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# Invited Reviews

## Carnitine Metabolism and Its Application in Parenteral Nutrition

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Fat is an important dietary component for all animals, including humans, by virtue of its high caloric density, its ability to supply essential fatty acids for essential structural components, and to carry fat-soluble vitamins. Fat is also the most important form of energy storage in the body because of its high caloric value, storage with minimum weight per unit of calorie, and without appreciable amounts of water or minerals. Energy derived from the oxidation of fat from exogenous or endogenous sources can maintain physiological processes essential to sustain life. The transformation of fat into utilizable energy involves lipolysis of lipid to free fatty acids (FFA) and the subsequent $\beta$-oxidation in the mitochondria; the entrance of fatty acids into mitochondria from cytoplasm requires the esterification of energy substrates with carnitine.[1]

Carnitine, chemically known as $\beta$-hydroxy-$\gamma$-trimethylaminobutyric acid, is a substance present in animal tissues, whose physiological function was unrecognized until recently. Carnitine was isolated from meat extracts as early as 1905,[2] and its chemical structure was established by chemical synthesis in 1927.[3] In spite of extensive biological and pharmacological studies, it was not until the late Forties and early Fifties that carnitine was shown to be an essential nutrient in the diet of the yellow meal worm, *Tenebrio molitor*, and thus called vitamin $B_T$ for its water-soluble properties, with the T standing for *Tenebrio*.[4] The physiological and biochemical functions of carnitine began to be elucidated in 1959 by Fritz who showed that carnitine had a fundamental effect on the oxidation of FFA by liver tissue.[5] This observation led the investigator to propose that carnitine could increase long chain fatty acid metabolism by facilitating transport of the energy substrates to the site of oxidation in the mitochondria. Further investigation by Fritz and his colleagues specifically showed that the oxidation of long chain fatty acids by heart muscle is highly dependent upon the presence of carnitine.[6]

Although the physiological functions of carnitine have been recognized, its essentiality as a dietary nutrient is emphatically neglected by most nutritionists. Carnitine can be synthesized endogenously in mammalian species and is high in foods of animal origin. Therefore, it is believed that carnitine deficiency is unlikely to occur in normal individuals who consume regular diets. In fact, no information in the literature has shown that lack of carnitine in an otherwise balanced diet leads to deficiency syndromes in humans. While a classical carnitine deficiency resulting from dietary manipulation has not been observed, a number of disease states, including those with disrupted lipid metabolism, which alters levels of carnitine in human biological fluids[7-10] and tissues,[11-14] have been reported. This suggests that the endogenous synthesis of carnitine may be impaired and that exogenous carnitine may be insufficient to meet total body needs under certain disease conditions.

Intravenous (IV) nutrition for patients who cannot consume foods, or in whom oral feeding is not advisable, has been used increasingly for critical patient care. Total parenteral nutrition (TPN), using chemically pure nutrients, has proven life-saving, and can be employed to sustain life for relatively long periods until normal oral feeding can be resumed. Fat emulsion is currently available as part of the TPN regimen. Abnormal hepatic fat deposition has been observed in TPN patients and laboratory animals, indicating an altered fat metabolism. In well-nourished patients undergoing elective surgery, protein-sparing therapy using IV amino acid (AA) infusion has been instituted to conserve body proteins while oxidizing stored body fat for energy during the brief postoperative period. Therapeutic roles of carnitine in patients receiving IV nutrition have not been studied; the need, however, seems to be apparent.

It is the intent of this review to summarize the physiological role of carnitine in fat metabolism, its biosynthesis, and clinical manifestation of carnitine deficiency in disease states. The period between 1976-1978 was searched using the Medlars II National Library of Medicine National Interactive Retrieval Service. Review articles and key references cited in articles retrieved from the Medlars search were also used to obtain earlier information concerning the subject. Although it is recognized that this process does not constitute a complete literature search, it is felt that this review represents the current "state-of-the-art" of the subject.

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### *Fatty Acid Activation*

Fatty acids are essential energy substrates in most cells, particularly those of heart and skeletal muscles. Contrary to fat cells of adipose tissues, normal cells do not store significant quantities of fat. Fatty acids undergoing oxidation in cells of higher animals come largely from extracellular fluid in the form of triglycerides, and very small amounts of FFA noncovalently bound to serum albumin are present in the circulation.

Although the mechanism by which triglycerides cross the cellular membrane is not well understood at the present time, it is known that intracellular triglycerides must first undergo hydrolysis by intracellular lipase to yield FFA prior to activation. Once FFA are formed in the cytoplasm, they are activated through enzymatic esterification by fatty acid thiokinases with extramitochondrial CoASH at the expense of ATP:

Fatty acid + ATP + CoASH

$$\longrightarrow \text{Fatty acyl-CoA} + \text{AMP} + \text{PPi}$$

Activation of long chain fatty acids occurs in the endoplasmic reticulum or on the external surface of mitochondrial membrane because the activating enzymes are found to be localized at these cellular components.[14, 15] The activated fatty acids in the form of acyl-CoA are impermeable to mitochondrial membrane[16] and must be transported into mitochondria in the form of acyl carnitine through a series of enzymatic reactions prior to oxidation for energy.[17]

### *Carnitine and Fatty Acid Transport*

Carnitine plays a major role in the transport of activated long chain fatty acyl groups from sites of activation in the cytoplasm to sites of $\beta$-oxidation in the mitochondria. Figure 1 illustrates a schematic representation of the transport mechanism. The investigation carried out by Fritz and colleagues was instrumental in determining the physiological role of carnitine in fatty acid oxidation at the cellular level.[18, 19] It was shown that L-carnitine is necessary for transfer of acyl groups across mitochondrial membrane, and that several enzymes, collectively called carnitine acyl transferases, are responsible for the reversible reaction:[20]

$$\text{Acyl-CoA} + \text{Carnitine} \rightleftharpoons \text{Acyl carnitine} + \text{CoASH}$$

Carnitine acyl transferases are thought to exist in the mitochondrial membranes. Using rat liver mitochondria and palmitic acid, Hoppel and Tomec[21] reported that the enzyme carnitine palmityl transferase (responsible for the transport of the long chain fatty acids) exists in two forms, carnitine palmityl transferase A and B: The transferases A and B are similar to the carnitine acyl transferases I and II as reported by others.[22, 23] Carnitine acyl transferase I is loosely bound to the external surface of the cytoplasmic side of the mitochondrial membrane where it catalyzes the transfer of the acyl moiety from CoA to carnitine:

$$\text{Acyl-CoA} + \text{Carnitine} \xrightarrow[\text{Transferase I}]{} \text{Acyl carnitine} + \text{CoASH}$$

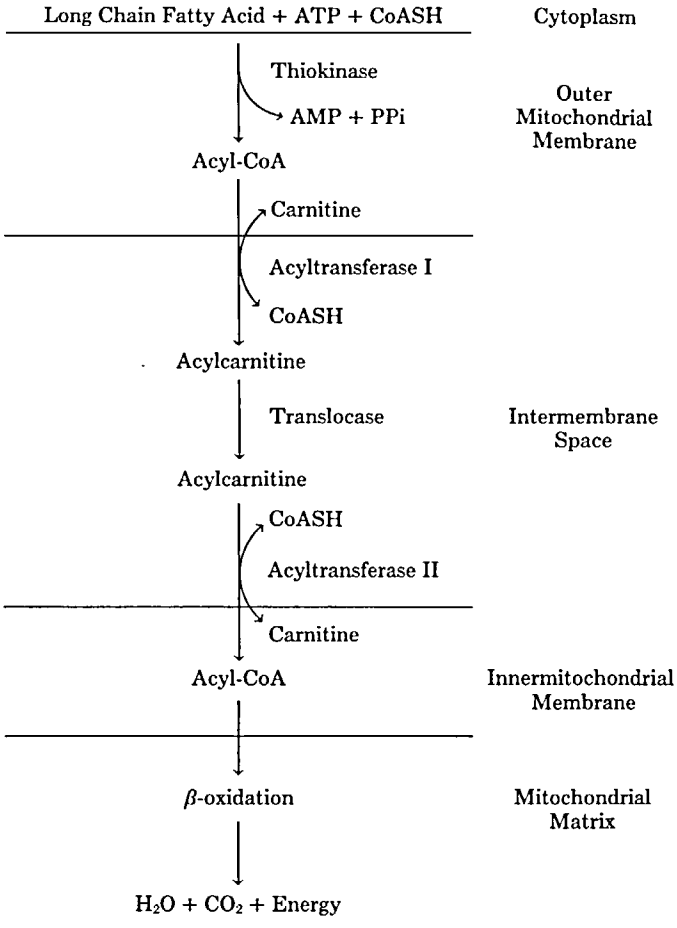



FIG. 1. Proposed mechanism of carnitine-mediated transport of long chain fatty acids into mitochondria.

Once acyl carnitine is formed, the question arises as to how the formation of the ester can facilitate the penetration of fatty acid across the inner mitochondrial membrane. Pande[24] proposed a mitochondrial acyl carnitine translocase system, which carries acyl carnitine from the cytoplasmic side to the inner side within the mitochondrial membrane while translocating free carnitine in the reverse direction (see Carnitine and Control of Fatty Acid Oxidation).

