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# Pharmacokinetics of L-Carnitine

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## Abstract

L-Carnitine is a naturally occurring compound that facilitates the transport of fatty acids into mitochondria for β-oxidation. Exogenous L-carnitine is used clinically for the treatment of carnitine deficiency disorders and a range of other conditions.

In humans, the endogenous carnitine pool, which comprises free L-carnitine and a range of short-, medium- and long-chain esters, is maintained by absorption of L-carnitine from dietary sources, biosynthesis within the body and extensive renal tubular reabsorption from glomerular filtrate. In addition, carrier-mediated transport ensures high tissue-to-plasma concentration ratios in tissues that depend critically on fatty acid oxidation. The absorption of L-carnitine after oral administration occurs partly via carrier-mediated transport and partly by passive diffusion. After oral doses of 1–6g, the absolute bioavailability is 5–18%. In contrast, the bioavailability of dietary L-carnitine may be as high as 75%. Therefore, pharmacological or supplemental doses of L-carnitine are absorbed less efficiently than the relatively smaller amounts present within a normal diet.

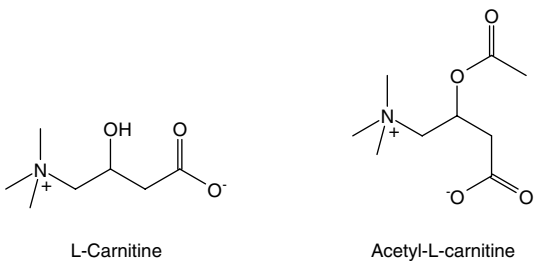
L-Carnitine and its short-chain esters do not bind to plasma proteins and, although blood cells contain L-carnitine, the rate of distribution between erythro-

cytes and plasma is extremely slow in whole blood. After intravenous administration, the initial distribution volume of L-carnitine is typically about 0.2–0.3 L/kg, which corresponds to extracellular fluid volume. There are at least three distinct pharmacokinetic compartments for L-carnitine, with the slowest equilibrating pool comprising skeletal and cardiac muscle.

L-Carnitine is eliminated from the body mainly via urinary excretion. Under baseline conditions, the renal clearance of L-carnitine (1–3 mL/min) is substantially less than glomerular filtration rate (GFR), indicating extensive (98–99%) tubular reabsorption. The threshold concentration for tubular reabsorption (above which the fractional reabsorption begins to decline) is about 40–60 μmol/L, which is similar to the endogenous plasma L-carnitine level. Therefore, the renal clearance of L-carnitine increases after exogenous administration, approaching GFR after high intravenous doses.

Patients with primary carnitine deficiency display alterations in the renal handling of L-carnitine and/or the transport of the compound into muscle tissue. Similarly, many forms of secondary carnitine deficiency, including some drug-induced disorders, arise from impaired renal tubular reabsorption. Patients with end-stage renal disease undergoing dialysis can develop a secondary carnitine deficiency due to the unrestricted loss of L-carnitine through the dialyser, and L-carnitine has been used for treatment of some patients during long-term haemodialysis. Recent studies have started to shed light on the pharmacokinetics of L-carnitine when used in haemodialysis patients.

L-Carnitine (3-hydroxy-4-*N*-trimethylammonium butyrate; figure 1) is a naturally occurring compound that is found in all mammalian species. This small, polar molecule forms the principal component of what is referred to as the 'carnitine pool' – other components of this pool include short-, medium-, and long-chain esters collectively referred to as 'acyl-carnitines'. A range of biological roles have been proposed for endogenous L-carnitine, but its primary function is to facilitate the transport of fatty acids across the inner mitochondrial membrane, making them available for mitochondrial β-oxidation. Mitochondrial fatty acid oxidation is a fundamental source of cellular energy, particularly in cardiac and skeletal muscle. L-Carnitine is also believed to be important for acting as an acyl group acceptor in order to maintain sufficient cellular levels of free coenzyme A (CoA) and it may act as an osmoprotectant in organs such as the kidney and as a general cell membrane stabiliser.[1-5]




L-Carnitine Acetyl-L-carnitine

**Fig. 1.** L-Carnitine and its short-chain ester acetyl-L-carnitine. At physiological pH, L-carnitine exists as a polar zwitterion.

Over the past 30 years, there have been thousands of published articles dealing with the biological role of L-carnitine, its endogenous synthesis, and its movement across biological membranes. However, there has also been a growing interest in the potential uses of L-carnitine as a medicinal agent and as a nutritional supplement. Interest in the importance of L-carnitine as a medicinal agent dates back to the discovery of deficiency syndromes that respond dramatically to L-carnitine supplementation. For example, patients with primary systemic carnitine deficiency develop progressive cardiomyopathy, encephalopathy and muscle weakness, leading to

death from heart failure unless L-carnitine supplementation is provided.[6] In these individuals, orally administered L-carnitine is a life-saving medicine. L-Carnitine has also been used for secondary carnitine deficiencies that are associated with a wide range of genetically determined metabolic disorders (often associated with disturbed oxidation of acyl-CoA intermediates) and acquired medical conditions associated with reduced intake or biosynthesis of the compound, increased renal loss, or alterations in its transport across membranes.[6,7] L-Carnitine has also received attention as a pharmacological agent in the treatment of a range of cardiovascular disorders,[8,9] for the prevention or treatment of drug-induced myopathies in patients with HIV infection,[10] and for the treatment of valproate toxicity.[11,12] The diverse range of conditions that have been purported to benefit from L-carnitine administration also include anorexia, chronic fatigue, male infertility, diphtheria and drug-induced carnitine deficiencies.[13]

L-Carnitine is administered orally, in the form of an oral solution, tablets or effervescent tablets, and intravenously as a bolus or infusion. Typical doses range from 10–50 mg/kg/day. The use of exogenous L-carnitine for supplementation or pharmacological purposes represents a source of the compound over and above dietary intake and *de novo* synthesis. Therefore, while the focus of this review is to describe the pharmacokinetics of oral and intravenous doses of L-carnitine that are used medicinally, any attempt to describe the pharmacokinetics of exogenous L-carnitine must also take into consideration the homeostatic mechanisms that control the endogenous carnitine pool.

## 1. Measurement of L-Carnitine and its Esters in Biological Samples

The most important and common analytical methods for the measurement of L-carnitine may be summarised as enzymatic, chromatographic and mass-spectrometric methods.

### 1.1 Enzymatic Assays

Enzymatic assays (spectrophotometric or radioenzymatic assays) have for many years been widely used for quantitative determination of L-carnitine and (after hydrolysis of esters) total carnitine in plasma, urine, tissue and red blood cells of humans and animals. The methods have also been adapted for determination of acetyl-L-carnitine.[14-18]

The first major advancement in the analysis of L-carnitine occurred when Fritz et al.[19] isolated and purified from pig heart the enzyme responsible for the reversible acetylation of L-carnitine from acetyl-CoA, carnitine acetyltransferase (CAT). From that moment, all the enzymatic assays that were developed for the analysis of L-carnitine were based on the reaction between L-carnitine and acetyl-CoA:

L-carnitine + acetyl-CoA ↔ acetyl-L-carnitine + CoASH

Marquis and Fritz[20] described the first enzymatic assay based on this reaction in the presence of 5,5′-dithiobis-2-nitrobenzoic acid (DTNB, also known as Ellman's reagent) producing a chromogenic compound that can be detected at 412nm. The method was subsequently been modified[21-28] and automated for pharmacokinetic studies in humans.[21-30]

In order to increase the specificity of the method, Pearson et al.[14,31] coupled the CAT reaction with acyl-CoA synthetase or thiokinase to convert free CoA into sorboyl-CoA, which could be detected at 300nm:

L-carnitine + acetyl-CoA ↔ acetylcarnitine + CoASH

CoASH + ATP + sorbate ↔ sorboyl-CoA + AMP + pyrophosphate

This method was adopted for analysis of L-carnitine in plasma and muscle of patients enrolled in one of largest clinical studies of the effect of L-carnitine on muscle symptoms of patients on dialysis.[32]

A significant development in the analysis of carnitine was the development by Cederblad & Lindstedt[33] of a radio-enzymatic assay for the determination of L-carnitine at picomole levels:

L-carnitine + [$^{14}$C]acetyl-CoA ↔ [$^{14}$C]acetyl-carnitine + CoASH

Chromatographic separation of reacted and unreacted acyl-CoA followed by scintillation counting provided a sensitivity that was about 100 times higher than that of the CAT/DTNB method. Unfortunately, short-chain acyl-carnitines (in particular high concentration of acetyl- L-carnitine) interfered with the assay due to the reversibility of the reaction. Due to its very high sensitivity, this method soon became the preferred assay in the area of carnitine, and several modifications were implemented to improve linearity and performance.[34-40] De Sousa et al.[41] described a method that incorporated a range of modifications to establish the control ranges of L-carnitine and total carnitine in plasma and urine of healthy children and adults, and the levels in liver and muscle of adult patients.

### 1.2 High Performance Liquid Chromatography

Many metabolic diseases are due to mitochondrial enzyme defects, and these conditions are often characterised by an accumulation in plasma and urine of specific carnitine esters. Since that the presence of abnormal levels of these carnitine esters in urine and/or in plasma may be a sign of an altered fatty acid metabolism, there was a need for new analytical assays capable to identify and quantify these possible 'metabolic markers' in order to facilitate an early diagnosis of metabolic disorders.

