Disposition and Metabolite Kinetics of Oral L-carnitine in Humans

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*The pharmacokinetics of L-carnitine and its metabolites were investigated in 7 healthy subjects following the oral administration of 0, 0.5, 1, and 2 g 3 times a day for 7 days. Mean plasma concentrations of L-carnitine across an 8-hour dose interval increased significantly (*P < *.05) from a baseline of 54.2 ± 9.3 μM to 80.5 ± 12.5 μM following the 0.5-g dose; there was no further increase at higher doses. There was a significant increase (*P < *.001) in the renal clearance of L-carnitine indicating saturation of tubular reabsorption. Trimethylamine plasma levels increased proportionately with L-carnitine dose, but there was no change in renal clearance. A significant increase in the plasma concentrations of trimethylamine-*N*-oxide from baseline was evident only for the 2-g dose of L-carnitine (from 34.5 ± 2.0 to 149 ± 145 μM), and its renal clearance decreased with increasing dose (*P < *.05). There was no evidence for nonlinearity in the metabolism of trimethylamine to trimethylamine-*N*-oxide. In conclusion, the pharmacokinetics of oral L-carnitine display nonlinearity above a dose of 0.5 g 3 times a day.*

Keywords: *L-carnitine; trimethylamine; trimethylamine-*N*-oxide; gas chromatography;* N*-nitrosodimethylamine*

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L-carnitine is an endogenous compound derived from the diet (eg, meat and dairy products) or synthesis in the human body from the essential amino acids lysine and methionine.[1,2] L-carnitine has important roles in intermediary metabolism, including the transport of long chain fatty acids across the mitochondrial inner membrane; more than 90% of the human body's store is present in skeletal muscle.[1,2]

Primary L-carnitine deficiency is an autosomal recessive disorder associated with genetic mutations in the organic cation transporter (*OCTN2*) gene, resulting in reduced gastrointestinal absorption and tissue uptake, and increased renal excretion of L-carnitine.[3-6] Infants or young children with this condition usually present early in life with extremely low concentrations of L-carnitine in plasma and muscle and associated symptoms, including cardiomyopathy, myopathy, and hypoketotic hypoglycemia.[4,5,7,8] Secondary L-carnitine deficiency has been implicated in persons with metabolic disorders (eg, propionic acidemia)[9,10] or renal Fanconi syndrome[11] and in end-stage renal disease patients undergoing long-term hemodialysis.[12,13] Secondary L-carnitine deficiency may also be precipitated by drugs, including cephaloridine, verapamil, valproic acid, and sulfonylureas, usually by competitive inhibition of its transport by OCTN2, although mechanisms involving alterations in the expression of OCTN2 cannot be excluded.[4,14-17]

Oral L-carnitine is often used to alleviate symptoms associated with primary and secondary L-carnitine deficiency, although other pharmacologic indications include the management of cardiac and neurologic conditions as well as infertility.[9,18] The recommended dose of oral L-carnitine in adults with primary L-carnitine deficiency or secondary L-carnitine deficiency due to an inborn error of metabolism is 990 mg 2 to 3 times a day.[19,20] Currently, oral L-carnitine is not indicated for patients with end-stage renal disease because of the potential for the accumulation of its metabolites and associated toxicity.[19]

When administered orally to humans, L-carnitine undergoes absorption via carrier-mediated transport

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and passive diffusion, with the oral bioavailability reported as approximately 16% and 5% following single oral doses of 2 and 6 g, respectively.[21-23] The unabsorbed fraction may pass to lower segments of the gastrointestinal tract where enterobacteria convert L-carnitine to trimethylamine and γ-butyrobetaine.[24,25] After absorption from the gastrointestinal tract, trimethylamine undergoes extensive *N*-oxidation to trimethylamine-*N*-oxide in the liver by the flavin-containing monooxygenase (isoform 3) enzyme (EC 1.14.13.8),[26] whereas γ-butyrobetaine is primarily excreted in the feces.[27,28] Trimethylamine-*N*-oxide is removed by renal excretion although retroreduction to trimethylamine has been shown to occur.[29] The clinical interest in trimethylamine and trimethylamine-*N*-oxide is related to the possible role of these compounds in the formation of the carcinogen *N*-nitrosodimethylamine.[30-32] In addition, patients with impaired flavin-containing monooxygenase form 3 activity have altered disposition of these amines, resulting in deleterious symptoms such as body malodor, psychosocial reactions, hypertension, and neurologic toxicity.[33-37]