The long chain fatty acid acyl carnitine translocated to the inner side of the mitochondrial membrane undergoes a second enzymatic reaction catalyzed by carnitine acyl transferase II whereby acyl-CoA is made available for $\beta$-oxidation within the mitochondrial matrix.[21-23]

$$\text{Acyl carnitine} + \text{CoASH} \xrightarrow[\text{Transferase II}]{} \text{Acyl-CoA} + \text{Carnitine}$$

The location of the carnitine transferases and translocase system for long chain fatty acid transport is not without controversy. Haddock et al[25] failed to detect a localized dual distribution of carnitine acyl transferase in mitochondrial subfractions. Lumeng and coworkers[26] consider the carnitine acyl transferase II to be part of the $\beta$-oxidation system in the matrix of liver mitochondria. The presence of carnitine in the mitochondrial matrix after formation of acyl-CoA was not detected in rat heart muscle by Oram et al.[27, 28] Based upon initial findings, the

investigators proposed that carnitine exists between the outer and inner mitochondrial membrane without actually entering the mitochondrial matrix. However, more recent investigation from the same institution has led to the acceptance of carnitine in the mitochondrial matrix.[29]

### *Carnitine and Control of Fatty Acid Oxidation*

In addition to the long chain acyl transferases, the presence of a two-carbon enzyme active in acyl transfer to and from carnitine was also inferred from work on whole mitochondria and from the substrate limitation of an acyl transferase studied by Fritz et al.[19] The enzyme, carnitine acetyl transferase, catalyzes the reaction to form acetyl carnitine for transporting the acetyl group across the mitochondrial membrane.[19, 30-32]

$$\text{Acetyl-CoA + Carnitine} \underset{\text{CoASH}}{\rightleftharpoons} \text{Acetyl carnitine +}$$

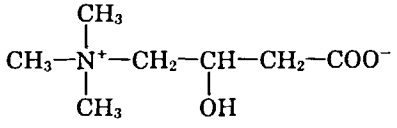
Oram et al[27, 28] and Hochachka et al[29] proposed that the regulation of fatty acid oxidation is regulated indirectly by the enzyme carnitine acetyl transferases. Acetyl-CoA produced within the mitochondria, for example from $\beta$-oxidation, is either oxidized via the tricarborylic acid cycle or leaves the matrix space as acetyl carnitine through the enzymatic reaction catalyzed by carnitine acetyl transferase located in the inner mitochondrial membrane. Acetyl carnitine is permeable to mitochondrial membrane and can form carnitine and acetyl-CoA at the outer mitochondrial membrane with the consumption of cytosolic CoA. The enzymes catalyzing the reaction at the inner and outer mitochondrial membrane were thought to be different and specific.[31] Under the circumstances where uptake of FFA and transfer of acyl carnitine into mitochondria are increased, intermitochondrial acetyl CoA from $\beta$-oxidation can turn off additional FFA activation by lowering the extramitochondrial CoA content. Conversely, sudden decreases in mitochondrial acetyl-CoA may enhance FFA activation due to excess extramitochondrial CoA content. Thus, the carnitine acetyl transferases are thought to couple extramitochondrial uptake and activation of fatty acids to intramitochondrial fatty acid oxidation.

In isolated rat heart, maximal mitochondrial rates of acyl-CoA oxidation require a carnitine concentration of about 1.5 mM.[33] Reduced levels of carnitine and CoASH in the extra-matrix space could limit carnitine acyl transferase activity.[27]

## CARNITINE METABOLISM

### *Chemistry*

Since the discovery of carnitine in meat extract in 1905, its chemical structure was studied intensely thereafter. In 1957, Fraenkel and Friedman[4] summarized the early research on carnitine chemistry. The chemical structure of carnitine is shown in the following:



Carnitine ($\beta$-hydroxy-$\gamma$-trimethylaminobutyric acid)

Carnitine is hygroscopic and very soluble in water. Like other asymmetric compounds, carnitine exists in the D- and L-forms, and only the L-form is biologically active. Biological assay, using *Tenebrio molitor* indicates that DL-carnitine has half the activity of L-carnitine.[4] Information concerning the calorie content of carnitine has not been obtained from this literature search. Metabolizable energy of carnitine is expected to be insignificant since approximately 1% of the injected radioactive carnitine was recovered as expired $CO_2$ in rats.[34]

Early chemical and biological methods for carnitine determination have been reviewed by Fraenkel and Friedman.[4] Results of carnitine in biological samples obtained from these methods were not accurate due to presence of interfering substances. A more specific enzymatic assay was developed in 1964 by Marquis and Fritz,[35] which was based upon the following reaction catalyzed by carnitine acetyl transferase.

$$\text{Acetyl-CoA + L-Carnitine} \rightleftharpoons \text{Acetyl-L-carnitine + CoASH}$$

The CoASH thus produced reacts with DTNB (5,5'-dithiobis-2-nitrobenzoic acid) and is measured colorimetrically; however, this method is relatively insensitive to low levels of carnitine in certain biological samples. The deficiency was overcome by Cederblad and Lindstedt[36] who introduced a radioisotopic method based upon the same enzymatic reaction using labeled acetyl-CoA. Further modification of this method was made by McGarry and Foster[37] to yield a linear response over a wide range of carnitine concentrations without the need of excessive amounts of labeled acetyl-CoA. This method has been successfully employed to determine carnitine in tissue extracts.[38]

### *Biosynthesis*

In 1929 Linneweh[39] found that $\gamma$-butyrobetaine ($\gamma$-trimethylaminobutyrate) injected into dogs led to increased urinary excretion of carnitine. This work, repeated in rats by Lindstedt and Lindstedt using [$^{14}$C]carboxy-labeled $\gamma$-butyrobetaine,[40] showed that within 30 hr over 70% of the labeled compound had been converted into carnitine. Further work[41, 42] led to the purification of an enzyme from rat liver which is capable of hydroxylating $\gamma$-butyrobetaine in the presence of oxygen, ascorbate, and $Fe^{2+}$ to yield carnitine.

The methyl groups of carnitine were thought to be derived from a common methyl pool. In rats, it was shown that the methyl groups of carnitine were derived from the essential AA methionine in the diet.[43] Addition of 0.7% L-methionine to a basal diet resulted in enhanced growth of rats and an increase in the creatinine content of the liver.[44] Using choline- and methionine-deficient rats in which liver carnitine was only 30% of normal, Strength and Yu[45] found that injection of methyl-labeled methionine increased the radioactivity of carnitine to more than 15 times that of carnitine from control rats injected with the same compound. Incorporation was maximal at 3 hr postinjection with all the activity in the methyl groups of carnitine.

The origin of the carbon chain of carnitine was identified as derived from lysine, a dietary essential AA, by

Tanphaichitr and Broquist.[46, 47] Rats fed a carnitine-free, lysine-depleted diet showed 30% decrease in muscle carnitine compared with animals consuming a carnitine-free, lysine-supplemented diet. On the other hand, laboratory chow containing a normal level of carnitine resulted in 30–40% higher tissue carnitine, compared with animals given the carnitine-free, lysine-supplemented diet.[46] A recent study by Khan and Bamji[48] in rats confirmed the observation that a diet deficient in lysine results in low carnitine levels in plasma and skeletal muscle. However, carnitine levels in liver and heart were not altered. Borum and Broquist[49] also reported minor reduction of carnitine in plasma, heart, and skeletal muscle but elevated carnitine in the liver of rats fed a lysine-deficient diet.

It has now been established by appropriate isotope-labeling studies that carbon 3, 4, 5, and 6 of lysine and the ε-nitrogen (N) atom form the carbon and N backbone of carnitine, and that the *S*-methyl group of methionine furnishes the methyl groups of carnitine.[50] While most tissues are able to carry out early steps in carnitine biosynthesis, the final production in the pathway, hydroxylation of γ-butyrobetaine, occurs primarily in the liver, and not at all in the skeletal muscle and heart of the rat.[51] The metabolic pathways leading to the biosynthesis of carnitine are shown in stepwise reactions in Figure 2.

The first reaction involves the formation of ε-*N*-trimethyllysine from methionine and lysine. Methylation of free lysine seems to be questionable since mammals lack the enzyme which methylates free lysine. However, methylation of protein-bound lysine by methylases is well documented.[52] Therefore, the suggestion was made that protein-bound lysine is methylated by *S*-adenosyl methionine; the resulting trimethyllysine could then be released upon proteolytic degradation of the methylated protein.[53, 54] This hypothesis was demonstrated experimentally by LaBadie et al[55] who showed that carnitine biosynthesis involves trimethyllysine as a peptide-bound precursor. In the intact rat, over 21% of injected trimethyllysine was converted to carnitine,[54] whereas isolated tissues yielded only small amounts of carnitine when incubated with trimethyllysine. Haigler and Broquist[51] found that kidney or liver slices can convert 7% of trimethyllysine to γ-trimethylaminobutyrate (γ-butyrobetaine), the immediate precursor of carnitine. This low rate of conversion may be due to impermeability of trimethyllysine to cell membranes.

Hulse et al[56] recently succeeded in establishing the complete pathway from trimethyllysine to carnitine by breaking the cell and working with subcellular fractions. The first reaction is a hydroxylation of trimethyllysine to yield β-hydroxy-ε-trimethyllysine. The hydroxylase was found to be located in the outer mitochondrial membrane. The interesting feature of this enzyme was its required cofactors, α-ketoglutarate, $Fe^{2+}$, and ascorbic acid. The cofactor requirement is identical to that for the hydroxylation of peptide-bound proline to hydroxyproline and of γ-trimethylaminobutyrate (γ-butyrobetaine), the last step in the carnitine biosynthetic pathway.

The next step in the metabolic pathway is the formation of glycine and γ-trimethylaminobutyraldehyde from β-hydroxytrimethyllysine,[57] a reaction analogous to that catalyzed by threonine aldolase, whereby threonine is split into glycine and acetaldehyde. This reaction is followed by an NAD-linked dehydrogenation to oxidize the γ-trimethylaminobutyraldehyde to γ-trimethylaminobutyrate (γ-butyrobetaine). The conversion of γ-trimethylaminobutyrate to carnitine, by a ketoglutarate-$Fe^{2+}$ ascorbate-dependent hydroxylation, has been found in rat liver.[42] As shown in Figure 2, the pathway for carnitine synthesis has been established. Carnitine biosynthesis requires only two essential AA, lysine and methionine, and other vitamins serving as cofactors.