One of the first high performance liquid chromatography (HPLC) methods described in the literature was developed for separation and quantification of short-chain acyl-carnitines ($C_2$–$C_5$) in tissue extracts.[42] The method was based on enzymatic equilibration of radioactive L-carnitine with CAT (called radioisotopic exchange method) followed by HPLC separation, using ion pair chromatography with gradient elution, and scintillation counting of individual acyl-carnitine fractions. Since CAT is a substrate for several short-chain acyl-L-carnitine derivatives and catalyses reversible reactions, when the equilibrium is reached, the radioactivity of the L-carnitine is incorporated into each short-chain acyl-carnitine. Schmidt-Sommerfeld et al.[43-46] modified the method and applied it to the analysis of acyl-carnitines in urine, plasma, and blood spots from children affected with metabolic diseases. The method was shown to be particular sensitive in the detection of the defects of fatty acid metabolism, in particular for the diagnosis of medium-chain acyl-CoA dehydrogenase deficiency from plasma and blood spots.

HPLC with precolumn derivatisation generally involves the following four steps: solid-phase extraction, precolumn derivatisation to produce a chromogenic substance, reverse-phase separation (generally on a C18 column), and UV or fluorescence detection. An HPLC method for the complete separation and quantification of acyl-carnitines in urine was developed by Milkier et al.[47] Pre-column derivatisation was carried out with 4′-bromophenacyl-trifluoro-methanesulfonate and *N*,*N*-diisopropylethylamine and HPLC separation was performed in reverse phase with a ternary gradient; UV detection was at 254nm. The authors were able to demonstrate the complete separation, in a single analytical run (60 minutes), of 16 different carnitine 4′-bromophenacyl esters. The method was successfully tested for the determination of specific acyl-carnitines in the urine of patients with particular metabolic disorders. The method was later modified for the quantitative determination of L-carnitine, total carnitine, and specific short-and medium chain acyl-L-carnitines in human urine and plasma.[48-51]

Although other HPLC methods have been described for the analysis of acyl-L-carnitines,[52-55] most of them are modifications of the HPLC method developed by Minkler. However, Longo et al.[56] developed a unique method for the quantification of L-carnitine, acetyl-L-carnitine and propionyl-L-carnitine in human plasma, involving pre-column derivatisation with 1-aminoanthracene in aqueous solution, elution under isocratic conditions, and fluorescence detection. Standard calibration curves were constructed using dialysed plasma to remove endogenous L-carnitine and acyl-carnitines. The authors developed this method specifically for L-

carnitine, acetyl-L-carnitine and propionyl-L-carnitine, since these three compounds are under clinical development as therapeutic agents for the treatment of metabolic, neurological and cardiovascular diseases. The advantages and/or disadvantages of isotopic-exchange/HPLC method in comparison with HPLC with pre-column derivatisation have been debated;[49,57-59] the major problem of the isotopic-exchange technique is the limited substrate specificity of CAT.

### 1.3 Mass Spectrometry

Despite the fact that mass spectrometry (MS) requires more sophisticated and expensive equipment than the enzymatic and HPLC methods, the technique offers the advantages of simple sample preparation, high sensitivity and specificity, together with versatility and the ability to measure hundreds of sample per day.

L-Carnitine and its short-chain esters are polar, nonvolatile, thermo-unstable substances, unsuitable for direct injection into a gas chromatography (GC) system; therefore, derivatisation is required if GC is to be used. For these reasons, GC and GC-MS techniques found very limited applications in the analysis of carnitine.[60-63] In contrast, alternative MS methods have found wide acceptability, particularly for the diagnosis of inborn errors of metabolism. These methods include fast atom bombardment (FAB) MS,[64] liquid chromatography MS,[65] continuous-flow FAB MS[66] and tandem MS (MS/MS). The latter technique, with all the possible modifications, can be considered the most successful, versatile and powerful method for the analysis of acyl-L-carnitines in biological specimens. Independently of the technique adopted, the principle of MS/MS is to use a double and sequential ionisation source to produce a characteristic and specific fragmentation pattern; the first 'soft ionisation' generates a molecular ion that is subsequently fragmented by a collision with a second gas, producing specific daughter ions from the parent ion. The first MS/MS application for the analysis of carnitine was described by Gaskell et al.[67] for the analysis of acyl-L-carnitines in urine of patients with organic acidurias. The technique was also applied for quantitative analysis of L-carnitine and total carnitine in plasma and urine.[68] Johnson et al.[69] compared FAB-MS/MS and electrospray ionisation (ESI) MS/MS, showing that the latter technique did not produce artefact ions typical of glycerol adducts, and this ultimately resulted in an increased sensitivity for long-chain acyl-carnitines. The ESI-MS/MS method has been used for a number of applications.[70-72] Automated ESI-MS/MS analysis of blood spots[69,73] has become a routine technique for newborn screening in several countries worldwide.[74,75] Currently, more than 40 rare inborn errors of metabolism can be easily identified with this technique, and others will be detectable in the future. The ESI-MS/MS technique remains unsurpassed in term of qualitative information that can be obtained from the analysis of the biological samples; furthermore, very simple sample preparation, high sensitivity, fast analysis and automation are important factors for a routine use of this technique. Despite its analytical power and versatility, MS/MS also has its limitations and pitfalls.[69,76-78]

### 1.4 Application to Pharmacokinetic Studies

The choice of assay for any particular study should be dictated by the form of carnitine that needs to be quantified. For the determination of L-carnitine and total L-carnitine, the spectrophotometric and radioenzymatic assays are suitable provided that the most recent procedures are used to avoid the pitfalls and limitations (such as non-linearity) of older versions of the assay. For accurate quantification of L-carnitine and specific short-chain acyl-L-carnitines in pharmacokinetic studies, a sensitive HPLC method or MS/MS should be considered, provided that issues such as stability during storage and analysis (particularly of the acyl-L-carnitines, which are susceptible to hydrolysis) are thoroughly evaluated prior to use. For qualitative and semiquantitative data regarding L-carnitine and the entire spectrum of acyl-L-carnitines (in particular for long-chain esters), and especially when diagnostic information is sought, the selection of an MS assay is almost mandatory.

Of the relatively few traditional pharmacokinetic studies that have evaluated the disposition of exogenous L-carnitine after oral or intravenous administration, the most frequently used methods are the spectrophotometric enzyme assay[30,79-82] and the radioenzymatic assay.[30,83-87] However, more recently, HPLC has been used to quantify the short-chain esters of L-carnitine in addition to the administered compound.[88,89] Although there are no published pharmacokinetic studies that have utilised MS, the technique has been used during the clinical development of acetyl-L-carnitine and propionyl-L-carnitine and has found widespread use in the diagnosis of metabolic disorders.

## 2. Endogenous L-Carnitine

As an endogenous compound, L-carnitine is a vital cofactor for the mitochondrial oxidation of fatty acids. In this capacity, L-carnitine serves to accept short-, medium- and long-chain acyl groups from the respective acyl-CoA thioesters (figure 2). The enzymes that catalyse these reversible transfer reactions are carnitine acyltransferases.[2-4,90] Acyl-carnitines are then transported across the inner mitochondrial membrane via specific translocase enzymes, and transesterification within mitochondria serves to generate the corresponding CoA thioester, which is then available for intra-mitochondrial β-oxidation (figure 2). Because acyl-CoA thioesters themselves and unchanged fatty acids are incapable of moving into and out of the inner mitochondrial environment, the presence of L-carnitine is obligatory for fatty acid oxidation.

In addition to delivering acyl groups to the inner mitochondrial environment, L-carnitine serves to accept short-chain acyl groups from the corresponding acyl-CoA, so as to aid in the removal of the short-chain acyl groups from the mitochondria and maintain adequate mitochondrial levels of free coenzyme A.[1-4,6] A wide range of additional functions have been ascribed to L-carnitine[3] and while it is clear that our understanding of the biological role of this small polar molecule is far from complete, its importance for mammalian survival is well established.

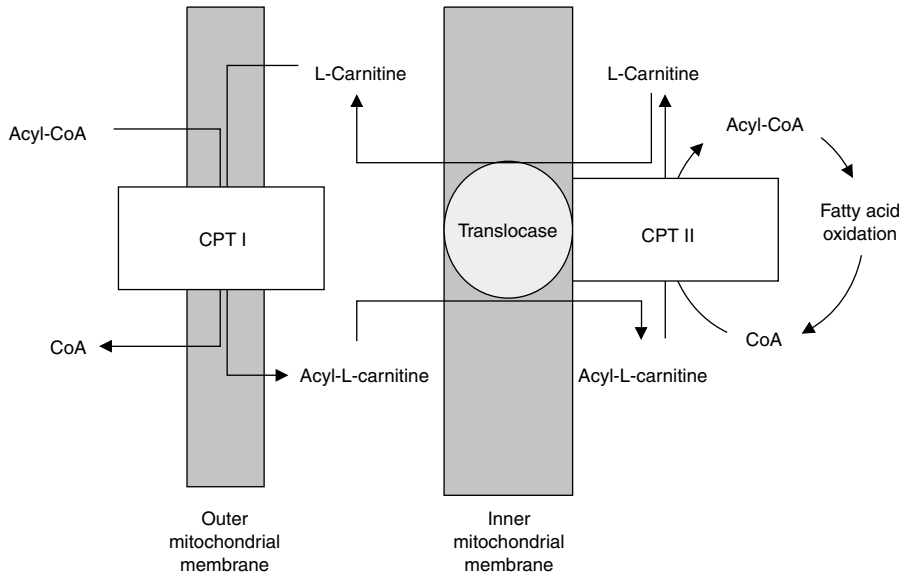



**Fig. 2.** The main biological role of L-carnitine is to facilitate the transport of fatty acids across the inner mitochondrial membrane, permitting intramitochondrial oxidation. This facilitation involves transesterification with acyl-CoA esters through the action of carnitine acyltransferases, of which there are various forms, including the carnitine palmitoyltransferases (CPT I and CPT II). The movement of acyl-L-carnitines across the inner mitochondrial membrane is mediated by membrane-bound L-carnitine/acyl-L-carnitine translocase.