Previous studies investigating the pharmacokinetics of oral L-carnitine have not concomitantly measured the concentrations of trimethylamine and trimethylamine-*N*-oxide in plasma. Moreover, although the linearity of L-carnitine pharmacokinetics after single oral doses have been examined, there have been no evaluations of linearity for L-carnitine and its 2 metabolites. In this study, steady-state plasma concentrations of L-carnitine were achieved by the oral administration of 0.5, 1, and 2 g of L-carnitine 3 times a day to 7 healthy subjects for 7 days using a dose-escalation design. The mean plasma concentrations, area under the plasma concentration-time curve, amount excreted in urine, and renal clearance were determined for L-carnitine, trimethylamine, and trimethylamine-*N*-oxide at each dose level (including baseline). The aim of the study was to determine whether the pharmacokinetics of L-carnitine, trimethylamine, and trimethylamine-*N*-oxide at steady state were dependent on the oral dose of L-carnitine.

SUBJECTS AND METHODS

Subjects

The University of South Australia Human Research Ethics Committee gave approval for the study, and signed informed consent was obtained from 7 adult male subjects. Subjects who suffered from any of the following medical conditions were excluded from the study: seizures, epilepsy, thyroid or liver dysfunction. Other exclusion criteria included subjects who had taken oral antibiotics or L-carnitine in the past month. Eight subjects were enrolled in the study, with 7 proceeding to completion. One subject was withdrawn from the study before the administration of oral L-carnitine was commenced, as the subject had been prescribed a medication for a chronic medical condition.

Study Design

The study involved 4 periods of 7 days duration with dose escalation of oral L-carnitine at the following doses: 0 (baseline), 0.5, 1, and 2 g 3 times a day (total daily dose of 0, 1.5, 3, and 6 g). On day 7 of each period, blood samples (9 mL) were taken at 0 (immediately prior to the morning dose) and then 1, 2, 4, and 8 hours after the morning dose of L-carnitine. Each subject voided their bladder immediately prior to collection of the first blood sample on day 7 of each period; urine was subsequently collected over the 8-hour interval, with the subjects emptying their bladder immediately following the collection of the last blood sample at 8 hours. The total volume of urine collected for each subject was recorded. Subjects were asked to avoid eating any food containing fish (rich in trimethylamine) for 24 hours prior to blood collection. All blood samples were collected into a vacuette (Greiner Bio-one, Kremsmunster, Austria) and immediately placed on ice prior to centrifugation (3000*g*) at 4°C for 10 minutes.

Analytical Methodology

Concentrations of L-carnitine in plasma were determined using reverse-phase high-performance liquid chromatography with fluorescence detection as described by Longo et al.[13,38] Briefly, this method involved extraction of the plasma sample (100 μL) with Bond Elut SAX-Isolute solid-phase extraction cartridges (100 mg/mL, Varian, Palo Alto, Calif), followed by precolumn derivatization with 1-aminoanthracene. The calibration curve for plasma was constructed in the range 2.5 to 160 μM of L-carnitine. Analyses of plasma and urine samples for trimethylamine and trimethylamine-*N*-oxide were performed using a solid-phase microextraction fiber with gas chromatography-mass spectrometry as described previously.[39,40] Calibration curves for trimethylamine and trimethylamine-*N*-oxide were constructed in the range 0.169 to 84.6 μM and 6.66 to 6.66×10^4 μM, respectively. Quality control samples

Table I Mean Plasma Concentrations of L-carnitine, Trimethylamine, and Trimethylamine-*N*-oxide