## *Absorption*

Carnitine is a naturally-occurring substance in foods. In a review article, Mitchell[58] lists free carnitine, lipid-bound carnitine, and total carnitine in foods. There is a wide discrepancy noted in the reported values, primarily attributed to assay methodologies. In general, carnitine is low in foods of plant origin and high in those of animal origin. It is interesting to note that plant materials are also most likely to be low in lysine and methionine, two

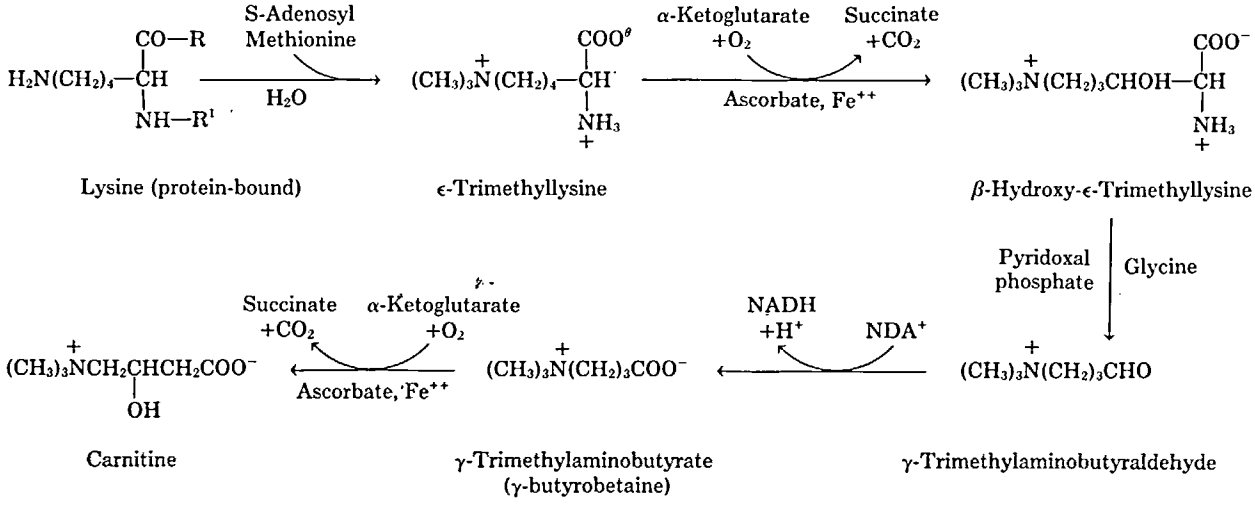



FIG. 2. Currently proposed biochemical pathway of carnitine biosynthesis.

essential AA identified as being essential for carnitine biosynthesis. Mikhail and Mansour[59] reported low serum carnitine levels in Egyptian subjects consuming predominantly cereal diets. Thus, a pure vegetarian diet may be low not only in preformed carnitine but also may contain limited amounts of the AA precursors of carnitine.

Literature information concerning the absorption of carnitine is very limited; a survey conducted by Mitchell[58] disclosed no studies on the absorption of carnitine, in either animals or humans. In view of its water-solubility, it is assumed that carnitine can be absorbed as readily as other water-soluble vitamins. The chemical forms in which creatinine is absorbed, however, are not clear at this time. Carnitine appears in foods as water-soluble free carnitine, short chain fatty acid esters, and water-soluble long chain fatty acid esters of carnitine. The latter forms can be hydrolyzed by esterases present in pancreatic juice, if the free form is required for absorption. The hypothesis that carnitine is absorbed in the free form is supported by the evidence that free serum carnitine levels rose within 30 min following oral administration of free carnitine.[60, 61]

The forms in which carnitine is transported in the circulation following absorption are not well documented; however, it is known that most tissues derive their carnitine from the circulation. In a study investigating the influence of age and diet on plasma carnitine levels in rabbits, Gillies and Bell measured different forms of carnitine present in the circulation.[62] They found that the majority of carnitine in blood was in the water-soluble forms, free carnitine, and short chain acyl carnitine; the long chain fatty acyl carnitine was not detectable. Results obtained from patients undergoing dialysis[63] showed that plasma carnitine decreased 66% from pre- to postdialysis, and that the loss of carnitine into the dialysate exceeded the normal loss in urine in most cases.[64] This observation suggests that carnitine in the circulation consists of small molecules that can be removed by dialysis. In a study using isolated rat liver cells, Christiansen and Bremer[65] found that the predominant form of carnitine released from liver into the incubation medium was acetyl carnitine. Based upon the findings discussed above, it is quite possible that the transporting forms of absorbed carnitine are free carnitine and acetyl carnitine.

### *Blood and Tissue Concentrations*

A great deal of variation has been found in the literature about the concentration of carnitine in blood and tissues of animals. The observed variation may partially be attributed to the difference in assay methods. Table I summarizes reported carnitine values found in normal laboratory rats. The variation in these values reflects numerous factors which can affect carnitine content.

Tissue concentrations and distribution of free and esterified carnitine in animals are influenced by diet and nutritional status.[66-68] In rabbits, the addition of 5% lard and 1% cholesterol resulted in significant increases in plasma and arterial tissue carnitine levels.[62] Corredor et al[70] demonstrated that rats fed a choline-deficient diet have reduced levels of carnitine in liver, heart, and skeletal muscle. Intraperitoneal injection of choline to rats fed a choline-deficient diet caused a prompt increase of carnitine in liver, whereas injections of other methyl donors, such as L-methionine, betaine, and sarcosine did not result in any changes.[71] Reduction of carnitine in muscle and plasma was also observed in rats fed a lysine-deficient diet.[35, 46, 47, 49] Starvation in rats (48 hr) resulted in significant elevation of carnitine in liver, skeletal muscle, and heart[71, 72] but a reduction in plasma.[71]

Age and sex of animals also contribute to the variation of tissue carnitine levels. The distribution of free carnitine and short chain acyl carnitine was altered and the aortic tissue content of carnitine increased 5-fold during the first 8 weeks postpartum in rabbits.[62] In newborn rats, plasma carnitine increased rapidly during the first 2 days after birth; carnitine in both heart and skeletal muscle increased, whereas liver carnitine decreased during the first week of life. In weanling rats, no differences in tissue carnitine were observed. In adult rats, carnitine levels in plasma, heart, and muscle were higher in the male rats, whereas levels in liver and urine were higher in the female rats.[73]

Increases of tissue carnitine levels also correlate with conditions in which accelerated fat metabolism in animals is expected to occur. Therriault and Mehlman[74] reported increases in the total amount of carnitine in the body and striated muscle of cold-acclimatized rats. In a later study in cold-acclimatized rats, Delisle and Radomski[72] found that levels of heart- and muscle-free carnitine and fatty acyl carnitine did not increase, but acetyl carnitine did. All three forms of carnitine increased in the liver of the cold-exposed rats. In a brief article, Van Alstyne et al[75] reported that plasma concentration of carnitine was greater in 20% body surface, full thickness burned rats than in control animals.

In humans, scattered information of carnitine content in blood and muscle are available in the literature. Information about carnitine content in tissues, such as liver, heart, and kidneys, are very limited. In a review of carnitine metabolism in humans, Mitchell[76] summarized literature values of carnitine in biological fluids and tissues of normal control subjects. Table II shows selected values of carnitine in plasma, skeletal muscle, and liver. Patients who served as controls in many studies were hospitalized for diseases other than the one under study; unfortunately, the investigators reporting these values as controls did not describe in detail the patients from whom the values were obtained so that it becomes difficult to compare the values reported in the literature from different studies.

Cederblad[77] found that women had significantly lower plasma concentrations of carnitine, and that there was a positive and significant relationship between age and plasma carnitine; similar observations were found in rats.[49, 73] In another study, Cederblad et al[78] obtained two pupillary muscle samples at open heart surgery from two female patients, aged 11 and 73 years. The concentration in heart muscle was lower than that in skeletal muscle measured in the same laboratory. This is in line with the data obtained in rats from one laboratory[47] but contradictory to others.[49, 69] Liver carnitine was determined in autopsy samples,[60] and the mean concentration was lower

TABLE I
*Total carnitine content in serum or plasma and various tissues in laboratory rats*

| Tissue | Carnitine | Animals (n) | Methodology | Ref. no. |
|---|---|---|---|---|
| Serum or plasma (nmol/ml) | 198 ± 7.8 | Weanling male (8) | Colorimetric | 35 |
| | 100 ± 3 | Adult male (8) | Radiochemical | 38 |
| | 51 ± 2 | Weanling male (16) | Radiochemical | 49 |
| | 20 ± 1 | Weanling female (10) | Radiochemical | 49 |
| Skeletal muscle (nmol/g wet tissue) | 1640 ± 140 | Weanling male (8) | Colorimetric | 35 |
| | 1616 ± 169 | Adult male (6) | Radiochemical | 38 |
| | 951 | Adult male, fed (6) | Radiochemical | 69 |
| | 1373 | Adult male, fasted (6) | Radiochemical | 69 |
| | 679 ± 24 | Weanling male (16) | Radiochemical | 49 |
| | 584 ± 25 | Weanling female (10) | Radiochemical | 49 |
| Liver (nmol/g wet tissue) | 440 ± 10 | Weanling male (8) | Colorimetric | 35 |
| | 698 ± 951 | Weanling male (3) | Colorimetric | 44 |
| | 369 ± 28 | Adult male (6) | Radiochemical | 38 |
| | 306 | Adult male, fed (6) | Radiochemical | 69 |
| | 417 | Adult male, fasted (6) | Radiochemical | 69 |
| | 79 ± 6 | Weanling male (16) | Radiochemical | 49 |
| | 117 ± 17 | Weanling female (10) | Radiochemical | 49 |
| Heart (nmol/g wet tissue) | 1900 ± 160 | Weanling male (8) | Colorimetric | 35 |
| | 1371 | Adult male, fed (6) | Radiochemical | 69 |
| | 1403 | Adult male, fasted (6) | Radiochemical | 69 |
| | 812 ± 16 | Weanling male (16) | Radiochemical | 49 |
| | 556 ± 21 | Weanling female (10) | Radiochemical | 49 |
| Kidney (nmol/g wet tissue) | 621 | Adult male, fed (6) | Radiochemical | 69 |
| | 776 | Adult male, fasted (6) | Radiochemical | 69 |

