increase in TG with an increase in platelet aggregation was reported in one study (104). Another study reported no clinical improvement (105). While not all studies agree with the summary of these findings (106, 107), they show that the effects of a dose of D,L-carnitine may not always be equivalent to those of half the dose of L-carnitine. D,L-Carnitine, but not L-carnitine, has been reported to cause a myasthenialike syndrome in hemodialysis patients (108–110).

The kinetics of D,L-carnitine should not be ignored in looking for a basis for this effect. While D,L-carnitine may eventually result in increased plasma and tissue levels of L-carnitine (the D isomer not measured by the enzymatic assay) for tissues containing low levels of carnitine, exchange with the 50% mixture of D and L isomers would result in a transient decrease in tissue L-carnitine. The uptake and half-life of D-carnitine are less than those of L-carnitine, with

the time to peak plasma level following oral dosing occurring after that of L-carnitine (111). An additional possibility is that D-carnitine is metabolized to an active molecule, which does not happen with L-carnitine (112).

In nonhemodialysis patients the level of high-density lipoprotein (HDL) increases, and serum cholesterol decreases slightly following L-carnitine supplementation. In two patients normal except for low HDL levels, L-carnitine increased HDL levels and decreased TG and total plasma cholesterol levels in one, but not the other, patient (113). Supplementation of carnitine may help prevent the decrease in very low density lipoproteins (VLDL) apoprotein CII to apoprotein CIII ratio observed in hemodialysis patients with elevated TG levels (114).

Myocardial Protection

Carnitine protects or improves the performance of the heart under certain laboratory conditions. Although not proven, it is assumed that carnitine in the heart acts analogously to carnitine in other tissues.

ANGINA Effort-induced angina pectoris improves with L-carnitine administration. In a study of patients with angiographically proven coronary artery disease, paced human hearts that were lactate producers extracted lactate from plasma following carnitine infusion (115). In patients with stable angina pectoris carnitine significantly increased the time of multistage-treadmill exercise over the placebo time during a 12-week study (116).

ISCHEMIA During zero-flow ischemia (cessation of coronary flow) futile and excessive production of lactic acid occurs as the glycolytic pathway is utilized to prevent depletion of ATP and irreversible damage to the heart. L-Carnitine does not temper the decrease in performance and the cellular damage caused by this type of ischemia (117). During low-flow ischemia, ATP decreases as long-chain acyl-CoA levels increase and finally plateau. Long-chain acyl carnitines accumulate in the heart cell, bound loosely to membrane structures and protein, as less acetyl carnitine is produced and carnitine levels of the heart decrease.

Arrhythmias develop during ischemia, especially with increased levels of free fatty acids, as the ischemic area becomes electrically and mechanically uncoupled from the rest of the heart (117). Acetyl carnitine decreases the incidence of arrhythmias at a lower concentration than carnitine (118). These arrhythmias might be caused by the long-chain acyl carnitine esters that accumulate when their production is not limited by acetyl carnitine's sequestering CoA. The long-chain acyl carnitines are not passed to the blood in exchange for carnitine and cannot be readily consumed by the mitochondria.

Reperfusion with oxygen-containing solutions increases mitochondrial activity as the backlog of acyl carnitine and mitochondrial acyl-CoA levels decrease. By perfusing with carnitine during low-flow ischemia, acyl carnitine exchange for free carnitine is at least partially maintained (119).

In the dog-heart model of low-flow ischemia, levels of long-chain acyl-CoA's, ATP, and related compounds do not change with acetyl L-carnitine, but recovered performance earlier (117). Acetyl carnitine reduces the rate of fatty acid activation by sequestering the necessary cytosolic CoA. Long-chain acyl carnitine production is then limited by the cytosolic production of long-chain acyl-CoA. The amount of acyl carnitine accumulation may have a significant impact on both the time to recovery and the extent of recovery. Significantly, smaller infarct size has been reported when nicotinic acid and/or oxfenicine was added to the perfusion medium of dogs with a ligated branch of the coronary artery (120). Nicotinic acid decreases concentrations of free fatty acids in plasma. Oxfenicine decreases fatty acid oxidation at a step before CPT II. Both agents reduce long-chain acyl-CoAs, long-chain acyl carnitines, and infarct size in treated hearts in this model of low-flow ischemia. The level of long-chain acyl-CoAs was reduced to or reduced below normal control levels, but long-chain acyl carnitine levels were significantly higher (120). Although it appears that infarct size could correlate with levels of long-chain acyl carnitines, another study did not find any such correlation (121). The studies may not be comparable because of differences in the model of ischemia and in the species. Since both carnitine and acetyl carnitine may prevent accumulation of acyl carnitines, they may have an effect on infarct size for both the dog and the rat heart. Tetradecylglycidic acid inhibits the long-chain fatty acid transfer from CoA to carnitine in the cytosol (122). This agent was developed to decrease the inappropriately overaggressive gluconeogenesis of diabetes, but by preventing LCFA-carnitine formation its use might help clarify the mechanism of myocardial damage during ischemia.