Given the fundamental importance of L-carnitine in fatty acid oxidation, it is not surprising that plasma and tissue levels of the compound (the carnitine pool) tend to be maintained within relatively narrow limits. Processes involved in this homeostasis include endogenous synthesis, absorption from dietary sources, extensive but saturable renal tubular reabsorption and carrier-mediated distribution processes that maintain high tissue-to-plasma concentration ratios, particularly for those tissues that rely on fatty acid metabolism as a source of cellular energy. The reversible nature of carnitine metabolism means that at any time a fraction of the body's L-carnitine content is in the form of short, medium and long-chain esters, with acetyl-L-carnitine being the predominant ester in plasma and other tissues.[3,91]

### 2.1 Dietary Intake and Synthesis

Recent findings on the biosynthesis of L-carnitine in mammals have been reviewed recently.[92] The main dietary sources of L-carnitine are meats, particularly red meats, and dairy products, whereas fruit and vegetables contain negligible quantities of the compound. Consequently, dietary L-carnitine intake can vary enormously – strict vegetarians consume less than 0.1 μmol/kg/day, representing about 1 mg/day for a 70kg adult, whereas the average omnivorous diet provides a daily intake of 2–12 μmol/kg/day, or 23–135mg per day for an average adult.[93-95] Although the endogenous levels of L-carnitine can be affected by long-term changes in the level of dietary intake and nutritional status,[93,96-98] under normal conditions healthy humans can synthesise sufficient amounts of the compound,[92] which is why it is not regarded as a true 'vitamin'.

Plasma and urinary levels of L-carnitine have been reported in adults and children for whom meat and dairy products was a normal part of the diet and in lacto-ovo-vegetarians and strict vegetarians.[93] Those individuals who consumed diets that were low in L-carnitine (vegetarian diets) had lower plasma L-carnitine levels than subjects consuming a mixed diet, with children being affected to a greater extent than adults. However, despite large variations in dietary intake, the mean plasma levels of the compound varied by only about 20%. Notably, renal excretion of L-carnitine was substantially less in those individuals with low dietary intake.[93] It was therefore suggested that carnitine biosynthesis and renal conservation mechanisms are generally adequate to prevent overt carnitine deficiency in individuals who self-select diets that are low in carnitine.[93] The rate of urinary excretion of total carnitine in strict vegetarians (for whom intake of L-carnitine was negligible) was about 1–2 μmol/kg/day (10–20 mg/day for an average adult), and given that renal excretion is the primary route of L-carnitine elimination, this is likely to represent the rate of endogenous biosynthesis.[91,94,95]

By comparing the amount of carnitine ingested per day with the amount recovered in urine and feces, it was found that subjects on a low-carnitine diet excreted more L-carnitine than they ingested, while those on the high-carnitine diet excreted less than they ingested. The results with the latter group suggested that humans do not absorb all of the L-carnitine that they consume.[99] In the same study, subjects on a low-carnitine diet excreted about 25% of an oral tracer of L-[*methyl*-$^3$H]carnitine as metabolites of the compound (mainly trimethylamine-*N*-oxide and γ-butryobetaine, which appear to be formed within the gastrointestinal tract prior to absorption; see section 3.3). If it is assumed that there was negligible excretion of the tracer via expired air, the extent of absorption in the subjects fed the low-carnitine diet may have been as high as 75%, on average.[99] In those subjects on a high-carnitine diet, 37% of the dose could be accounted for as excreted metabolites, meaning that the extent of absorption might have been about 63%. Importantly, the results suggest that the efficiency of absorption tends to diminish as the carnitine content of the diet increases,[91,99] reflecting the involvement of specific transporters that can be saturated even with normal dietary intake. As described below (section 3.1), the bioavailability of supplemental or medicinal oral doses of L-carnitine tends to be even lower, at 5–18%.

In humans, several tissues can convert trimethyl-lysine (derived from protein bound lysine) into γ-butyrobetaine, but the complete synthesis of L-carnitine, which involves the final hydroxylation of butyrobetaine, takes place primarily within the liver.[3,4,91] The kidney and brain are also involved in L-carnitine synthesis, but cardiac and skeletal muscle, despite being critically reliant on L-carnitine for fatty acid utilisation, are not capable of synthesising the molecule.[91,92] Instead, high muscle levels are maintained by carrier-mediated uptake of L-carnitine from the bloodstream.[1-3,91]

Besides its reversible conversion to the various acyl-carnitines, there is no appreciable systemic metabolism of L-carnitine, with the exception perhaps of a minor degree of decarboxylation.[2,91] However, unabsorbed L-carnitine within the gastrointestinal tract undergoes bacterial metabolism to trimethylamine and γ-butyrobetaine, and the extent of bacterial metabolism appears to increase as the oral intake of L-carnitine increases.[91,99] This situation arises because as the dose is increased, a higher fraction of the dose reaches the regions of the gastrointestinal tract that are colonised by bacteria capable of breaking down L-carnitine.

### 2.2 Renal Tubular Reabsorption

Loss of endogenous L-carnitine from the body primarily occurs via renal excretion in the form of L-carnitine, acetyl-L-carnitine and longer chain esters. In a 24-hour period, a healthy human consuming a normal diet excretes between 100 and 300 μmol of total carnitine, although the overall rate of excretion varies according to dietary intake.[93,96,97,100] The ratio of acetyl-L-carnitine to L-carnitine in urine tends to exceed that in plasma, possibly due to slightly less efficient renal tubular reabsorption of the ester derivative or as a consequence of renal acetylation of L-carnitine followed by leakage of the locally formed acetylated product into urine.[89,101]

Because L-carnitine is not bound to plasma protein,[102] it is extensively filtered at the glomerulus (filtration clearance = glomerular filtration rate [GFR]). However, tubular reabsorption ensures that only a small fraction of the filtered load is excreted in urine. In healthy individuals, the fractional tubular reabsorption of L-carnitine (and acyl-L-carnitine derivatives) exceeds 90% and probably is greater than 98% under normal homeostatic conditions.[89,91,103] However, if the tubular reabsorption of L-carnitine is impaired due to disease or the administration of compounds that inhibit the renal tubular transport of the compound, the result is an increased urinary loss, and possibly a systemic deficiency may develop.[104,105] In renal Fanconi syndrome, a significant reduction in the tubular reabsorption of L-carnitine results in a secondary deficiency of L-carnitine in plasma and muscle.[106]

### 2.3 Tissue Uptake and Transporters

L-Carnitine is present within most, if not all, tissues of the body, and there is a large body of data on the transport of the compound across cell membranes within liver, kidney, intestine, brain and cardiac muscle. However, the majority of research on the cellular transport of L-carnitine has focussed on skeletal muscle cells and mitochondria, reflecting the vital role of the compound in muscle fatty acid oxidation.

The high tissue-to-plasma concentration ratio of L-carnitine within muscle (up to about 100 **:** 1) appears to be maintained in part by a $Na^+$/L-carnitine cotransporter. Recent studies indicate that the primary transporter involved in this uptake of L-carnitine into tissues is the carnitine organic cation transporter (OCTN2).[107-111] This transporter, which is also involved in the extensive renal tubular reabsorption of L-carnitine, is relatively nonspecific and is inhibited by several substances that are known to induce systemic carnitine deficiency, including emetine and pivalic acid.[109] Patients with primary systemic carnitine deficiency have been reported to have mutations in the OCTN2 gene, which leads to disturbances in renal tubular reabsorption as well as muscle uptake.[112,113]

Published Michaelis-Menten (Km) values (concentration at which membrane transport is half-maximally saturated) for the tissue uptake of L-carnitine vary widely, but the pattern of data suggests that the

Km values for uptake into skeletal and cardiac muscle is in the low-to-medium micromolar range (20–100 μmol/L), whereas that for the kidney is intermediate (0.1–0.5 mmol/L) and that for the liver and brain is substantially higher (1–5 mmol/L).[2,6,114] According to Bieber,[2] it is not surprising that the Km values for liver, which can synthesise the compound, and brain, which has low L-carnitine levels, are substantially higher than for the high-affinity process that is involved in skeletal and cardiac muscle uptake, given that these latter tissues have a high reliance on fatty acid oxidation for cellular energy and are incapable of L-carnitine synthesis. The presence of multiple transporters with differing Km values and maximum rates ($V_{max}$), together with a component of passive transport, complicates comparisons of kinetic parameters between tissues and between experimental systems. For example, within muscle, there appears to be a low-affinity and high-affinity component to L-carnitine uptake, with reported Km values of 60 μmol/L and 0.6 mmol/L, respectively.[114] Possible contributors to the low-affinity transport of L-carnitine include OCTN1 but it is uncertain what, if any, transported are involved in the efflux of L-carnitine from the cells involved in biosynthesis.[92]

### 2.4 The Carnitine Pool

Table I summarises the concentrations of L-carnitine in human tissue samples and expresses the content of each organ and tissue as a percent of the total L-carnitine pool. It is notable that the concentration in skeletal muscle (2000–4000 μmol/kg wet weight) is as much as 100 times higher than that in plasma.[114,115] Organs such as kidney, liver and brain contain intermediate levels of 300–1000 μmol/kg,[114,116] with cardiac levels being at the higher end of this range. The highest concentration of L-carnitine is in the epididymis.[117]

**Table I.** Carnitine content of various tissues and organs of the human body

| Compartment | Concentration (μmol/L or μmol/kg)[114] | Estimated content (mmol)[118] | Estimated content (% of listed compartments) |
|---|---|---|---|
| Plasma and extracellular fluid | 40–50 | 0.5 | ~0.4 |
| Liver | 500–1000 | 1.3 | ~1 |
| Kidney | 330–600 | 0.2 | ~0.2 |
| Skeletal muscle | Up to 4000 | 126.4 | >98 |
| Total of listed compartments | | 128.4 | |

The reported concentrations of L-carnitine in plasma, muscle and other tissues vary depending on the assay methodology, as described earlier. Moreover, comparisons of muscle content are also complicated by the fact that values are expressed with reference to wet weight, dry weight or per amount of non-collagen protein, and variations are expected depending on the method of muscle tissue collection and the site of the biopsy.[32,114-116,119-121] Nevertheless, the ratios of tissue-to-plasma levels are impressively high and point to the importance of maintaining adequate levels of the compound within the primary sites of fatty acid oxidation.