TABLE II
*Total carnitine content in serum or plasma and various tissues of normal human subjects*

| Age | Sex | n | Carnitine | Methodology | Ref. no. |
|---|---|---|---|---|---|
| *yr* | | | | | |
| | | | *Serum or plasma, nmol/ml* | | |
| Adult | M, F | 23 | 23 - 70 | Colorimetric | 9, 79 |
| Child | M, F | 13 | 23 - 60 | Colorimetric | 9, 79 |
| 21-64 | M | 16 | 57 ± 13 | Radiochemical | 77 |
| 19-67 | F | 45 | 46 ± 12 | Radiochemical | 77 |
| 16-81 | M | 26 | 47 | Radiochemical | 78 |
| 16-81 | F | 21 | 47 | Radiochemical | 78 |
| 21-30 | M | 11 | 43 | Radiochemical | 78 |
| 21-30 | F | 8 | 37 | Radiochemical | 78 |
| | | | *Skeletal muscle,[a] nmol/g wet tissue* | | |
| 16-81 | M, F | 48 | 3887 ± 1032 | Radiochemical | 78 |
| 35-77 | N/A | 18 | 4220 ± 907 | Colorimetric | 61 |
| | | | *Liver, nmol/g noncollagen protein* | | |
| N/A | N/A | 10 | 6800 ± 700 | Radiochemical | 136 |

[a] Carnitine in muscle is calculated by assuming 76% water content, and by the equation $Y = 1.2x + 4.5$ where $x$ = μmol/g dry weight, and Y = μmol/g noncollagen protein.

than in skeletal muscles. Liver concentrations in laboratory rats are considerably lower than in skeletal muscle;[38, 49, 69] thus, it appears that the use of laboratory rats for carnitine research is well justified.

In the study reported by Cederblad et al[78] there was no relationship between muscle and plasma carnitine concentrations in the human. Plasma carnitine by itself is unlikely to be a satisfactory index of the state of carnitine storage in the body. Low carnitine plasma levels may or may not signify a depletion of body carnitine.

There is no difference between plasma carnitine (prepared either with heparin or EDTA as anticoagulants) and serum carnitine.[7] It was also found that carnitine is stable in plasma kept at room temperature for 4 hr and frozen at −35°C for one month.[80]

### *Carnitine Transport*

In the rat, carnitine biosynthesis occurs in the liver, and not at all in skeletal muscle and heart.[51] Exogenous carnitine from dietary sources is absorbed into the circulation. The carnitine content in skeletal muscle or heart is much greater than that in plasma, which is the carrier of carnitine from liver or dietary sources to muscle or heart. Therefore, it is likely that carnitine enters muscle cells by an active transport mechanism against a concentration gradient.

In order to determine whether carnitine enters muscle cells by an active transport mechanism, investigators at two separate institutions conducted in vitro experiments simultaneously, using rat extensor digitorum longus muscle. Rebouche[81] reported that the mechanisms involved with L-carnitine uptake are consistent with the presence of a carrier-mediated active transport system associated with the sarcoplasmic membrane. The transport system is saturable and has a degree of specificity for L-carnitine. The apparent $K_m$ value obtained for L-carnitine was 60 μM. The investigator further speculated, based upon the $K_m$ value, that under normal physiological conditions, L-carnitine transport would be slightly less than half maximal capacity of the system as the average serum concentrations in normal rats are slightly below the $K_m$ value. Results obtained with various metabolic inhibitors and from anaerobiosis indicate that metabolic energy is required for transport.

Willner et al[82] conducted a similar study as that reported by Rebouche[81] to determine whether a concentration gradient between skeletal muscle and blood is maintained by an active transport mechanism, using soleus and extensor digitorum longus muscles. Results from this study support the conclusion that L-carnitine is actively transported across muscle cell membrane from lower concentrations in blood. Observations consistent with the existence of an active transport mechanism include: a) anaerobic incubation reduced intracellular carnitine accumulation by approximately 30%; b) the rate of carnitine accumulation was saturated at high concentrations of substrate; and, c) transport was competitively inhibited by γ-butyrobetaine (a structure analog). The $K_m$ values determined in this study were 0.259 and 0.585 mM for soleus muscle and extensor digitorum longus muscle, respectively. The value for extensor digitorum longus muscle, 0.585 mM, is approximately 10-fold higher than the 60 μM reported by Rebouche.[81]

The specificity and characteristics of the uptake mechanism have also been studied in cultured human heart cells, using radiolabeled L-carnitine.[83] The investigators suggested that the carnitine transport depends upon free sulfhydryl groups and is not linked to the transport of AA or glucose. Variation in osmolality in the incubation medium within 225–450 mOsm/kg did not influence carnitine uptake. A change in pH from 7 to 8, however, did reduce the uptake by approximately 40%. Similar to the results obtained in rat skeletal muscles,[81, 82] structurally-related compounds containing a quaternary ammonium, and a carboxylic group such as γ-butyrobetaine significantly reduced carnitine uptake. In this study, radiolabeled L-carnitine was also actively transported into the cells. Since a significant portion of serum total carnitine is in the form of acetyl carnitine (30%), both L-carnitine and L-acetyl carnitine can be physiological substrates for this active transport mechanism in vivo.

In isolated liver cells from rats, Christiansen and Bremer[65] reported that both carnitine and γ-butyrobetaine are taken up by an active transport mechanism. The latter compound is a precursor in the last step in carnitine biosynthesis, which is localized mainly in the liver.[42, 53] Data obtained in this study indicate the existence of a common carrier in the cellular membrane that mediates the transport of both carnitine and γ-butyrobetaine; the carrier was found to have a high affinity for γ-butyrobetaine ($K_m$ = 0.5 mM) and a lower one for carnitine ($K_m$ = 5.6 mM). Additional results showed that both carnitine and acetyl carnitine are released from the liver cells and that release of both compounds is probably physiological since acetyl carnitine constitutes a similar fraction of the total acid-soluble carnitine in the blood and liver of the intact rat.

### *Carnitine Storage and Turnover*

Results obtained from animal studies suggested that carnitine is primarily synthesized in the liver and stored in skeletal muscles.[47] The highest concentrations of carnitine are in adrenal glands, followed by heart, skeletal muscle, adipose tissue, and liver; smaller concentrations are found in kidney and brain. This pattern of carnitine concentration seems to reflect the utilization of fatty acids by the tissues as primary energy sources. Tissues such as brain, that utilize mainly glucose under normal physiological conditions, have the least amount of carnitine.

The total carnitine pool of rats has been estimated either by direct analysis of carcass carnitine or by radioisotopic dilution technique. Using the direct chemical determination, Tsai et al[84, 85] reported a figure around 40 μmol/100 g body weight (6.4 mg/100 g). A similar figure of 35 μmol/100 g (5.67 mg) was estimated by Cederblad and Lindstedt,[34] using a two-compartment metabolic model following intraperitoneal injection of L-[Me-$^{14}$C] carnitine. Mehlman et al[86, 87] reported earlier that the body pool of rats contained 159 μmol/100 g body weight (25.76 mg/100 g), using a radioisotope technique. This figure is considerably higher than that reported by other investigators; the differences may be attributed to the use of a one-compartment model as well as the analytical methodology for carnitine determination.

The turnover time of carnitine has been estimated in a single rat to be 97 days.[43] Later studies,[86] performed in a number of animals injected with physiological doses of carnitine, showed that the turnover time is in the order of 16–12 days (based upon data from 8–10 day urine collections). Using DL-carnitine, Tsai et al[84] obtained results suggesting two apparent carnitine turnover pools with turnover times of 4.6 and 24.5 days. These results were obtained from an extended urinary collection period of 42 days. By comparing the total radioactivity excreted from the slower turnover pool, the investigators estimated the relative size of the two pools to be approximately 1:5. Based on this information, the average turnover time of body carnitine was calculated to be 21 days. In a later study,[85] the investigators repeated the turnover time estimation, using L-[$^{14}$C]carnitine instead of the DL-enantiomer reported earlier,[84] and found that the turnover time was longer (35 days) than that obtained for DL-carnitine (21 days). Cederblad and Lindstedt[34] estimated that about 7% (2.3 μmol/100 g body weight) of the total body pool of carnitine was excreted daily. Using data for specific radioactivity of plasma carnitine, Brooks and McIntosh[89] calculated a daily turnover of 25 μmol/100 g body weight.

Carnitine turnover time is influenced by factors that affect fat metabolism; in cold-exposed rats, carnitine turnover is enhanced.[85] The turnover of carnitine in alloxan-diabetic rats is approximately 4 times faster than in normal rats.[87] It was suggested that the enhanced carnitine turnover in diabetic animals may be associated with a more rapid utilization of FFA as a result of impaired carbohydrate metabolism. Delayed carnitine turnover has also been found in rats fed a choline-deficient and low-methionine diet.[84, 85]

Using the turnover time and total body carnitine pool, Tsai et al[84] estimated that rats fed a low methionine, choline-deficient diet would have to synthesize no more than 1.5 μmol carnitine/100 g body weight/day. This quantity was estimated to be approximately 5% of the methionine intake in rats consuming 7–8 g of a diet containing 0.22% methionine. This result is different from an earlier study,[44] wherein an estimate of up to 25% of the methionine present in an 8% casein diet was utilized for carnitine synthesis. A recent study by Cederblad and

Lindstedt[34] indicates that the dietary intake was about 0.7 μmol. Assuming that the rats were in steady state, the daily synthesis of carnitine would thus be about 3 μmol for rats weighing 105–220 g.