	Plasma Concentrations (μM)[a]				
	Baseline	**0.5 g**	**1 g**	**2 g**	***P*[b]**
L-carnitine	54.2 ± 9.3	80.5 ±12.5	79.6 ± 13.1	82.3 ± 15.9	.0004
Trimethylamine	0.8 ± 0.3	1.1 ± 0.07	1.3 ± 0.06	1.6 ± 0.5	.0004
Trimethylamine-*N*-oxide	34.5 ± 2.0	32.4 ± 21.1	35.2 ± 18.5	149 ± 145	.04

a. Arithmetic mean ± SD.
b. Calculated using analysis of variance with factor of dose.

were prepared for L-carnitine, trimethylamine, and trimethylamine-*N*-oxide and assayed during sample analysis. The interday precision and accuracy of the plasma assays for L-carnitine, trimethylamine, and trimethylamine-*N*-oxide were within 25%.

Calculations

Area under the plasma concentration-time curve from 0 to 8 hours (AUC_0^8) was calculated using the linear trapezoidal method, and dividing by the dosing interval (8 hours) gave the mean plasma concentrations for L-carnitine, trimethylamine, and trimethylamine-*N*-oxide.

The renal clearances for L-carnitine, trimethylamine, and trimethylamine-*N*-oxide were calculated by dividing the amounts excreted in urine over the 8-hour collection interval by their respective values for AUC_0^8.

Statistical Analysis

The influence of L-carnitine dose on average plasma concentrations, area under the plasma concentration-time curve, amount excreted in urine, and renal clearance of L-carnitine, trimethylamine, and trimethylamine-*N*-oxide was determined using analysis of variance (ANOVA) with $P < .05$ taken to represent significance at the $\alpha = .05$ level.[41] Post hoc analysis was performed using contrasts with $P < .05$ taken to represent significance; all data were logarithmically transformed prior to analysis.[41]

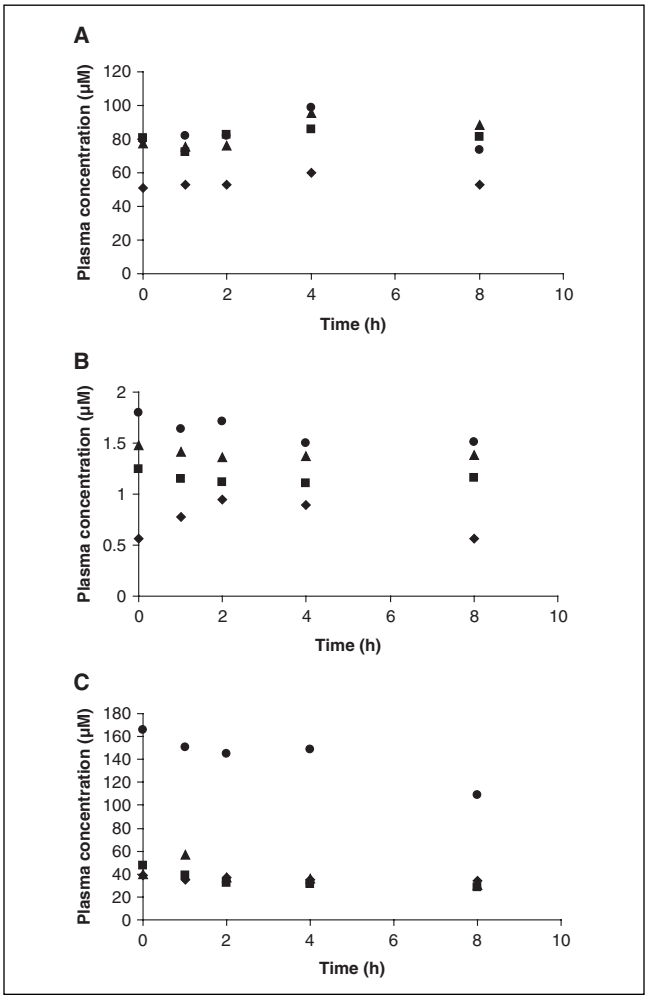

*Figure 1. Plasma concentration at steady state versus time profile for L-carnitine (A), trimethylamine (B), and trimethylamine-*N*-oxide (C). Healthy subjects had blood samples taken at 0 (immediately prior), 1, 2, 4, and 8 hours after the oral administration of L-carnitine at the doses of 0.5 g (■), 1 g (▲), and 2 g (●) 3 times a day, as well as baseline measurements (◆).*