The overall size of the total carnitine pool in a 70kg healthy male has been estimated to be about 128mmol, or 21g (table I). It is important to note that less than 1% of the carnitine pool is located within plasma, which explains why measurements of plasma L-carnitine levels do not necessary provide reliable information on the body's carnitine status.[94,118] However, in subjects who are relatively stable in terms of carnitine mass balance (i.e. a steady rate of input and a steady rate of elimination), statistically significant correlations between blood (plasma or serum) and tissue (skeletal muscle) levels have been found.[122,123]

The plasma levels of L-carnitine in healthy adults are generally reported to be 40–50 μmol/L, while plasma levels of acetyl-L-carnitine, the most abundant ester, are typically in the range of 3–6 μmol/L. The concentration of total L-carnitine (including L-carnitine and acetyl-L-carnitine) is about 50–60 μmol/L.[32,89,93,104,106] There does not appear to be a significant diurnal rhythm in terms of plasma (serum) and urinary L-carnitine and acetyl-L-carnitine levels in healthy subjects,[124] but variations between individuals can be significant. In patients with primary carnitine deficiency, plasma levels may be so low that they are undetectable, and tissue levels can be as much as 95% lower than normal.[6] Un-

corrected, such deviations lead to severe myopathies and ultimately death. The plasma levels of L-carnitine tend to rise in patients with renal dysfunction, possibly due to increased retention of the compound[125] whereas patients with end-stage renal disease (ESRD) undergoing chronic haemodialysis tend to have low levels of L-carnitine in plasma and skeletal muscle, as discussed in section 4. Exhaustive lists of conditions that lead to secondary carnitine deficiency have been published.[6,7]

L-Carnitine is present within red blood cells at a concentration that is comparable to that of plasma.[121,126] However, the acyl-L-carnitine to L-carnitine ratio is 2–3 times higher within erythrocytes, due to a comparatively higher concentration of acetyl-L-carnitine. Neither L-carnitine or acetyl-L-carnitine are able to enter or leave mature erythrocytes during prolonged incubations, suggesting that the compounds cannot move across erythrocyte membranes. Indeed, Cooper et al.[126] suggested that the presence of L-carnitine within erythrocytes, which lack mitochondria, is vestigial from the process of haemopoiesis, and that L-carnitine has no further function in mature red blood cells, except possibly in the stabilisation of erythrocyte membranes.[127] It was further suggested that the lack of transport of L-carnitine between plasma and erythrocytes explains the poor correlation between the levels of the compound in these two compartments.[126]

## 3. Pharmacokinetics of Exogenous L-Carnitine

Exogenous L-carnitine administered for supplementation or for therapeutic use displays the pharmacokinetic features that typify small, polar, endogenous compounds, including a reliance on carrier-mediated transport for movement across biological membranes. This reliance on specific transport systems leads to concentration-dependent disposition in certain circumstances, and this potential for non-linear behaviour must be taken into consideration when evaluating the results of studies on the pharmacokinetics of exogenous L-carnitine.

The plasma concentration of L-carnitine measured after an exogenous dose represents the sum of the endogenous and exogenous material, and as discussed above, endogenous levels can vary between individuals. This adds significant complexity to the derivation of descriptive pharmacokinetic parameters such as volume of distribution, clearance and bioavailability. A number of techniques have been used to 'distinguish' between endogenous and exogenous L-carnitine, including the use of simple baseline subtraction or through the use of stable or radiolabelled isotopes. Each of these methods carries certain assumptions and limitations. In the case of baseline subtraction, it is generally assumed that the baseline levels themselves are not perturbed by the presence of the exogenous material. This assumption is violated if, for example, the presence of the exogenous load leads to a saturation of renal tubular reabsorption and an increased fractional loss of the renally filtered load, and the presence of this phenomenon has been well established, particularly after intravenous administration of L-carnitine (see section 3.4). When stable or radio-isotopes are used, it must be assumed that the presence of the label does not influence the interaction of the administered compound with endogenous transporters and enzymes, causing a significant isotope effect.[128]

The key findings from studies that have evaluated the pharmacokinetics of pharmacological doses of L-carnitine are given in table II. In the first true pharmacokinetic study, Welling and colleagues[82] administered an intravenous infusion of (racemic) DL-carnitine. The results of this study need to be interpreted with some caution because the D-isomer might have influenced the pharmacokinetics of the natural isomer – indeed, we now know that D-carnitine is capable of interfering with the membrane transport of L-carnitine.[111] However, the findings of Welling et al.[82] indicated pharmacokinetic features that were typical of the studies that were to follow – multicompartmental behaviour with a short initial half-life, a relatively small central distribution volume, and high fractional recovery in urine.

**Table II.** Summary studies investigating the pharmacokinetics of L-carnitine after oral or intravenous administration

| Study | Design features | Pivotal findings |
|---|---|---|
| Brass et al. 1994[83] | 14 healthy males; 92.5–185 μmol/kg as a single IV dose; monitoring of muscle function | Sampling for only 2h after dose meant that only rapid kinetic events could be characterised; $V_c$ 0.2 L/kg; $CL_R$ 1–1.5 mL/min/kg |
| Evans et al. 2000[88] | 12 patients with end-stage renal disease requiring haemodialysis; 20 mg/kg infused IV over 2 min after each dialysis session | Baseline conditions studied; L-carnitine and acetyl-L-carnitine efficiently removed via haemodialysis (extraction >0.7) |
| Harper et al. 1988[30] | Six healthy individuals; low carnitine diet; single dose; 2 and 6g IV bolus and oral | Baseline subtraction used; CL 5–6 L/h; terminal half-life 4–7h; $CL_R$ 3 mL/min under baseline conditions, increasing to 100 mL/min after 6g IV; dose-dependent oral bioavailability 0.16 (2g) and 0.05 (6g) |
| Rebouche 1991[129] | Five healthy males on a high carnitine diet (2 g/day); oral tracer dose of L-[$^3$H]carnitine; urine and feces collected | Recovery of dose was as follows; 6.3% as carnitine in urine; 31% as trimethylamine-*N*-oxide in urine; 21% as γ-butyrobetaine in faeces |
| Rebouche & Engel 1984[130] | Six healthy individuals and five patients with carnitine deficiency; IV administration of L-[$^3$H]carnitine; radioactivity followed for 28 days | Tri-exponential fit; whole body turnover time 60–70 days; rate constant for muscle uptake reduced in patients with carnitine deficiency; impaired renal tubular reabsorption in patients with carnitine deficiency |
| Rizza et al. 1992[80] | Six healthy individuals; 30 and 100 mg/kg oral and IV bolus | Baseline subtraction used; tri-exponential fit; terminal half-life 9–12h; CL 8–10 L/h; $CL_R$ 7–8 L/h; $f_e$ 0.82–0.86; bioavailability 0.15 |
| Sahajwalla et al. 1995[85] | Fifteen healthy adults; single IV dose 20 mg/kg | Data analysed with and without baseline subtraction; bi-exponential fit; $V_c$ 11.2L; CL 4 L/h; terminal half-life 5–17h; $CL_R$ 3 L/h; $f_e$ 0.73 |
| Segre et al. 1988[87] | Six healthy individuals; 30 mg/kg IV; 100 mg/kg oral; 30 mg/kg oral (n = 3 only) | Baseline subtraction used; bi-exponential fit; $V_c$ 0.1 L/kg; $f_e$ 0.85 (IV); bioavailability 0.18; CL 10.85 L/h |
| Uematsu et al. 1988[81] | Ten healthy individuals each given a single IV dose of 0, 20, 40 and 60 mg/kg; IV infusion over 10 min | Tri-exponential fit; terminal half-life 10–23h; $V_c$ 0.1–0.2 L/kg; CL 0.1 L/h; $f_e$ 0.77–0.95 |

**CL** = systemic clearance with reference to plasma; **$CL_R$** = renal clearance with reference to plasma; **$f_e$** = fraction of an intravenous dose excreted unchanged in urine; **IV** = intravenous; **$V_c$** = volume of the central compartment.

### 3.1 Absorption and Bioavailability

A variety of test systems, including animals,[131-133] animal intestinal preparations,[133-135] human intestinal biopsy samples[136] and human intestinal epithelial cell lines[137] have found that the uptake of L-carnitine into the intestinal epithelium of the small intestine occurs partly via carrier-mediated transport and partly by passive diffusion. Uptake in the colon appears to be restricted to a passive component only,[136] adding to evidence suggesting that the small intestine is the main site of L-carnitine absorption.[133,138] The absorption of L-carnitine is characterised by slow mucosal uptake, prolonged mucosal retention and slow mucosal exit into blood.[131,138,139] Therefore, in humans the time to achieve maximum plasma concentrations after oral administration can be up to 4–6 hours, or longer. Evidence also suggests that acetylation of L-carnitine can take place during the absorption process.[131,138]

At this time, the relative importance of carrier-mediated transport and passive diffusion in the overall absorption of L-carnitine is not known. In a study in rats, Rebouche et al.[140] found that while only 4% of a tracer oral dose of radiolabelled L-carnitine (0.09 μmol) was recovered (as total radioactivity) in feces, a much larger recovery (53%) was observed in feces after a dose of 124 μmol. This suggested that the large dose of L-carnitine overwhelmed the carrier systems involved in the absorption of the compound. In humans, just as in rats, it appears likely that passive diffusion becomes the dominant absorption pathway as the oral dose of L-carnitine increases.[141]

Sahajwalla et al.[85] evaluated the pharmacokinetics of three oral formulations of L-carnitine (oral solution, tablets and chewable tablets) by comparing the plasma concentrations of L-carnitine observed during repeated administration of each formulation (2g every 12 hours for 4 days) with those obtained after a single intravenous dose (a 3-minute infusion of 20 mg/kg) of a proprietary injection. The study was conducted in 15 healthy volunteers and dietary intake was controlled for L-carnitine content. Measurement of predose plasma L-carnitine concentrations during each oral regimen indicated that an apparent steady state was achieved with respect to plasma levels by day 3 of each treatment (in view of the long terminal half-life of L-carnitine in muscle, it is unlikely that a true steady state had been achieved by that time, although the impact of additional accumulation on bioavailability estimates is likely to be minimal). Bioavailability estimates were based on area under the plasma concentration-time curve (AUC) values during a single dosage interval (0–12 hours). Using baseline-corrected AUC values, the mean (± SD) absolute bioavailability of L-carnitine was 15.9 ± 4.9% for the oral solution, 15.1 ± 5.3% for the tablet and 14.8 ± 5.1% for the chewable tablet. Mean unadjusted values for the maximum plasma concentration of L-carnitine ($C_{max}$), the predose plasma concentration ($C_{min}$), the time of $C_{max}$ ($t_{max}$) and AUC during a single dosage interval are in table III. Based on confidence interval testing procedures, the three oral formulations were found to be bioequivalent (i.e. all 90% CIs for the oral-oral comparisons were well within the permitted 80–125% range for establishing bioequivalence).