### *Urinary Excretion*

Using labeled L-[Me-$^{14}$C]carnitine, Lindstedt and Lindstedt[34a] found that only about 3% of the administered dose was recovered as respiratory $CO_2$ in rats during a 24-h period. This observation implies that the majority of body carnitine is excreted in the urine. It has been suggested that under normal physiological conditions, a large portion of the body carnitine is converted to metabolites of carnitine prior to excretion in the urine.[87] Khairallah and Wolfe[90] reported the presence of carnitine decarboxylase in rat liver, kidney, muscle, and adrenals, with the largest amount in heart. From 2 to 20% of the injected methyl-labeled carnitine was excreted in the urine of rats as β-methylcholine from decarboxylation of carnitine by the enzyme carnitine decarboxylase. In rats fed a choline-deficient diet, most of the body carnitine is converted to β-methylcholine before it is excreted.[87] Tsai et al[85] estimated between 10 to 20% of the carnitine was excreted intact in the urine of rats, and that the amount in the urine appeared to be affected by choline content in the diet. Based upon available information in the literature, it appears that not all carnitine catabolic products excreted in the urine have been investigated.

Since urine samples are readily accessible in human subjects, literature concerning urinary carnitine data under various physiological conditions are available. It has been reported[91] that free urinary carnitine is slightly lower in women than in men, indicating the lower muscle mass in women (28.7 mg/day for 20- to 47-year-old males and 13.8 mg/day for 38- to 60-year-old females). Results obtained from a large number of normal subjects (143 males, 135 females) indicate that males between 16 to 50 years excreted a mean value of 59.3 mg/day carnitine, whereas females excreted a mean value of 44.1 mg/day.[92, 93]

Friedman and Fraenkel[4] reported urinary carnitine in human subjects to be 56 μg/ml after consuming a high protein diet. The concentration increased to 132 to 264 μg/ml following 3 days of starvation. Maebashi et al[92] reported that, in fasted adult male subjects consuming water and fruit juices daily, urinary carnitine increased from 63 to about 330 mg on the 5th day and that the increases were progressive. Excretion decreased progressively to the original level at the end of the 10-day fast. Similar changes in urinary carnitine during a 5-day starvation were observed in adult subjects;[93] the maximum carnitine level was 352 ± 16 mg/day. Respiratory quotients (RQ) in these subjects during the fast period was negatively correlated to urine carnitine levels, perhaps indicating that an enhanced fat utilization requires increased amounts of carnitine.

Muscular activity, a process requiring long chain fatty acids as the primary energy source, was found to influence urinary carnitine excretion. Cederblad and Lindstedt[36] reported that more carnitine is excreted during periods of daily activity than during nocturnal sleep, and suggested that muscle activity occurring during the day time is the primary cause of this difference. In limited trained male students consuming a standard diet, physical exercise before each meal increased urinary carnitine from 55 ± 19 mg/day during a nonexercise control period to 94 ± 27 mg/day.[94]

### *Carnitine Toxicity*

Since carnitine is a natural compound present in food and can be synthesized in the liver from lysine and methionine,[51] very little information concerning its toxicity is available in the literature. In one study, carnitine and its related compounds were injected subcutaneously into mice to determine the median lethal doses.[95] Results are summarized in Table III. $LD_{50}$ value for L-carnitine is 8.9 g/kg and for D-carnitine, 10.3 g/kg, indicating that carnitine is a nontoxic substance, with $LD_{50}$ values approximately equivalent to AA.

Carnitine exerts specific effects on cardiac and peripheral hemodynamics. In anesthetized dogs, IV infusion of DL-carnitine HCl at rates of 10–70 mg/kg/min caused dose-related responses in vasodilation of coronary, pulmonary, and systemic vascular beds, as well as inotropic effects of muscles.[64] Improvements in myocardial ischemia in dogs[96] and improved stress tolerance of the ischemic myocardium in men[97] have been attributed to the vasodilating and inotropic effect of carnitine.

## CARNITINE AND DISEASES

### *Animal Experimentation*

*Growth retardation.* Although carnitine can be readily synthesized in the liver of rats from its precursors, methionine and lysine,[51] limited intakes of these AA can cause low tissue carnitine levels.[44, 48, 98] Dietary supplementation of 0.2% DL-carnitine to diets low in methionine has led to increased growth in rats;[44] augmentation of dietary methionine also resulted in enhanced growth and increased liver carnitine. The results of this study led to the suggestion that carnitine has a methionine-sparing action, and may thus be considered as a required nutrient in marginal diets for rats.

*Fatty liver.* In view of the role of carnitine in the mitochondrial transport of fatty acids and their subsequent oxidation as energy substrates, an impairment in lipid metabolism can occur under nutritional conditions, leading to tissue carnitine deficiency. Rats fed a rice diet limited in lysine and threonine developed fatty liver with retarded growth.[99] In another study, the liver lipid accumulation was found to be triglyceride and total cholesterol fractions.[98]

TABLE III
*Toxicity of carnitine compounds in mice injected subcutaneously*[a]

| Substance | n | $LD_{50}$ | | Confidence range (95%) | |
|---|---|---|---|---|---|
| | | *g/kg* | *mM/kg* | *g/kg* | *mM/kg* |
| L-carnitine | 823 | 8.9 | 55 | 8.1–9.8 | 50–61 |
| D-carnitine | 40 | 10.3 | 64 | 10.0–10.6 | 62–66 |
| Acetyl-L-carnitine | 38 | 8.4 | 41 | 7.3–9.7 | 36–48 |
| Acetyl-D-carnitine | 41 | 10.9 | 54 | 9.6–12.4 | 32–61 |
| Butyryl-L-carnitine | 29 | 12.0 | 52 | 10.9–13.2 | 47–51 |
| Decanoyl-L-carnitine | 33 | 2.6 | 8.2 | 2.4–2.8 | 7.6–8.9 |
| Decanoyl-D-carnitine | 28 | 2.1 | 6.7 | 1.9–2.3 | 6.0–7.3 |

[a] Data from reference (95).

Two possible causes for the production of fatty liver have been considered: a) an impairment in the formation of the lipoprotein complex from apoprotein and the lipid component which is necessary for triglyceride removal from the liver into the plasma;[100] and/or, b) an impairment of fatty acid oxidation related to the role of carnitine in the mitochondrial transport of fatty acids.[100, 101] Dietary supplementation of 2% DL-carnitine to a lysine- and threonine-deficient diet in rats reduced slightly the total lipid content, but greater reductions in liver lipid were seen in rats fed the carnitine-supplemented diets with either lysine alone or lysine and threonine.[98] Increases in tissue carnitine have been observed in all rats fed carnitine- and/or AA-supplemented diets. The observation that reduction in liver lipid content was greater in rats fed diets supplemented with carnitine, lysine, and threonine than carnitine alone may be attributed to the fact that the addition of the AA may enhance synthesis of enzymes necessary for fatty acid oxidation. In a recent study, Khan and Bamji[48] observed similar effects of dietary supplementation of DL-carnitine on the reduction in tissue lipid contents, including liver of rats. In addition to the role of carnitine in fatty acid transport across mitochondrial membrane, these investigators further suggested that an adequate supply of carnitine may be necessary to preserve the functional integrity of the fatty acid oxidation system.

*Ketosis.* Mild elevation of circulating ketone bodies as a result of enhanced fatty acid oxidation is not considered to be harmful; the ketone bodies can serve as fuel to extrahepatic tissues during starvation. In newborn rats, a surge of ketone body production was observed with a concomitant elevation in liver carnitine content during the transition from intrauterine to extrauterine period.[101] During starvation, significant ketosis can result from an accelerated fatty acid oxidation in the liver.[102] Carnitine enhances oxidation of acetoacetate in isolated mitochondria of heart muscle and kidney.[103] It has also been demonstrated in laboratory animals[104, 105] and children[106] that carnitine either stimulates or inhibits the uptake of ketone bodies, depending upon the nutritional state and degree of ketosis. In a recent study, Yeh[107] reported that oral administration of carnitine to rats reduced plasma concentration of ketone bodies; however, concentrations of plasma ketones below 1.2 mM were not affected by carnitine. The investigator suggested that carnitine has a dual function in ketone metabolism: stimulating hepatic ketogenesis on the one hand and stimulating ketone utilization by extrahepatic tissues on the other. Since fatty acid and ketone bodies are important energy substrates, the different actions of carnitine may provide a basis for treatment of various physiological conditions involving fat metabolism.

*Diabetes.* Carnitine levels are changed in fluids and tissues of rats that have been made diabetic with alloxan. Mehlman et al[86] reported that muscle carnitine levels in alloxan-treated rats were reduced to half of those found in control animals, while insulin treatment partially restored the carnitine level. McGarry et al[108] found that total liver carnitine increased from 92 in fed controls to 358 μg/liver in alloxan-diabetic rats, whereas rats fasted for 24 hr showed an immediate increase to 168 μg/liver. Khairallah and Wolfe[90] found increased urinary β-methylcholine excretion in alloxan-diabetic rats compared with animals fed a 9% casein diet. Results from these studies suggest that diabetic animals have an influx of carnitine from muscle to the liver and the accumulated carnitine is decarboxylated prior to excretion.

In diabetic animals, production of ketone bodies, acetoacetate, β-hydroxybutyrate, and acetone increases due to enhanced fatty acid oxidation in the liver. It is, therefore, of particular interest that liver carnitine increases in alloxan-diabetic animals. Fasting is another condition in which ketone bodies are produced. McGarry et al[108] showed that fasted rats also accumulate carnitine in the liver but not to the extent of alloxan-diabetic rats. It has been proposed that in fasting rats the activation of hepatic ketogenic capacity is brought about by elevation of the circulating glucagon to insulin ratio.[102] This change in ratio causes increased release of muscle carnitine with concomitant increase in liver carnitine either from increased liver carnitine synthesis or uptake, which consequently induces fatty acid oxidation and hepatic ketone body formation.[108] If the production mechanisms of ketone bodies are the same in fasting as in diabetes, it appears that the timing of carnitine manipulation would be crucial in determining the extent in which ketone body production could be reduced in uncontrolled ketotic diabetes. Based on our earlier discussion, it appears that carnitine plays a role in both the production and utilization of ketone bodies.