RESULTS

The mean age of the subjects included in the study was 32 ± 11 years. Although there were no serious adverse events reported, 1 subject at the 0.5-g dose and 2 subjects at the 2-g dose reported nausea; 2 subjects at the 2-g dose reported diarrhea; and 1 subject at the 2-g dose of L-carnitine reported a metallic taste. No subject received oral antibiotics during the study, although 1 subject consumed a

Table II Renal Clearance for L-carnitine, Trimethylamine, and Trimethylamine-*N*-oxide

	Renal Clearance (mL/min)[a]				
	Baseline	**0.5 g**	**1 g**	**2 g**	***P*[b]**
L-carnitine	4.1 ± 2.1	11.1 ± 3.7	13.4 ± 5.9	29.2 ± 12.6	<.00001
Trimethylamine	5.8 ± 3.7	10.9 ± 5.6	11.5 ± 8.9	57.8 ± 82.7	.6
Trimethylamine-*N*-oxide	28.1 ± 18.4	138 ± 44	103 ± 79	60.4 ± 41.1	.004

a. Arithmetic mean ± SD.
b. Calculated using analysis of variance with factor of dose.

fish-containing meal within 24 hours of blood collection while taking the 1-g dose of L-carnitine. This subject's plasma and urinary concentrations of L-carnitine, trimethylamine, and trimethylamine-*N*-oxide were excluded from the calculations for this period. The urinary concentrations of trimethylamine and trimethylamine-*N*-oxide for this subject were 0.12 mM and 19.3 mM, respectively. By comparison, the mean urinary concentration of trimethylamine and trimethylamine-*N*-oxide for the other subjects was 6.7 and 1868.8 µM, respectively. The concentrations of trimethylamine and trimethylamine-*N*-oxide in the plasma of the subject who consumed fish were 1.6 and 80.0 µM, respectively, whereas the corresponding data for the other periods are included in Table I.

There was a significant increase in the mean concentrations of L-carnitine in plasma at all dose levels (Table I, Figure 1) as compared with baseline ($P < .001$). However, there were no significant differences in mean concentrations between the 0.5-g and 2-g doses. A significant increase in the renal clearance for L-carnitine (Table II) was found following the oral administration of each dose of L-carnitine as compared with baseline ($P < .001$). Significant increases in this parameter were also observed when comparing the 0.5-g with 2-g and 1-g with 2-g doses ($P < .05$). The amount of L-carnitine excreted in urine at baseline and following the 0.5-g, 1-g, and 2-g doses were 102.7 ± 48.5, 427.0 ± 146.7, 541.8 ± 271.0, and 1150.1 ± 457.1 µmol, respectively (a 1-g dose corresponds to 6.2 mmol of L-carnitine). The amount of L-carnitine excreted in urine increased significantly from baseline after the administration of each dose of L-carnitine ($P < .005$), and a significant increase was also observed when comparing the 0.5-g with 2-g and 1-g with 2-g doses ($P < .05$).

The mean concentrations of trimethylamine and trimethylamine-*N*-oxide in plasma are shown in Figure 1, and the data are presented in Table I. The mean concentrations of trimethylamine increased significantly for each dose of L-carnitine as compared with baseline ($P < .01$). There were also significant differences ($P < .05$) between the 0.5-g and 2-g doses. A significant increase in the mean concentration of trimethylamine-*N*-oxide was observed between baseline and the 2-g dose of L-carnitine ($P < .05$). Significant differences in the mean plasma concentrations of trimethylamine-*N*-oxide were also noticed when comparing the 0.5-g with 2-g and 1-g with 2-g doses of L-carnitine ($P < .05$).