The results of Sahajwalla et al.[85] are in keeping with the results of Segre et al.[87] who reported an absolute bioavailability of 18% after a single oral dose of 100 mg/kg as L-carnitine solution, and Harper et al,[30] who reported a value of 16% (1.98g as a single oral dose of 6 × 330mg tablets with 200mL of water). Interestingly, in this latter study, the absolute bioavailability of a single 5.94g oral dose (18 × 330mg tablets with 400mL of water) of L-carnitine was only 5%, and these authors suggest-

**Table III.** Pharmacokinetic parameters determined during repeated administration of L-carnitine 2g every 12 hours to healthy subjects as three different dosage forms[85]

| Parameter and unit | Oral solution | Tablet | Chewable tablet |
|---|---|---|---|
| AUC (μmol • h/L) | 779.9 | 771.4 | 762.6 |
| $C_{max}$ (μmol/L) | 80.3 | 79.2 | 76.8 |
| $C_{min}$ (μmol/L) | 54.2 | 54.5 | 53.8 |
| $t_{max}$ (h) | 3.3 | 3.4 | 3.1 |

**AUC** = area under the concentration-time curve; **$C_{max}$** = peak plasma concentration; **$C_{min}$** = trough plasma concentration; **$t_{max}$** = time to $C_{max}$.

ed that the absorption of the compound is saturated at or beyond a 2g dose. However, the biopharmaceutical performance of such a large number of tablets in a relatively small volume of fluid might also have affected bioavailability, for example due to slow disintegration or dissolution of the dosage forms. Rizza et al.[80] reported absolute bioavailability values of 16 ± 3% and 14 ± 2% for oral doses of 20 mg/kg (approx. 2g) and 100 mg/kg (approx. 6g), respectively. Collectively, the results of pharmacokinetic studies with medicinal doses of L-carnitine suggest an absolute bioavailability of 10–20%, which is substantially less than the value of up to 75% or higher obtained after administration of a tracer dose of radiolabelled L-carnitine to people on a normal diet.[99] Because of its low bioavailability, oral administration of L-carnitine is a relatively inefficient way of elevating plasma L-carnitine levels unless daily doses of 2–4g are used, and even then the short-term changes in plasma levels are not marked.[85,142]

Although the aforementioned pharmacokinetic studies do indicate that the bioavailability of L-carnitine is incomplete, some comment should be made regarding experimental design. In all studies that have measured the absolute bioavailability of L-carnitine, the plasma level after intravenous administration exceeded those found after the oral dose. For example, in the study of Sahajwalla et al.[85] the plasma levels of L-carnitine were as high as 700 μmol/L immediately after intravenous administration, and were still about 200 μmol/L about 2 hours later. In contrast, the plasma levels observed after the oral doses reached a maximum values of 70–80 μmol/L. Given this disparity, and the well-established non-linearity in the renal clearance of L-carnitine (renal clearance increases as plasma concentrations increase), it is unlikely that the assumption of constancy of total clearance (which is of pivotal importance when measuring bioavailability from plasma concentrations) was valid. In these studies, if the clearance after intravenous administration was larger than that after the oral dose (and there is ample evidence of this in the literature, as described in section 3.4) then the absolute bioavailability of oral L-carnitine may have been overestimated (because the AUC after the intravenous dose would be lower than what would be obtained if clearance did not increase with dose).

Ideally, any future attempt to measure the absolute bioavailability of oral L-carnitine should select an intravenous dose and dose strategy (e.g. slow infusion over 2–4 hours) that provides plasma levels that are within the same range as those obtained after oral doses; in this case, the assumption of constancy of clearance is more likely to be valid.

The incomplete absorption of L-carnitine is likely to be due to the relatively high polarity of the compound, which impedes its free diffusion across lipid membranes, together with the limited capacity of intestinal transporters. In addition, acetylation of the compound during its movement across through intestinal epithelial cells may reduce the effective bioavailability. Its low nonrenal clearance (total clearance is close in magnitude to renal clearance, as described in table II) suggests that the hepatic extraction ratio of L-carnitine in humans is low, and this is supported by perfused rat liver experiments, which suggest that L-carnitine has a hepatic extraction ratio of less than 0.02.[143] These *in vitro* studies found that the low hepatic extraction of L-carnitine was primarily caused by limited uptake of the compound from the hepatic vasculature during a single passage through the liver.

The low hepatic extraction of L-carnitine supports the concepts that its low oral bioavailability of L-carnitine is caused by poor absorption across the intestinal epithelium, meaning that a significant fraction of the dose (up to 90%) might be expected to be excreted in the feces. However, findings from studies in which radio- or deuterium-labelled L-carnitine was administered to healthy volunteers, discussed in section 3.3, indicate that most unabsorbed L-carnitine undergoes bacteria-mediated metabolism within the gastrointestinal tract.

### 3.2 Distribution

L-Carnitine and its short-chain esters do not bind to plasma proteins (fraction unbound = 1)[102] and the rate and extent of distribution from plasma into

erythrocytes appears to be minor or negligible,[142] despite the fact that blood cells do contain L-carnitine (see section 2.4). After intravenous administration, the initial distribution volume of L-carnitine is typically about 0.2–0.3 L/kg (table II), which corresponds to extracellular fluid volume. The plasma levels of intravenous L-carnitine decline in a bi- (or tri-) exponential manner, with an initial half-life of about 0.5–1 hours, and a terminal half-life of 3–12 hours.[30,81,83,85-87] However, the value of the terminal half-life can vary depending on whether baseline correction is used or not.[86]

After intravenous administration to healthy subjects, the plasma concentrations of L-carnitine are indistinguishable from baseline levels after about 12–24 hours.[80,85-87] This does not imply that the entire dose has been completely eliminated by that time. Rather, while a fraction of the dose has been eliminated primarily via urinary excretion, the remaining fraction is likely to have been incorporated into the endogenous carnitine pool. It must be remembered that even an intravenous dose of 1g represents less than 5% of the carnitine pool in a healthy individual. Even if half of the intravenous dose was incorporated into the body pool (i.e. 50% escaped renal excretion during the first 12–24 hours), then the overall size of the pool would increase by only 2–3%, and because of normal fluctuations and assay variability a change of this magnitude would be virtually impossible to detect. Therefore, it is unlikely that the true terminal half-life of L-carnitine could be measured with a conventional pharmacokinetic study.

The movement of L-carnitine into skeletal muscle, the main storage compartment, is a very slow process and therefore difficult to characterise from a kinetic viewpoint, particularly if one is measuring plasma levels only. Studies that have monitored exogenous L-carnitine for relatively small time periods therefore have little ability to characterise slow distribution processes that may reflect tissue (muscle) uptake. Such problems associated with studying the pharmacokinetics of an exogenous dose of L-carnitine were overcome by Rebouche and Engel[130] who administered an intravenous tracer dose of L-[*methyl*-$^3$H]carnitine to six healthy volunteers and collected blood samples for up to 29 days. The results of this pivotal study indicated triexponential disposition. Pharmacokinetic modelling identified three distinct compartments for L-carnitine within the body: extracellular fluid, which represents the initial distribution volume; fast equilibrating tissues that are most likely to represented by organs such as liver and kidney; and slow equilibrating tissues that are likely to comprise skeletal and cardiac muscle (figure 3). The turnover times (or mean residence times) of L-carnitine in these three compartments were determined to be about 1, 12 and 191 hours, respectively, and the whole body turnover time was about 66 days.[130] Mean rate constants for the movement of L-carnitine between the three compartments are given in figure 3. Significantly, the rate constant for the exit of L-carnitine from muscle (into the central compartment) was about 0.005h$^{-1}$, meaning that the half-life of this process is about 5–6 days. However, even longer half-lives are feasible if the L-carnitine distributes within a deep compartment of muscle. Studies in animals also suggest that L-carnitine displays slow uptake and prolonged residence within peripheral distribution sites. For example, in dogs, the resi-

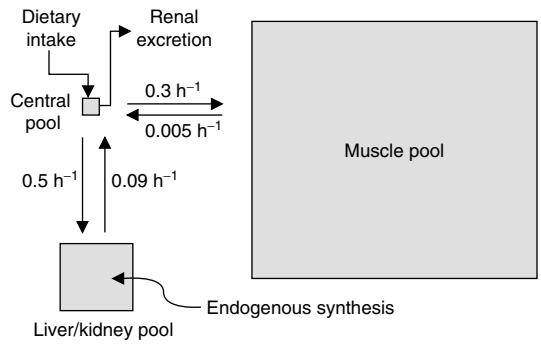



**Fig. 3.** Pharmacokinetic model for the disposition of L-carnitine using parameters reported by Rebouche & Engel.[130] The size of the central pool (extracellular fluid including plasma) and two peripheral pools (skeletal and cardiac muscle as the larger pool and other tissues such as liver and kidney in the smaller pool) are represented in proportion to the relative L-carnitine content. The rate constants represent the fractional content of each compartment moving into the receiving comportment per unit time. For example, the fraction of muscle content moving into the central pool per hour is 0.005 (0.5%).

dence time for L-carnitine was 232 hours for muscle, compared with about 8 hours in other tissues, and the whole body turnover time of L-carnitine in dogs was about 63 days.[144]

Positron emission scintigraphy, using L-[$^{11}$C]carnitine, has been used to study the uptake of the compound into human muscle. In one particular study,[145] it was shown that uptake of labelled L-carnitine into the thigh muscle of a patient with myopathic carnitine deficiency was improved after L-carnitine and fatty acid supplementation. Clearly, intravenous administration of L-carnitine results in a transient increase in the body carnitine pool, but the sheer size of the total pool coupled with slow intercompartmental movement means that pharmacokinetic parameters derived from plasma levels alone, such as the 'steady state volume of distribution' must be interpreted with caution.