*Myocardial ischemia.* The heart is known to utilize long chain fatty acids as its primary energy substrates.[109, 110] High levels of FFA have been found in acute myocardial infarction.[111, 112] Fritz et al[6] have shown that the oxidation of long chain fatty acids is highly dependent on the presence of carnitine. In a review of the role of carnitine in fatty acid metabolism of normal and ischemic myocardium, Opie[113] stated that whether or not carnitine plays a role in decreasing myocardial ischemic damage requires a knowledge of four postulates. First, tissue carnitine levels in the ischemic heart must be known. Secondly, the equilibrium constant for carnitine in the acyl transferase system should be such that carnitine concentration decrease in ischemic heart is significant. Thirdly, carnitine should be taken up by the ischemic tissue. Fourthly, rates of oxidation of long chain fatty acids should increase and overall tissue adenosine triphosphate (ATP) should increase after carnitine is provided to the ischemic heart.

Reported animal studies have provided relevant information on the above postulates. 1) Shug et al[114] have shown that the myocardial carnitine content decreased by one-third after 10 minutes of regional ischemia and, by 30 minutes, the content was reduced in half. 2) The maximal oxidation of long chain fatty acids by rat heart mitochondria was achieved by a carnitine concentration of 1.5 mM,[33] which is approximate to the value of 1.0 μmol/g wet tissue reported by Shug et al.[114] Hence, a fall of tissue carnitine by half in ischemia should significantly reduce oxidation of activated long chain fatty acids in mitochondria. 3) Infusion of L-carnitine into the coronary bed during regional ischemia in the dog was associated with a local increase in carnitine content to 2.2 μmol/g wet tissue.[96] 4) The infusion of L-carnitine increased tissue ATP, creatine phosphate, and adenine nucleotide

translocase activity in the ischemic tissue.[96] Furthermore, after the addition of carnitine directly to ischemic heart mitochondria, there was a partial restoration of the depressed oxygen uptake,[115] and increased the oxygen uptake of heart mitochondria after inhibition of mitochondrial adenosine diphosphate phosphorylation by long chain acyl-CoA esters.[116] Using isolated rabbit heart, Fanelli[117] reported that carnitine and derivatives at the concentration of 10 mg/L increased significantly the contractile force depressed by anoxia. The inotropic effect of IV administered DL-carnitine on myocardial muscles has also been demonstrated in dogs.[64]

*Others.* In renal patients undergoing dialysis, it has been observed clinically that a significant portion of plasma carnitine is lost to the dialysate.[63, 64] Bartel et al reported losses of carnitine in liver, heart, and muscle tissues, using a rat model simulating peritoneal dialysis in humans.[118] Results indicated a loss of approximately 30% in skeletal muscle (4.1 ± 0.25 vs 2.98 ± 0.30 μmol/g dry wt) and 40% in cardiac muscle (6.02 ± 0.40 vs 3.61 ± 0.62 μmol/g dry wt). Liver carnitine concentrations, however, did not change significantly during dialysis. Carnitine losses observed in skeletal and cardiac muscles may cause abnormal lipid metabolism in renal patients requiring frequent dialysis.

The role of branched chain amino acids (BCAA), leucine, isoleucine, and valine in such altered nutritional and physiological states as sepsis, stress, and trauma is currently under investigation at many institutions. In addition, BCAA have been found to play a special role in decreasing protein degradation and increasing protein synthesis.[119, 120] Continuous infusion studies in vivo using traumatized rats indicate that these AA also conserve N.[121] Recently, a number of investigators have identified acyl carnitine derivatives of BCAA in various tissues of rats.[122-124] The proportionate increase in isobutyryl and isovaleryl carnitine in muscle of fasted rats has also been observed, lending support to the observation that such peripheral tissues as skeletal muscle can metabolize BCAA.[125] In vitro studies, using gastrocnemius muscle and liver homogenates from fasted and diabetic rats, showed that addition of L-carnitine (0.5–2.0 mM) increased the rate of α-decarboxylation of leucine (125%) and valine (38%) in muscle but not in liver homogenate.[126] The activity of carnitine acyl transferase, using isovaleryl-CoA as a substrate, was 18 times higher in muscle than liver mitochondria. Furthermore, both starvation (48 hr) and diabetes increased the rate of α-decarboxylation of leucine by muscle without having a remarkable effect on the concentration of carnitine or the activity of carnitine acyl transferase. In a follow-up study, these investigators concluded that the stimulation of BCAA oxidation by carnitine is not due to the increased transport across mitochondrial membrane as that for long chain fatty acyl-CoA but to the activation of the decarboxylase moiety of the branched chain α-ketoacid dehydrogenase complex.[127] However, no information is currently available about N-conserving properties attributed to the oxidation of BCAA in traumatized animals administered carnitine. In anesthetized rats, subcutaneous injection of DL-carnitine at 6 mg/100 g body weight following 33 μg/100 g noreprinephrine injection (a condition likely to occur in trauma) caused increased oxygen consumption.[128]

### *Clinical Observation*

Even though the physiological role of carnitine in fat metabolism was recognized over a decade ago, clinical observations of its deficiency in human patients have not been reported until recent years. This may be partially attributed to the assumption that normal dietary intake and endogenous synthesis of carnitine can meet the total body needs. Mitchell[129] recently summarized cases in which carnitine deficiency was diagnosed and, in certain cases, where treatment with exogenous carnitine was instituted, clinical improvements were observed.

There appear to be two types of carnitine deficiency. In the first type, hepatic, serum, and extrahepatic tissue levels are all depressed, indicating a defect in carnitine biosynthesis.[12, 60] In the second type, serum carnitine levels are normal, whereas tissue levels are depressed, indicating a defect in carnitine uptake in tissue cells.[9, 61] In addition to these two distinct types of carnitine deficiency, mechanical removal of carnitine, such as that seen in dialyzed renal patients, may also render patients carnitine-deficient.[13] Protein-calorie malnutrition,[130] diabetes,[131] and thyroid disorders[10] may also lead to altered carnitine and lipid metabolism.

*Lipid storage myopathy.* The pathological presence of lipid in type I muscle fibers was first described by Bradley et al in 1969,[132] who defined this new pathological condition as lipid storage myopathy and speculated that the lipid accumulation was due to an impaired mitochondrial oxidation of FFA. In the following year, Engel et al[133] reported a skeletal muscle disorder associated with intermittent symptoms of muscle cramp in identical twins, who also had myoglobinuria and lipid accumulation in the muscle. The investigators ascribed these observations to a possible defect in long chain fatty acid metabolism. Bressler[134] in an editorial letter suggested that the pathological etiology of the observed lipid accumulation in muscle of the identical twins may have been related to a deficiency of either carnitine or carnitine acyltransferase I or II. Since then, two metabolic disorders of fat metabolism have been recognized. Engel and Angelini[135] in 1973 described one of the disorders to be carnitine deficiency. Muscle carnitine deficiency associated with weakness and triglyceride accumulation in muscle fibers has been referred to as type I lipid storage myopathy. The other disorder described by DiMauro and DiMauro,[136] also in 1973, is caused by carnitine palmityl (acyl) transferase deficiency, which is associated with attacks of myoglobinuria provoked by fasting or exertion, minimal or no lipid excess in the muscle fibers, and normal levels of muscle carnitine. Since then, a number of cases in both disorders have been reported (Table IV).

Although histochemical examinations may provide positive diagnosis of lipid storage myopathy, biochemical abnormalities leading to the pathogenesis of the carnitine deficiency syndrome are not clear at the present time. Angelini et al[61] suggested a number of biochemical alterations that may cause the deficiency: a) defect in carnitine transport from the extracellular fluid into muscle; b)

TABLE IV
*Summary of clinical observations of altered carnitine metabolism*

| Diagnosis | Age | Sex | Symptoms | Treatment | Ref. no. |
|---|---|---|---|---|---|
| | *yr* | | | | |
| Metabolic myopathy | 18 | F | Muscle cramps, myoglobinuria | None | 133 |
| Carnitine palmityl transferase deficiency | 29 | M | Muscle cramps, myoglobinuria | None | 136 |
| Lipid storage myopathy due to carnitine deficiency | 24 | F | Muscle weakness | Prednisone—improved | 135 |
| Muscle carnitine deficiency | 61 | F | Muscle weakness complicated by diabetes mellitus | None | 9 |
| Systemic carnitine deficiency | 11 | M | Muscle weakness, liver complication, fatigue, low appetite, confusion and stupor | DL-Carnitine, 2 g/day, showed improvement | 60 |
| Hereditary carnitine deficiency | 8 | M | Muscle weakness and atrophy, right ventricular hypertrophy | Prednisone—improved | 137 |
| Carnitine deficiency | 10 | F | Muscle weakness, anorexia | 15–23 g DL-Carnitine/day dietary management with improvement after 6 months | 61 |
| Carnitine deficiency | 20 | M | Muscular weakness, lipid accumulation, abnormal mitochondria | Prednisone 30 mg/day, slight improvement<br>Propranolol 40 mg 3 times daily, dramatic improvement | 138 |
| Systemic carnitine deficiency | 20 | F | Muscle weakness, vomiting, azotemia, cardiac arrest, fatal fatty liver, and lipid storage myopathy | None | 11 |
| Partial carnitine deficiency | 51 | F | Obesity, muscle weakness, lipid storage, liver morphological alteration | Prednisone 40–100 mg daily | 139 |
| Carnitine deficiency due to defect in biosynthesis | 8 | M | Lipid deposits in liver muscle, lethargy weakness, fatal | None | 140 |
| Carnitine deficiency, lipid storage myopathy | 28 | F | Lipid storage in muscle, liver, kidney, and myocardium; muscle weakness, cardiac failure, coma | DL-Carnitine 2 g/day (1 case), ineffective | 141 |
| Carnitine palmityl-transferase deficiency | 22 | M | Muscle pain, fatigue, high BUN, renal failure, increased plasma carnitine | None | 142 |
| Partial carnitine palmityltransferase deficiency | 28 | M | Myoglobinuria, muscle pain, accumulation of lipid in muscle, normal ketone | None | 143 |
| Cardiomyopathy due to carnitine deficiency | 30 months | M | Dyspnea, duskiness, muscle weakness, lipid storage, myopathy, fatal | DL-Carnitine 1.5 g/day | 144 |
| Carnitine palmityl-transferase deficiency | 20 | M | Muscle cramps, myoglobinuria, severe myalgias, dyspnea, lipid accumulation | None | 145 |
| Cardiac ischemia | 21 patients | | Coronary artery disease, exertional angina pectoris | DL-Carnitine 20–40 mg/kg IV, improved stress tolerance and heart rate | 97 |
| Carnitine palmityl-transferase II deficiency | 20 | M | Transitory muscle pain and pigmenturia | None | 146 |

lack of hypothetical protein receptor in tissues, especially in muscle; c) abnormal release from or abnormally accelerated breakdown by tissues; and d) defective hepatic carnitine synthesis.