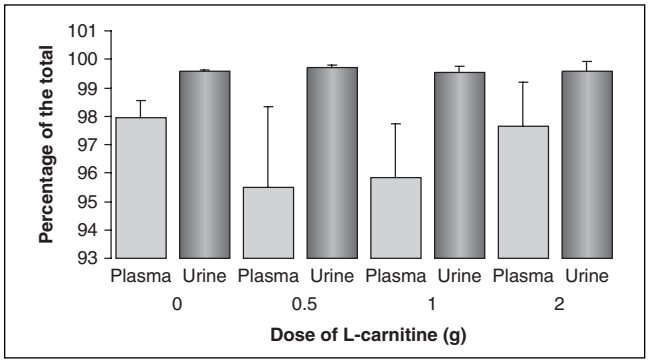

*Figure 2. Percentage of total trimethylamine-related material (trimethylamine and trimethylamine-*N*-oxide) as trimethylamine-*N*-oxide in plasma and urine, calculated using mean concentrations. Error bars indicate SD.*

The renal clearance for trimethylamine-*N*-oxide (Table II) increased significantly following the 0.5-g dose of L-carnitine from baseline ($P = .0005$), with a significant decrease in this parameter between the 0.5-g and 2-g dose ($P < .05$). No significant differences in the renal clearance of trimethylamine were revealed between the baseline phase and any of the phases when L-carnitine was administered. There was no significant correlation between urinary flow rate for the subjects and the renal clearance of trimethylamine and trimethylamine-*N*-oxide. Approximately 96% to 97% and 99% of total trimethylamine-related material (trimethylamine and trimethylamine-*N*-oxide) was present as trimethylamine-*N*-oxide in plasma and urine, respectively (Figure 2).

Table III Percentage of the Oral L-carnitine Dose Excreted in Urine as L-carnitine, Trimethylamine, and Trimethylamine-*N*-oxide (with baseline correction)

	Percentage L-carnitine Dose Excreted in Urine[a]			
	0.5 g	1 g	2 g	*P*[b]
L-carnitine	10.5 ± 5.0	7.0 ± 4.9	8.4 ± 3.7	.8
Trimethylamine	0.1 ± 0.1	0.08 ± 0.09	0.4 ± 0.7	.6
Trimethylamine-*N*-oxide	50.8 ± 37.3	25.0 ± 31.1	42.6 ± 66.7	.7

a. Arithmetic mean ± SD.
b. Calculated using analysis of variance with factor of dose.

The percentage of the oral dose of L-carnitine excreted in the urine as L-carnitine, trimethylamine, and trimethylamine-*N*-oxide, with baseline correction for endogenous levels of each substance, is presented in Table III.

DISCUSSION AND CONCLUSIONS

This is the first study to investigate the pharmacokinetics of oral L-carnitine when administered as a repeated dose regimen at multiple dose levels. A dose-escalation study design was employed to ensure that any subject who experienced serious adverse events with a particular dose would not progress in the study to a higher dose. To mimic the oral administration of L-carnitine under regular conditions, subjects were not required to consume a low L-carnitine diet. Fish are an abundant source of trimethylamine-*N*-oxide,[42] and there was a 10-fold increase in the urinary concentrations of this substance in the subject who consumed fish prior to sample collection as compared with the mean data from the remainder of the subjects. Although there was also an increase in the plasma concentration of trimethylamine-*N*-oxide in this subject, it was not to the same extent as in the urine suggesting that the kidneys readily excrete this metabolite.