The apparent steady-state volume of distribution of a compound ($V_{ss}$) represents the amount of compound in the body divided by the concentration in plasma. For L-carnitine, information on total body content (128.4 mmol – see section 2.4) and endogenous plasma levels (40–50 μmol/L) suggest a total $V_{ss}$ of about 3000L. The enormity of the value merely reflects the fact than more than 99% of L-carnitine is located outside of plasma. Based on conventional pharmacokinetic analysis, $V_{ss}$ values (with respect to plasma) of about 20–50L have been reported for exogenous L-carnitine.[81,86,87] The difference between the $V_{ss}$ estimates for endogenous (approximately 3000L) and exogenous (20–50L) L-carnitine reflects the difficulty of quantifying its distribution into slowly equilibrating tissues such as skeletal muscle (unless radiolabelled material is used). Importantly, the estimated $V_{ss}$ value is not a true 'steady state' volume of distribution, it is more of an 'early' distribution volume.

Muscle L-carnitine content changes very little, if at all, after acute or short-term administration of L-carnitine to healthy individuals.[118] This is because the movement of the compound into and out of skeletal muscle is a slow process with turnover times in the vicinity of weeks or months rather than hours.[130] However, chronic administration of intravenous and oral L-carnitine, if used for a sufficient period of time, has been reported to increase skeletal muscle L-carnitine levels.[118]

### 3.3 Metabolism

After intravenous administration, L-carnitine is mainly excreted via the kidneys, with about 70–90% of a 2g dose being recovered unchanged in urine within 24 hours.[30,80,81,85-87] The remainder of the dose is incorporated into tissue as L-carnitine or other components of the carnitine pool, including the various acyl-carnitines.[87,130] In keeping with findings from animal studies, investigations in humans have not detected metabolites of L-carnitine (other than acyl-carnitines) after intravenous administration, suggesting negligible systemic breakdown.[130] With the exception of acylation (a reversible process), the metabolism of intravenously administered L-carnitine is also negligible in rats[140,146,147] and dogs,[144] as it is in humans. However, a minor degree of decarboxylation to β-methylcholine has been reported.[148]

In 1991, Rebouche published a pivotal paper that provided a quantitative estimation of the fate of an oral tracer dose of L-[*methyl*-$^3$H]carnitine in five men who were receiving a high-carnitine diet and L-carnitine supplementation.[129] After administration of the tracer, urinary and fecal excretion was monitored for between 5 and 11 days. In addition, serum samples were collected to follow the time course of the tracer and labelled metabolites. It was found that the absorption of oral L-[$^3$H]carnitine was slow and incomplete, with $t_{max}$ values of 2–4.5 hours. Interestingly, between 20 and 50 hours after administration, the serum concentrations of L-carnitine remained virtually constant. This suggests prolonged retention of that fraction of the dose that had been incorporated into the body's carnitine pool. Only 6.3% of the oral dose was recovered unchanged in urine, with a further 34% recovered in urine as metabolites, mostly [$^3$H]trimethylamine-*N*-oxide. About 22% of the dose was recovered in feces, mostly as labelled γ-butyrobetaine. The only radiolabelled metabolite that could be detected in serum was [$^3$H]trimethylamine-*N*-oxide, and in some sub-

jects the serum concentrations of this metabolite were actually higher than those of L-[$^3$H]carnitine itself. However, 50 hours after administration, most of the [$^3$H]trimethylamine-*N*-oxide had been excreted into urine. Interestingly, after about 24–30 hours the serum concentrations of [$^3$H]trimethylamine-*N*-oxide decreased with a shorter half-life than those of L-[$^3$H]carnitine. This is consistent with the fact that L-carnitine within the systemic circulation is not converted to trimethylamine-*N*-oxide (if it was being formed from systemic L-carnitine, one would expect either a parallel decline in L-carnitine and trimethylamine-*N*-oxide, or a longer half-life for the metabolite). On the basis of this and earlier work[140] it was suggested that oral L-carnitine underwent degradation in the gastrointestinal tract via the actions of enteral bacteria, with the resultant formation of trimethylamine and γ-butyrobetaine. It was envisaged that the enterally-formed trimethylamine was absorbed into the bloodstream and converted to trimethylamine-*N*-oxide, within the liver, prior to excretion via the kidneys (figure 4). Although a substantial amount of labelled γ-butyrobetaine formed from oral L-[*methyl*-$^3$H]carnitine was recovered in faeces,[129] it is possible that some of the intestinally formed γ-butyrobetaine may be absorbed and potentially converted to L-carnitine in the liver.

Trimethylamine is a volatile aliphatic tertiary amine with a pungent, fishy, ammoniac odour. It is present within a range of foods and is also formed within the human gastrointestinal tract via the actions of enteral bacteria on a variety of dietary chemicals, including choline and lecithin, in addition to L-carnitine.[149,150] As shown by Rebouche[129] and Zhang et al,[150] pharmacological doses of L-carnitine are also extensively converted to trimethylamine and its *N*-oxide.

Trimethylaminuria, also called 'fish-odour syndrome', is a condition in which patients exhibit a

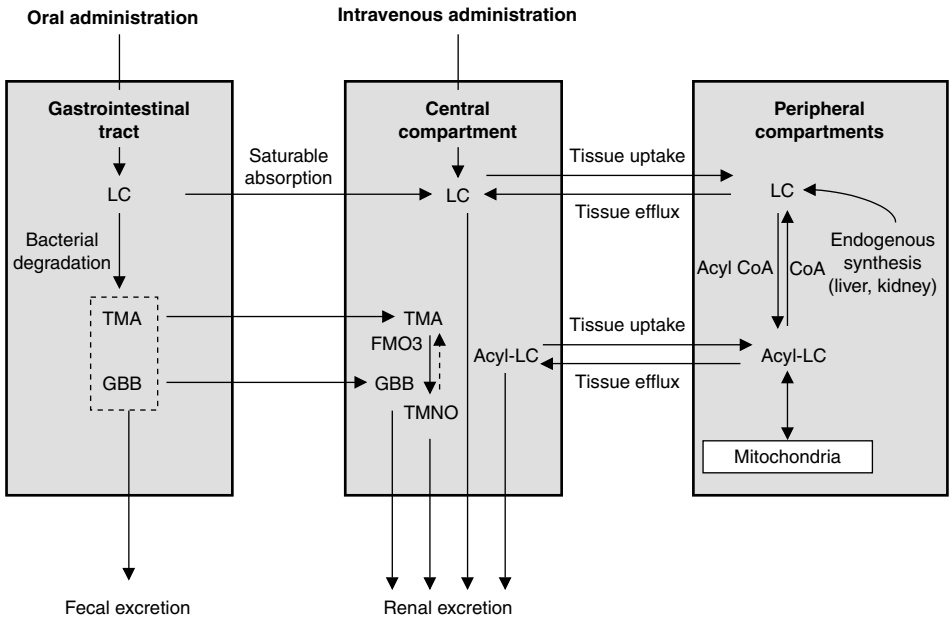



**Fig. 4.** Summary of the disposition of L-carnitine in humans. Unabsorbed oral L-carnitine (LC) undergoes bacterial degradation to trimethylamine (TMA) and γ-butyrobetaine (GBB). TMA is absorbed and converted to trimethylamine-*N*-oxide (TMNO) by flavin-containing monooxygenase form 3 (FMO3). While GBB is excreted mainly via the feces, a large portion of TMA is excreted in urine as TMNO. The LC absorbed from the gastrointestinal tract, and that injected into the blood after intravenous administration, can undergo saturable uptake (and efflux) into (and out of) the various peripheral compartments, including liver and kidneys (where it is also biosynthesised) and muscle. Active transport systems maintain high tissue-to-blood concentration ratios. Within tissues, LC performs its various biological roles, primarily mediating the transport of acyl groups into and out of the inner mitochondrial compartment.

distinctive odour of stale or rotting fish, emanating from urine, breath and sweat.[151-153] This odour is due to accumulation of trimethylamine within blood, sweat and urine and is worsened by increased dietary intake of trimethylamine precursors such as L-carnitine. Primary trimethylaminuria is caused by defects in the human FMO3 gene, which encodes the flavin-containing monooxygenase (form 3) that is responsible for conversion of trimethylamine to trimethylamine-*N*-oxide. In these patients, there is deficient conversion of trimethylamine into the odourless *N*-oxide. However, transient trimethylaminuria can also occur in the absence of such defects, with possible causes including hepatic, renal or gastrointestinal disturbances as well as dietary L-carnitine overload.[153,154] Interestingly, hepatically formed trimethylamine-*N*-oxide can undergo metabolic reduction to reform trimethylamine, setting up a cycle of reversible metabolism.[155] In addition, trimethylamine is also converted to dimethylamine,[156] which is excreted unchanged in urine.[157,158]

Because the kidney plays an important role in facilitating the excretion from the body of trimethylamine *N*-oxide, trimethylamine and other associated metabolites, the plasma and tissue concentration of these compounds is elevated in patients with ESRD.[159-161] Indeed, it has been proposed that elevated trimethylamine may contribute to the fishy breath ('uraemic breath') of such patients.[160,161] The use of large oral doses of L-carnitine in patients with renal failure therefore carries with it the potential for accumulation of trimethylamine within the body, and the potential adverse effects of this,[162] and on this basis, intravenous administration is the preferred route of L-carnitine administration in these patients.