Metabolic acidosis has also been observed in patients with systemic carnitine deficiency,[11, 60, 141] at least partially attributable to lactic and ketoacidosis. The lactic acidosis might be a consequence of accelerated glycolysis when fatty acids are not available as energy substrates and impaired use of lactate by liver for gluconeogenesis.[140] Liver dysfunction, as evidenced by marked elevation of serum liver enzymes and fatty liver, has been reported in fatal cases ascribed to carnitine deficiency.[141] The mechanism of the ketoacidosis, which occurs during exacerbation of systemic carnitine deficiency, is of special interest. It is unlikely to arise from the excessive $\beta$-oxidation of long chain fatty acids because of impaired transport in the absence of carnitine. Boudin et al[11] and Engel et al[140] have suggested that the acidosis may, in part, be due to excessive $\beta$-oxidation of fatty acids, resulting in high serum and urinary levels of dicarboxylic acids. This metabolic change has been demonstrated in one patient suffering from carnitine deficiency.[60] The $\beta$-oxidation is extramitochondrial and not carnitine-dependent, and is believed to occur in the endoplasmic reticulum of liver cells. In addition, ketone bodies derived from the oxidation of ketogenic BCAA (leucine, isoleucine, and valine) have also been suggested to contribute to the acidosis.[11] The investigators speculated that, since these AA are preferentially oxidized in muscles, the initial step in the metabolism consists of the transfer of their amino group to pyruvic acid. The alanine thus formed is released from muscle, taken up by liver, and deaminated for gluconeogenesis. The carbon skeleton of the BCAA

can contribute to the total ketone production. If fatty acids are stored in muscle and are unavailable as energy sources, increased utilization of glucose yielding pyruvic and lactic acids, as well as increased oxidation of ketogenic BCAA, could result in metabolic acidosis.

Reported treatment of lipid storage myopathy has not been consistent. Exogenous carnitine can be effective if carnitine deficiency is due to impaired hepatic biosynthesis. Treatment of a carnitine-deficient patient with high doses of prednisone resulted in dramatic improvements;[147] subsequent treatment with DL-carnitine in combination with a medium chain triglyceride diet also maintained the patient's strength and normalized her blood carnitine level. Karpati et al[60] administered DL-carnitine at a dose of 0.1 g/kg body weight to an 11-year-old boy; Angelini et al[61] also treated a 10-year-old girl with DL-carnitine at a dose of 15 g/day for replacement therapy followed by 3–4 g/day. Both patients showed clinical improvement in strength and general health. Although spontaneous improvement could not be excluded, it is possible that oral carnitine represents an effective treatment for the disease.[61]

*Cardiac myopathy and ischemia.* In the generalized systemic form of carnitine deficieny, cardiac involvement in terms of lipid storage and/or decreased carnitine content has been reported.[11, 137, 141, 144] In the 3 cases reported by Boudin et al,[11] cardiac failure was identified to be the immediate cause of death. However, not in all reported cases of systemic carnitine deficiency, such as the one reported by Karpati et al,[60] are there clinical signs of cardiac dysfunction. The reason for the varying degrees of myocardial involvement in the different cases of carnitine deficiency described to date is not yet known. Boudin et al[11] suggested that cardiac involvement may be less extensive than that of skeletal muscle because the myocardium takes up carnitine preferentially from a common pool.

Myocardial ischemia can also be a symptomatic manifestation associated with carnitine deficiency. In experimental animals with myocardial ischemia and anoxia, myocardial L-carnitine initially decreased with concomitant increases in the concentrations of acetyl and acyl carnitine, and later decreased.[114] The concentration of long chain fatty acid acyl-CoA was found to increase in the ischemic myocardium; this increase was thought to inhibit adenine nucleotide.[115] Infusion of L-carnitine into the coronary bed in dogs resulted in increases in tissue carnitine and adenine nucleotides (see section on Animal Experimentation). Results in human patients with myocardial ischemia are difficult to obtain and are generally not available in the literature. Thomsen et al[97] have demonstrated that the IV administration of DL-carnitine in doses of 20–40 mg/kg improved the tolerance for stress in ischemic human patients, evidenced by increases in heart rate, pressure-rate product, and improved myocardial lactate metabolism. These investigators proposed that the beneficial effect of carnitine in myocardial ischemia is due to restoration of L-carnitine levels, which stimulates pyruvic oxidation and acetyl carnitine production with concomitant reduction in myocardial lactate concentration. Furthermore, the production of long chain acyl carnitine through increased carnitine, decreases the concentration of accumulated long chain acyl-CoA in the ischemic heart, which in turn results in the reversal of adenine nucleotide translocase inhibition. They discussed the pharmacokinetics of IV infusion of DL-carnitine HCl and indicated that L-carnitine appears to distribute into a rapidly perfused, then into a slower perfused, body space after administration. The overall apparent volume of distribution is equivalent to approximately 30% of total body weight. Experimental evidence in this study suggests that carnitine may improve metabolism in the ischemic heart and that the availability of oxygen may not be the only factor that limits myocardium performance before the administration of carnitine.

*Cirrhosis.* Since the biosynthesis of carnitine occurs chiefly in the liver (see section on Biosynthesis) it is not surprising that patients with liver dysfunction can develop carnitine deficiency. Only one study of carnitine deficiency in cirrhotic patients has been reported; Rudman et al[12] studied normal subjects and hypocarnitinemic cirrhotics who received oral and parenteral nutrition without carnitine, followed by an oral supplement of 500 μmol (81 mg) DL-carnitine/day. Levels of carnitine in liver, kidney, muscle, and brain were determined in the cirrhotic patients after death and compared with values obtained from normally nourished, nonhepatic patients who died after an acute illness. Results from this study showed that intake of exogenous carnitine by normal subjects ingesting a diet selected from 25 common foods was 380–450 μmol (62–73 mg); a similar amount was excreted in the urine. Therefore, endogenous synthesis in normal individuals with about 400 μmol (65 mg) carnitine intake was estimated to be close to zero, assuming that carnitine was excreted intact without substantial degradation of β-methylcholine. When exogenous intake was reduced to < 10 μmol/day (1.62 mg) and lysine and methionine were supplied, urinary excretion by healthy subjects stabilized at 100 μmol/day (16.2 mg), which may represent endogenous synthesis of carnitine. In cirrhotic patients with subnormal serum carnitine, urinary carnitine excretion was in the range of 15–30 μmol (2.43–2.86 mg)/day when dietary intake of carnitine was restricted to < 10 μmol/day, and lysine and methionine were supplied from the oral and parenteral regimen at levels from 2 to 4 times the RDA. Dietary supplementation of carnitine promptly augmented serum carnitine to normal levels and urinary carnitine excretion increased progressively to 150 μmol/day (24.3 mg). The capacity of cirrhotics to synthesize carnitine was estimated to be approximately 10% of that in normal subjects. In all analyzed tissues, carnitine content was significantly lower in cirrhotic patients than in those from nonhepatic controls. Although serum carnitine was reduced in cirrhotics, it did not correlate significantly with tissue carnitine. Thus, the investigators suggested that serum carnitine in cirrhotic patients may have resulted from a) reduced intake of exogenous carnitine due to anorexia, ie, 29 in cirrhotics vs 380–450 μmol in controls; b) inadequate intake of lysine and methionine, and c) reduced endogenous biosynthesis of carnitine due to liver dysfunction. They concluded that the abnormalities of myopathy, fatty liver, and cerebral disorder are common manifestations of acquired liver disease, and that carnitine deficiency

could play an important role in the pathogenesis of this decrease. Therefore, correction of the carnitine deficiency by oral or IV administration of carnitine may prove beneficial.

*Uremia.* Cardiomyopathy develops in some patients with renal failure treated by intermittent hemodialysis; cardiac failure remains one of the most important causes of death in these patients.[13] This cardiomyopathy does not improve on an intensive dialysis regimen, indicating that accumulation of toxic metabolites is not responsible for the clinical manifestation, so that it is likely that low molecular weight compounds of nutritional importance, such as carnitine, become deficient following dialysis.

Results obtained on 6 patients undergoing dialysis showed that plasma carnitine declined from 335 to 114 nmol/ml, a 66% reduction between pre- to postdialysis.[7] Subsequent data showed that the concentration of carnitine in the dialysate was 10–15% of that in plasma and increased with time. In 8 of 9 patients, muscle carnitine concentrations were reduced to 10% of those in controls, although plasma carnitine concentrations in some patients were higher than controls. During hemodialysis patients lost 135–2100 μmol (32–340 mg) of carnitine, considered to be significant in comparison with a normal daily urinary loss of 87 ± 77 μmol (14.1 ± 12.5 mg) in males and 175 ± 80 μmol (28.4 ± 13.0 mg) in females.[91] It is plausible that low muscle carnitine is caused by this loss. In a later study, Battistella et al[148] reported that prolonged longitudinal plasma carnitine measurement in patients undergoing hemodialysis can be subdivided into two groups: one in which there is a return to a normal or higher plasma carnitine, and another in which chronic carnitine deficiency is established by dialysis. In the latter group, exogenous carnitine treatment may be of value to prevent irreversible cardiomyopathy.