Limitations in the study design must be considered during the analysis and interpretation of the data contained herein. The main purpose was to gain preliminary information on the disposition of oral L-carnitine and its metabolites in healthy persons; hence, a small number of subjects were included in the present study. It was envisaged that further work would employ a larger number of subjects depending on the outcomes and findings of this present study. There were differences between subjects in the plasma concentrations of trimethylamine-*N*-oxide and in the renal clearances of each substance. Although it was assumed that subjects had normal liver and kidney function, it is conceivable that interindividual differences as well as environmental influences may explain the variation seen in some of the data. The latter influence could arise from certain vegetables that may decrease the activity of flavin-containing monooxygenase (isoform 3)[43] or from the abundance of L-carnitine in meat and dairy products.[2] Finally, women were excluded from the study, and future, more extensive studies will also include female subjects because the impact of menstrual cycles on the activity of flavin-containing monooxygenase (isoform 3) and the relative amounts of trimethylamine and its oxide may well be apparent.[36,44]

The baseline mean concentration of L-carnitine in plasma was 54.2 ± 9.3 μM, which is slightly higher than previously found in healthy subjects (40 to 50 μM).[45] After the oral administration of 0.5, 1, and 2 g 3 times a day, there were significant ($P < .0005$) increases above baseline in the mean concentrations of L-carnitine. Similar to previous studies,[27,46] maximum concentrations of L-carnitine were observed at approximately 4 hours after dosing, reflecting its slow absorption from the intestinal lumen. However, the mean concentrations of L-carnitine did not differ across the 3 doses. This finding could have been due to saturation of absorption and/or tubular reabsorption (the latter accompanied by an increase in the renal clearance). Harper et al found that the oral biovailability of L-carnitine was 16% and 5% following single doses of 2 and 6 g, respectively.[21] They concluded that saturation of the carrier-mediated transport systems involved in the oral absorption of L-carnitine had occurred at single oral doses of 2 g and above.[21] However, these authors had not considered the possibility of nonlinearity in the renal clearance of L-carnitine.

The disposition kinetics of L-carnitine following intravenous administration have been characterized as polyexponential (bi- or triexponential), which has been attributed to slow equilibration of L-carnitine

between plasma and the tissue compartment (ie, skeletal and cardiac muscle).[47-49] The terminal half-life of L-carnitine has been reported as 17 hours, and therefore steady state would have been achieved in this current study following 3 days of oral dosing with L-carnitine.[47] L-carnitine is eliminated predominantly by the kidney, but there is extensive active tubular reabsorption mediated by the OCTN2 protein.[3,14,17,50] L-carnitine does not bind strongly to plasma proteins,[51] and therefore its filtration clearance is similar to glomerular filtration rate.[52] The baseline renal clearance for L-carnitine in this current study was 4.1 ± 2.1 mL/min, and given that healthy persons have a glomerular filtration rate of approximately 120 mL/min,[53] more than 90% of the filtered L-carnitine was subject to active tubular reabsorption. Saturation of this transport process with increasing concentrations of L-carnitine was likely to have occurred, as there was a significant increase in the renal clearance between the 0.5-g and 2-g oral dose of L-carnitine from 11.1 ± 3.7 to 29.2 ± 12.6 mL/min, respectively ($P = .002$). Similar findings have been found previously with increasing intravenous doses of L-carnitine and concomitant increases in its renal and nonrenal clearance (eg, biliary excretion).[21,49]

The baseline amount of L-carnitine excreted in urine (over an 8-hour interval) and its renal clearance in this study were 102.7 ± 48.5 μmol and 4.1 ± 2.1 mL/min, respectively; these values correspond well to baseline values of approximately 110 μmol/24 h and 3.1 mL/min found by Harper et al.[21]

After the oral administration of L-carnitine, trimethylamine is formed by enterobacteria, and after being absorbed, it is metabolized efficiently in the liver to trimethylamine-*N*-oxide.[25-27] The 2 substances subsequently become involved in a "futile" metabolic cycle, where trimethylamine-*N*-oxide is retroreduced to trimethylamine.[29] Therefore, the extent of formation of trimethylamine and trimethylamine-*N*-oxide would be expected to increase with increasing doses of L-carnitine. Significant increases in the mean concentrations of trimethylamine in plasma were observed following each dose of L-carnitine as compared to baseline ($P < .01$). Furthermore, given that the renal clearance of trimethylamine remained relatively constant during the study, a significant increase in the mean concentrations of trimethylamine as the dose of L-carnitine increased from the 0.5 to 2 g ($P < .05$) may be attributed to its increased formation.