### 3.4 Excretion and Elimination Kinetics

Intravenous L-carnitine is cleared almost exclusively by renal excretion, and estimates of total and renal clearance are very similar in magnitude.[30,80] Because L-carnitine is not bound to plasma proteins, the filtration clearance is similar to GFR (100–120 mL/min), and at normal plasma concentration of 40–50 μmol/L about 8–9 mmol of L-carnitine is filtered per day. Given that the total body pool is about 128 mmol, and daily input via biosynthesis and absorption from the diet is 0.1–0.3 mmol, it is clear that deficiency would develop if filtered L-carnitine was not efficiently reabsorbed.

In healthy adults, under baseline conditions, the renal clearance of L-carnitine (1–3 mL/min) is substantially less than GFR,[30,100,103,104] indicating that the renal handling of L-carnitine involves extensive tubular reabsorption, The extent of reabsorption typically is about 98–99% under baseline conditions. However, the threshold concentration for tubular reabsorption in healthy adults, about 40–60 μmol/L, is similar in magnitude to the endogenous plasma L-carnitine concentration,[100,104] meaning that a medicinal dose of L-carnitine is likely to lead to a change in renal clearance.

Although modest increases in the renal clearance of L-carnitine have been observed after oral administration,[85,87,100] dramatic increases have been reported after intravenous administration. For example, Harper et al.[30] reported renal clearance values of 78 and 100 mL/min after intravenous doses of 2 and 6g, respectively, and Sahajwalla et al.[86] reported values of about 50 mL/min after a single 20 mg/kg intravenous dose. Similar values were reported by Rizza et al.[80] Therefore, as the plasma concentrations of L-carnitine increase, renal clearance becomes closer in magnitude to creatinine clearance, signifying that reabsorption approaches full saturation.

There are a number of implications of the nonlinear renal clearance of L-carnitine. The first, as discussed in section 3.1, is that we cannot rely on previous estimates of absolute bioavailability because the assumption of constancy of clearance between oral and intravenous administration was unlikely to have been valid. The second is that the half-life of L-carnitine after intravenous administration might actually decrease as the concentration in plasma increases (half-life is given by 0.693V/CL, where V is volume of distribution and CL is clearance; as clearance increases at high L-carnitine plasma levels, due to saturation of tubular reabsorption,

half-life would be expected to decrease). Therefore, the multi-exponential behaviour of L-carnitine discussed previously may, in part, be due to a prolongation of half-life during the disappearance profile.

After intravenous administration, most of the discernible renal excretion of exogenous L-carnitine is completed within the first 12–24 hours.[30] Indeed, Brass et al.[83] found that 42% of an intravenous dose of L-carnitine was recovered in urine within just 2 hours of administration. The rapid excretion of intravenously administered L-carnitine does not imply that endogenous L-carnitine is dealt with in a similar manner. Indeed, based on a total body L-carnitine content of 128 mmol,[118] and a daily excretion rate of about 5 μmol/kg/day (0.3 mmol/day for a 60kg adult), as reported by Lombard et al,[93] it appears that less than 0.25% of the total body store of L-carnitine is excreted in urine per day. The reason that endogenous L-carnitine is well conserved by the kidney, while an intravenous dose is efficiently excreted within 12 hours of administration, simply reflects the consequences of saturation of renal tubular reabsorption.

During repeated oral administration (2g twice a day) of L-carnitine (oral solution, chewable tablets and tablets), the urinary recovery of L-carnitine and short-chain acyl-carnitine during a single administration interval accounted for about 9% of the dose.[85] This decreased to about 4–5% after adjustments are made for endogenous excretion.[85] The low urinary recovery after oral administration is primarily due to the low bioavailability of the compound.

In rats, the renal clearance of L-carnitine involves extensive tubular reabsorption,[163] just as it does in humans. Saturation of reabsorption had an indirect but dramatic impact on the uptake of the compound into tissues. Thus, after a tracer oral dose of radiolabelled L-carnitine, only 3.86 ± 1.56% of the dose was recovered in urine to 48 hours, with the majority of the tracer dose (66.4 ± 3.6%) residing in tissues. In contrast, after a bolus intravenous dose that caused saturation of tubular reabsorption and an increase in renal clearance, 67.4 ± 12.0% of the dose was recovered in urine up to 48 hours and only 15.1 ± 1.3% was located within tissues.

## 4. Pharmacokinetics in Patients

Primary carnitine deficiency, which can be categorised into systemic, myopathic and cardiomyopathic forms, may have a variety of causes, including defective carnitine biosynthesis, excessive renal loss, impaired tissue uptake, excessive carnitine catabolism and malabsorption of dietary L-carnitine.[6,7,164-166] Secondary carnitine deficiencies can arise from causes such as excessive loss due to haemodialysis or altered metabolism due to total parenteral nutrition. The administration of certain drugs has also been associated with the development of secondary carnitine deficiency.[7] For example, Rose et al.[167] reported on a patient who developed a skeletal muscle myopathy associated with a carnitine deficiency after 3 months of pivampicillin therapy. Pivampicillin is now known to inhibit OCTN2, which is involved in L-carnitine movement into skeletal muscle and tubular reabsorption, and the drug is eliminated as pivaloyl-carnitine, suggesting that it can interfere with L-carnitine metabolism.[168] Pivampicillin-induced carnitine deficiency failed to respond immediately to removal of the antibiotic, which is in keeping with the fact that the half-life of L-carnitine in muscle is in the vicinity of many weeks. Even with long-term oral L-carnitine administration, the plasma L-carnitine levels increased very slowly.[167]

The long-term administration of valproic acid can also lead to decreased plasma levels of L-carnitine,[7] as can the administration of zidovudine[6,169] and ibuprofen[170] and a range of other compounds.[7] With our increasing understanding of the role of OCTN2 in the membrane transport of L-carnitine, and the potential for a diverse range of drugs to interfere with the transport of L-carnitine via OCTN2 (β-lactam antibiotics, quinidine, verapamil), it is likely that additional clinical interactions will become apparent in the near future.[107,171]

Although a very large body of data is available on the use of L-carnitine in primary and secondary carnitine deficiency, very few studies have been

conducted to assess the pharmacokinetics of exogenous L-carnitine in these patients. Invariably, the alterations in absorption/distribution/excretion that led to the deficiency would also be expected to change the pharmacokinetics of medicinal doses of L-carnitine.[104,105,172]

Given the role of the liver in L-carnitine synthesis and the importance of the kidney in L-carnitine conservation, it is not surprising that alterations in the disposition of L-carnitine occur in patients with diseases of the liver[173] and kidney.[118] However, the only conditions in which the pharmacokinetics of medicinal doses of L-carnitine have been studied involve patients with ESRD requiring long-term haemodialysis.

As kidney function deteriorates, the plasma concentrations of endogenous L-carnitine increase and there are disturbances in the acyl-carnitine-to-L-carnitine ratios.[174,175] However, significant elevations in plasma L-carnitine concentrations (>100 μmol/L) appear only when creatinine clearance decreases below 20 mL/min. Once such patients develop ESRD requiring haemodialysis, plasma levels at first return to normal and subsequently decline to levels that are 30–60% of normal.[118,176]

L-Carnitine is efficiently removed from blood during haemodialysis.[79,88,116,177-180] The extraction ratios of L-carnitine and acetyl-L-carnitine were reported to be about 0.74 and 0.70, respectively.[88] During the interdialysis period, there seems to be a net movement of the compound out of slowly-equilibrating tissue stores, such as skeletal muscle, into the plasma compartment, and with time this leads to depleted tissue stores and a secondary carnitine deficiency.[116,118,176,181,182] In addition, patients undergoing long-term haemodialysis have an elevated acyl-carnitine-to-L-carnitine concentration ratio in plasma.[32,118,176,177,179,181,183]

In addition to the progressive decline in the plasma levels of L-carnitine with increasing number of months on haemodialysis[182] skeletal muscle levels are also reduced. In one of the earliest studies to demonstrate this, Bohmer[177] reported that skeletal muscle levels of L-carnitine decreased by 50% during a single dialysis session. This results is not in keeping with the findings from more recent studies that it can take many months of haemodialysis therapy for muscle levels of the compound to decrease by this extent.[118] Moreover, for muscle levels to decrease by 50%, the loss of L-carnitine via dialysate would need to be significantly greater than what is normally observed.

Where researchers have performed valid comparisons of muscle L-carnitine levels (i.e. studies involving age-matched control subjects and sufficient subject numbers) it has been found that haemodialysis causes a reduction in the concentrations of L-carnitine in skeletal muscle.[32,115,122,123,179,184] For example, Bertoli et al.[115] showed that plasma and muscle L-carnitine levels were reduced by about 30% compared with control subjects. Savica et al.[123] found a correlation between plasma and muscle levels of L-carnitine in patients who had been undergoing haemodialysis for an average period of 23 months. Most recently, Debska-Slizen et al.[122] also found that the muscle levels of L-carnitine were significantly lower, by about 30%, in 37 haemodialysis patients compared with 29 age-matched controls.