*Others.* During the late Fifties, when the involvement of carnitine in fat metabolism was identified, extensive work on the effect of carnitine malnutrition was reported in European countries. Early claims of positive results on appetite and growth have been well accepted. Borniche and Canlorbe[149] in France reported that, in infants and children of ages 1.5–6 months who suffered from malnutrition due to infection, treatment with an oral 20% DL-carnitine solution to provide 200–500 mg/day resulted in recovery of appetite, acceleration of growth, and restoration of plasma proteins to normal levels.

Although recent clinical research revealed the impact of carnitine deficiency on the function of such vital organs and tissues as the heart, muscle, and liver, only a few studies have been reported on carnitine in malnutrition. Khan and Bamji[130] reported that, in children with protein-calorie malnutrition, plasma carnitine levels were depressed as a result of limited intake of methionine and lysine. Their rehabilitation with a high protein diet for 4 weeks resulted in restored levels of plasma carnitine as well as concentration of albumin. In adult malnourished patients infected with schistosomiasis, dietary repletion with an adequate diet containing both carnitine and the AA precursors, lysine and methionine, led to increased serum carnitine.[59]

In contrast to the results obtained from alloxan-diabetic rats, concentrations of carnitine in leg or abdominal muscle tissues from patients with diabetes mellitus were not significantly different from appropriate controls, although the diabetic patients were expected to have enhanced fat metabolism as a result of limited carbohydrate utilization.[131] These results suggest that carnitine derived from exogenous intake and endogenous synthesis is sufficient to handle the increased fatty acid oxidation.

Since carnitine partially antagonizes the effect of thyroxine in animal experiments,[150] its influence on patients with hyperthyroidism has been investigated. In still another review of carnitine, Reynier[151] summarized experimental evidence that carnitine retained N equilibrium in rats treated with thyroxine and that in patients with hyperthyroidism carnitine reduced an elevated basal metabolism and improved clinical response. Recent studies indicate that urinary carnitine excretion increased in hyperthyroid patients while excretion was markedly reduced in hypothyroid patients.[10] There was an inverse correlation between urinary excretion of carnitine and serum triglyceride concentrations. Urinary carnitine excretion, serum carnitine, and FFA were greatly increased in normal subjects injected with ACTH, whereas patients with adrenocortical insufficiency, hypothyroidism, and hypopituitarism did not show these responses.[152] It was suggested that carnitine and lipid metabolism response to ACTH requires the presence of intact adrenocortical and thyroid functions.

Recently, Maebashi et al[153] reported that exogenous carnitine at a dose of 900 mg/day administered orally reduced hyperlipoproteinemia in patients with serum triglycerides and cholesterol concentrations > 200 and 250 mg/100 ml, respectively. It was also interesting to note that there was no anorexia, nausea, vomiting, change in bowel habits, or discomfort during carnitine treatment. Laboratory findings, including urinalysis, leukocyte count, urea N, uric acid, and liver function tests, remained stable throughout the course of this study. study.

## FAT METABOLISM IN IV NUTRITION

IV nutrition has been used successfully for critical patient care during recent years. Nutrients provided in the infusates include AA, carbohydrates, fat, electrolytes, and vitamins in their chemically purified forms. Carnitine has not been added to any commercially available large-volume parenteral fluids. The question, therefore, arises as to whether the need for carnitine in patients receiving IV nutrition can be met entirely by endogenous synthesis from lysine and methionine in order to insure normal fat metabolism. In a recent study, newborn patients on a TPN regimen showed approximately 50% reduction in plasma carnitine compared to when they are fed orally with expressed human milk or a proprietary formula.[154] Although plasma carnitine does not necessarily correlate with tissue carnitine,[71, 78] depressed plasma levels may signify carnitine depletion in the body. Border et al[155] reported that carnitine levels in skeletal muscle decreased in septic patients without starvation; however, muscle level was maintained in starved septic patients. Similar changes were observed in dogs with peritonitis. The investigators suggested that these changes are in the

direction expected for limited fat oxidation and associated protein catabolism.

The recent practice of protein-sparing therapy, using near-isotonic AA solutions with or without hypocaloric amounts of energy for adequately nourished postoperative patients, is rationalized upon an enhanced endogenous fat mobilization and utilization while conserving body proteins. It thus becomes important to have tissue carnitine concentrations high enough to maximally transport the FFA derived from lipolysis across mitochondrial membrane for subsequent oxidation during stress and trauma. In addition, enhanced utilization of ketone bodies by high concentrations of carnitine[106, 107] may also contribute to the conservation of proteins.

In TPN, when sufficient calories in the form of concentrated dextrose are provided, abnormal results from liver function tests have been noted clinically.[156–158] We have observed hepatic fatty changes in rats receiving 30% dextrose with an AA solution containing tryptophan and sodium bisulfite, as well as in those receiving 30% dextrose alone.[159] No fatty changes were observed in rats infused with the AA solution alone or 5% dextrose. There were higher serum liver enzymes and bilirubin in the two groups of rats receiving 30% dextrose. These results suggest that the higher concentration dextrose solution used in TPN is the primary cause of hepatic fatty infiltration, an implication which is in agreement with others.[160] In TPN patients, Lowry and Brennan[156] found a strong correlation of abnormal liver function and the infusion of carbohydrate calories and N in excess of needs for N balance. Intrahepatic cholestasis in TPN patients has also been reported.[158] Fatty infiltration of the liver may result from either increased deposition or decreased removal of fat from the liver. The current recommendation for liver dysfunction in TPN patients entails reduction of dextrose and/or AA in TPN fluids.[157, 158]

Recently, two fat emulsion preparations (Intralipid and Liposyn) have become available commercially for use in TPN in the United States. Rat studies indicate that increasing fat to levels > 50% of total calories in TPN fluids results in progressively more severe hepatomegaly.[161] Paradis et al[162] found that a 10% fat emulsion (Intralipid) maintained normal body cell mass but did not replete patients with pre-existing malnutrition. Long et al[163] observed that fat emulsion did not reduce N excretion in TPN patients receiving various levels of dextrose. In postoperative patients receiving protein-sparing therapy, addition of fat to the infusate did not significantly improve N balance;[164] Brennan et al[165] indicated that the protein-sparing effect of fat emulsion can be attributed to glycerol used in the fat emulsion preparation. Postmortem examination of 3 patients using fat emulsion (20% Intralipid) revealed fat deposition in the heart with fatty acid composition resembling that of the emulsion.[166] These results suggest that in vivo utilization of IV fat emulsion may be limited, as evidenced by the inability to reduce protein catabolism and fat deposition in tissues.

Based upon the physiological role of carnitine in fat metabolism, it is hypothesized that exogenous carnitine can enhance fat utilization in patients supported by IV nutrition. These include: a) increased utilization of endogenous fat to conserve body proteins in postoperative patients on protein-sparing therapy; b) to eliminate fat deposition in the liver in patients receiving concentrated dextrose solutions; and, c) to enhance utilization of fat emulsion as part of a TPN regimen.

## SUMMARY

Since the first recognition of the interrelationship between carnitine and fat metabolism, knowledge has accumulated to give us insight into its biosynthesis, physiological roles, deficiency symptoms, and possible applications as a therapeutic agent and/or to enhance nutrient utilization. Normally, little attention has been devoted to carnitine as an essential nutrient because it is either consumed in the diet or synthesized endogenously in sufficient amounts to meet body needs.

Carnitine is necessary to transport activated long chain fatty acids across mitochondrial membrane; 2 enzymes, carnitine acyltransferase I and II are involved. The long chain fatty acids transported into mitochondria can undergo $\beta$-oxidation and liberate biologically utilizable energy. Carnitine is an innocuous compound with $LD_{50}$ values similar to AA.

Carnitine can be synthesized from two essential AA, lysine and methionine. It has been speculated that only protein-bound lysine can serve as substrate for carnitine biosynthesis. From a number of animal studies, it has been shown that diets deficient in one of these two precursors can lead to retarded growth, fatty liver, and low tissue carnitine. In the rat, the final step in carnitine biosynthesis, hydroxylation of $\gamma$-butyrobetaine, occurs primarily in the liver, then is transported through the circulation to various organs in two major forms, free carnitine and acetylcarnitine. The uptake of carnitine by organs is accomplished by an active transport mechanism.

Dietary supplementation of carnitine can promote growth and reduce hepatic lipid content in rats fed lysine- and methionine-deficient diets. Animal models induced with myocardial ischemia showed decreased fatty acid oxidation and low carnitine in heart tissues. Exogenous carnitine increases oxygen uptake and contractile forces of cardiac muscle, and enhances BCAA oxidation. These experimental evidences indicate that carnitine is an essential nutrient whose functions are intimately associated with energy metabolism of vital organs and tissues.

The clinical importance of carnitine can be seen in various diseases resulting from altered fat metabolism. Lipid storage myopathy due to incapability of muscles to utilize fatty acids has been attributed to either impaired carnitine synthesis or deficient carnitine acyltransferase activity. A deficiency of carnitine in cardiac muscle can cause severe physiological consequences; fatal cases as a result of carnitine deficiency have been reported in the literature. Treatment with exogenous carnitine has also been found beneficial in terms of clinical improvement in some cases.

In TPN, wherein the carnitine precursors lysine and methionine are probably provided in adequate quantities, dietary carnitine intake is reduced to zero. Under these conditions, fatty infiltration and liver dysfunction have

been observed in TPN patients receiving concentrated dextrose solutions as part of their TPN regimen. Fat deposition from IV fat emulsion in tissues has also been observed. These untoward effects of TPN may be, on a theoretical basis at least, connected to the patients' free and bound carnitine supply. In adequately nourished patients receiving IV protein-sparing therapy, optimal tissue carnitine levels are necessary to insure maximal mobilization and utilization of stored body fat. Based upon reported information, it is hypothesized that exogenous carnitine may enhance endogenous fat utilization under hypocaloric conditions and increase hepatic fatty acid oxidation, thereby minimizing fat deposition and facilitating the utilization of IV fat emulsion as part of a TPN regimen.

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