Experimental animal models of diabetes show diabetic hearts have low levels of total carnitine, increased levels of long-chain acyl carnitine with long-chain acyl-CoA and ATP levels the same as in the ischemic heart (123). Chronic lower plasma levels of free carnitine and increased levels of acyl carnitine may contribute to these effects. In humans treated for diabetes these altered levels are less of a problem. During a period of relatively good control of blood glucose levels they do not show the altered level of plasma and tissue carnitine seen in the untreated experimental animal (124, 125).

Di Lisa et al (126) studied the molecular mechanism of carnitine stabilization of energy-linked processes in the rat liver mitochondria and concluded that it was caused by removal of membrane-bound long-chain acyl-CoAs. The effect may have been due to the removal of acyl carnitine ester rather than

acyl-CoA ester. Also, carnitine itself may have had a direct stabilizing activity. Di Lisa et al pointed out that carnitine acted primarily as a stabilizing factor rather than as a specific antidote for a particular noxious factor or condition.

Adriamycin's effect as an anticancer drug is limited by its cardiac toxicity. When carnitine is coadministered with adriamycin there is no evidence of the acute cardiotoxicity in humans as measured by EKG (127). This finding may be another example of a circumstance in which carnitine acts as a stabilizing factor.

CONCLUSION

Carnitine, as an obligatory cofactor of LCFA oxidation, plays an important role in lipid metabolism. Carnitine and CoA esters generated in LCFA oxidation regulate multiple enzymes. A transport system maintains a gradient between low plasma levels and high cellular level. Acyl groups that are difficult to metabolize or that are only slowly metabolized are transferred from CoA to carnitine. The transporter exchanges these cytosolic acyl carnitines for free carnitine of the plasma before they are finally eliminated via the kidney. Carnitine deficiency states result in greater reliance on glucose for energy production. The basis for the pharmacological actions of carnitine is in large measure accounted for by the increased utilization of LCFA for energy production. Also, these pharmacological actions are effected by the elimination of acyl carnitine that could potentially interfere with the integrated regulatory system comprising the intermediary metabolism.

The development of a rapid assay for carnitine and the availability of significant quantities of L-carnitine help to accelerate the rate at which knowledge has been acquired. The relatively new tools of inhibitors of carnitine-related enzymes are also beneficial. These inhibitors include tetradecylglycidic acid (122); 2[5(4-chlorophenyl)pentyl] oxirane-2-carboxylic acid (128); CPT inhibitors, preventing transfer of LCFA from CoA to carnitine in the cytoplasm; 2–(3–methylcinnamyl) hydrazonal-propionic acid (129); translocase inhibitor, blocking carnitine esters from entering the mitochondria; and methoxycarbonyl-CoA disulfide (130), a substrate directed at the SCAT enzyme that transfers acetate between CoA and carnitine in the cytosol. Molecular biology, with its rapid advances in many aspects of cellular regulation, may be of assistance in explaining what factors regulate the production of the proteins found in the biosynthetic pathway and the one or more proteins that make up the transporter.

To date limited specific patient populations have been identified that benefit from carnitine supplementation. However, it is appropriate to ask and determine if chronic excessive elevation of carnitine might not result in

chronic choline loss. Potentially, choline loss could induce the functional deficits both centrally and peripherally. Twenty years ago it was shown that a rat injected with carnitine had brain levels of acetylcholine that were only one-half those of control animals (131). On the other hand, if competition for methyl groups normally limits choline availability to the brain, low-dose supplementation of carnitine might result in the opposite, and potentially therapeutic, effect. No compound is more efficacious in the treatment of a patient with a carnitine deficiency. However, there is no evidence that carnitine in reasonable doses is of benefit or harm to a healthy individual.

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