There is some conjecture over the cause of reduced carnitine levels (in plasma and tissues) in patients undergoing chronic haemodialysis. However, possible causes include impaired biosynthesis or reduced dietary intake in renal failure patients, or more efficient removal of carnitine from the body by the haemodialysis procedure in comparison with normal renal excretion. Because renal excretion is the primary route of carnitine elimination in healthy individuals, the rate of recovery of total carnitine in urine provides an index of the total rate of input into the body via gastrointestinal absorption and endogenous synthesis.[91] Similarly, in patients with ESRD undergoing long-term haemodialysis, the primary route of carnitine elimination is removal via the dialysis fluid. It has been claimed that haemodialysis is unlikely to be a cause of carnitine depletion because the overall weekly recovery of L-carnitine in an individual undergoing haemodialysis (or other forms of dialysis) is not significantly different from that in a healthy individual.[185-187] However, such

statements are pharmacokinetically unsound because, under steady-state conditions, the rate of elimination only reflects the rate of input and, if the rate of input (dietary intake plus endogenous synthesis) is the same between the two populations (healthy individuals and patients receiving haemodialysis) then so too must be the total rate of elimination (in healthy individuals this is the rate of urinary recovery, while in haemodialysis patients this is the overall dialytic loss). The critical issue here is not so much the rate of recovery in urine versus that in dialysate but the efficiency of the respective removal (elimination) processes (i.e. the clearance of the compound), since this dictates the relationship between rate of input (and elimination) and the level of the compound in plasma and presumably tissues.

If a group of haemodialysis patients were to have lower plasma levels of L-carnitine than healthy subjects, yet both groups were found to have the same rate of loss (via urine or dialysate), then this in itself would indicate that the cause of any secondary deficiency may indeed be the efficient removal of L-carnitine via haemodialysis. Conversely, if it was found that the dialytic loss of L-carnitine was much lower than the normal urinary loss of L-carnitine in healthy individuals, this would simply mean that the rate of input (dietary intake and biosynthesis) was lower in the dialysis patients. Importantly, the rate of recovery of L-carnitine via dialysis fluid alone cannot in itself be used to gauge whether dialysis contributes to carnitine deficiency.

In a recent study, Evans et al.[88] measured the dialytic loss of L-carnitine plus acetyl-L-carnitine in ESRD patients. These patients had been stabilised on dialysis for at least 6 months and most for more than 12 months. Under baseline conditions, the loss of L-carnitine plus acetyl-L-carnitine was about 350 μmol per dialysis session, or 1 mmol per week. This equates to an average loss of about 2 μmol/kg/day. This is about half the rate of urinary recovery of total carnitine in healthy adults, as reported by Lombard et al,[93] but is close to that observed in lacto-ovo-vegetarians. However, although the lacto-ovo-vegetarians had relatively normal plasma L-carnitine levels, the haemodialysed patients had levels that were substantially lower than normal (mean pre- and post-dialysis levels were 19.5 and 5.6 μmol/L, respectively). Based on an area-under-the-curve approach, Evans et al.[88] estimated an 'average' plasma concentration of about 16 μmol/L under baseline conditions in the haemodialysed patients.[88] Using the mean rate of recovery of unchanged L-carnitine in dialysis over a 1-week period (750 μmol)[88] and an average plasma concentration of 16 μmol/L, one can estimate a weekly dialysis clearance (rate of elimination divided by plasma level) of about 47 L/week. As discussed in section 3.4, the renal clearance of L-carnitine in healthy individuals is about 1–3 mL/min, which equates to 10–30 L/week. Therefore, when considered on the basis of an entire week, the healthy kidney serves to conserve L-carnitine more efficiently than does haemodialysis.

Carnitine supplementation is used in dialysis patients showing signs of secondary carnitine deficiency, typically at a dose of 10–20 mg/kg intravenously after each dialysis session,[176] although oral administration has also been used.[118] Although a large number of studies have assessed the plasma (and/or tissue) levels of L-carnitine and acyl-carnitine in patients undergoing haemodialysis with or without L-carnitine administration, most of these are clinical studies and very few have formally assessed the pharmacokinetics of exogenous L-carnitine.[84,88,118,176,179,183,188] Vacha et al.[179] studied five patients who had been receiving L-carnitine as a 2g intravenous dose at the end of dialysis for about 30 months. The predialysis plasma concentration of L-carnitine was 431 ± 205 μmol/L, and this decreased to 150 ± 40 μmol/L at the end of dialysis. These concentrations were well above predialysis concentrations in haemodialysis patients who were not receiving L-carnitine (30–40 μmol/L). When L-carnitine administration was discontinued, the predialysis plasma concentrations of the compound decreased over time, and postdialysis levels were well below normal after 4 months. The L-carnitine content of muscle biopsy samples decreased by more than 50% once L-carnitine administration was discontinued. This result is in keeping with the results of clinical studies reporting that intravenous

administration of L-carnitine leads to a 60–200% increase in muscle L-carnitine concentrations, as reviewed by Brass.[118]

In children receiving L-carnitine 5 mg/kg postdialysis, L-carnitine was found to undergo biexponential disposition with an initial volume of distribution of 0.1–0.2 L/kg and a terminal half-life of 2–3 hours. However, the results of this study are somewhat limited because blood sampling was conducted for only 3 hours after administration.[84]

In adults with ESRD, intravenous administration of L-carnitine 20 mg/kg at the end of each dialysis session led to a progressive increase in the pre-and postdialysis levels of the compound in plasma, reaching constant values after about 6–8 weeks (figure 5). After multiple administration, only 50% of each dose was recovered in dialysate during the subsequent haemodialysis session, which suggests that there was ongoing incorporation of L-carnitine into the endogenous pool. As expected, upon discontinuing L-carnitine, plasma levels declined over a 5-week period. However, even after 5 weeks, the pre- and postdialysis levels were still higher than the baseline levels measured at the start of the study. It was suggested that during the washout period there was movement of L-carnitine out of a slowly equilibrating pool (probably muscle) into a pool that is in rapid equilibrium with plasma.

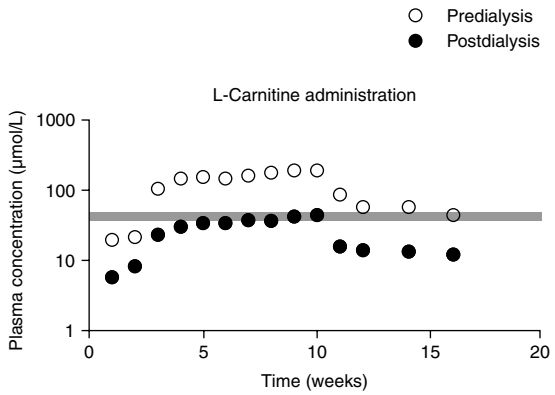



**Fig. 5.** Predialysis and postdialysis plasma concentrations of L-carnitine in patients with end-stage renal disease undergoing thrice-weekly haemodialysis. Levels are shown under baseline conditions, during the administration of 20 mg/kg of L-carnitine at the end of each dialysis session, and during a 5-week washout period.[88] The shaded region represents the endogenous levels of L-carnitine in healthy human adults.

The use of intravenous L-carnitine at doses of up to 20 mg/kg leads to plasma L-carnitine levels that greatly exceed the normal endogenous levels. This dosage regimen appears to have been established on empirical grounds based on correction of muscular symptoms of secondary carnitine deficiency.[118] Given the fact that L-carnitine moves very slowly into and out of skeletal muscle and, presumably cardiac muscle, it could be argued that the higher plasma levels are required to speed up the replenishment of tissue stores. Clearly, however, there is a need to extend our understanding of L-carnitine distribution in humans and only then can the merits of different administration strategies be considered on theoretical rather than empirical grounds.[118]

## 5. Conclusions

Figure 4 provides an overview of the disposition of L-carnitine after oral and intravenous administration of supplemental or pharmacological doses. Because of low gastrointestinal membrane permeability and the limited capacity of intestinal transporters, the absolute bioavailability of oral doses greater than 1g is less than 20%. However, due to the limitations of previous studies, the true absolute bioavailability of oral L-carnitine is not known. Of the oral L-carnitine that is not absorbed, most, if not all, is broken down in the intestine to γ-butyrobetaine and trimethylamine. Although γ-butryobetaine is excreted primarily in the feces, trimethylamine is absorbed and converted to trimethylamine-*N*-oxide prior to urinary excretion. Although this pattern of metabolism is well established, the toxicological implications in patients with an impaired ability to metabolise trimethylamine, or to effectively excrete the *N*-oxide, are only now starting to be recognised.

Systemically available L-carnitine is incorporated into the endogenous carnitine pool, but the movement of L-carnitine between plasma and the peripheral pools is so slow that the plasma concentrations of L-carnitine do not always reflect the total body carnitine status. Although many studies have

demonstrated high variability within and between patients in terms of the plasma-to-tissue levels of L-carnitine and the ratios of acyl-L-carnitine to L-carnitine, very few attempts have been made to explain this variability. Consequently, there is enormous scope for using pharmacokinetics to gain a clearer understanding of this variability.

Many forms of secondary carnitine deficiency are caused by alterations in the body's handling of endogenous L-carnitine, notably its distribution and renal excretion, and as a consequence the pharmacokinetics of exogenous L-carnitine are also likely to be altered in these patients. Nevertheless, very few studies have examined the pharmacokinetics of L-carnitine in patients with carnitine deficiency and there is little information on the effectiveness of different administration strategies for restoring tissue levels to normal. At the present time, administration strategies are based upon empirical judgements rather than sound pharmacokinetic theory, and the basis for this has been the excellent tolerability of the compound. However, by increasing our understanding of the pharmacokinetics of L-carnitine, in particular its movements into and out of muscle tissue and the relationships between levels in plasma and muscle, it is likely that the compound can be used in a more effective manner to avoid the potential complications of primary and secondary carnitine deficiency.

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