In a similar manner, there were significant increases in the mean concentrations of trimethylamine-*N*-oxide above those for the baseline and the 0.5-g and 1 g-dose when a 2-g dose of L-carnitine was administered for 7 days ($P < .05$). The renal handling of trimethylamine-*N*-oxide during the study revealed a significant increase in its renal clearance between baseline and the 0.5-g dose ($P = .0005$) and a subsequent decrease in this parameter with higher doses of L-carnitine ($P < .05$). It is possible that the renal disposition of trimethylamine-*N*-oxide is complex, involving both carrier-mediated tubular secretion and carrier-mediated reabsorption, with the secretory process being saturated at higher concentrations than the reabsorptive process. The 5-fold increase in the plasma concentrations of trimethylamine-*N*-oxide from the 0.5-g and 1-g dose to the 2-g dose suggests that saturation of a carrier-mediated process may have occurred. The continual decline in plasma concentrations of trimethylamine-*N*-oxide during the 8-hour interval for each period could be attributed to its movement from plasma into tissue and/or elimination by the kidneys: either or both processes may be saturable. Further work may offer insight as to whether a saturable process in the renal elimination of trimethylamine-*N*-oxide would explain the observations seen in this present study.

The extent to which trimethylamine is metabolized to trimethylamine-*N*-oxide in the body was assessed by the percentage of the mean plasma concentrations for trimethylamine-*N*-oxide to total trimethylamine-related material (trimethylamine and trimethylamine-*N*-oxide). This percentage ranged from 96% to 97% throughout the study, with no evidence of saturation of liver metabolism occurring (which would be expected to lead to a reduction in this value). Interestingly, the percentage of urinary concentrations of trimethylamine-*N*-oxide to total trimethylamine-related material (trimethylamine and trimethylamine-*N*-oxide) was 99%. This higher percentage (in urine versus plasma) is consistent with previous studies and highlights the differences that may exist in the renal handling of these 2 metabolites.

The percentage of the oral L-carnitine dose recovered in urine as L-carnitine, trimethylamine, and trimethylamine-*N*-oxide was similar to previous findings.[21,27,54] After oral doses of 1.98 g every 12 hours for 3 days, 8.5% of the dose was excreted in the urine as L-carnitine.[54] However, this value may be a slight overestimate, as baseline correction for endogenous urinary levels of L-carnitine was not conducted.[54] Harper et al recovered 8% and 4% of single oral doses of 2 and 6 g, respectively, of L-carnitine in urine as unchanged drug,[21] the former recovery being comparable to that observed in the present study. Because

the bioavailability of oral L-carnitine may be reduced at higher doses, decreased recovery of this substance in urine would be expected.[27] As discussed above, another important consideration is that there is an increased renal excretion of L-carnitine due to saturation of active tubular reabsorption with increased oral doses of L-carnitine.[50]

After the oral administration of radio-labeled L-carnitine, Rebouche et al recovered 8% to 49% of the oral dose in urine as trimethylamine-*N*-oxide.[27] By comparison, our current study recovered between 25% to 51% of the oral L-carnitine dose in urine as trimethylamine-*N*-oxide, with a significant correlation between dose and amount of trimethylamine-*N*-oxide excreted in urine ($P < .05$). Factors likely to affect urinary recovery as trimethylamine-*N*-oxide include diet, extent of enterobacterial generation from L-carnitine, and renal clearance. As noted above, the exact processes involved in the renal elimination of trimethylamine-*N*-oxide may be complex and future studies are needed to examine this.

In conclusion, mean concentrations of L-carnitine in plasma increased from baseline following the oral administration of 0.5 g, 1 g, and 2 g 3 times a day of L-carnitine, reaching a plateau at the 0.5-g dose. It is likely that this plateau can be ascribed to increases in the renal clearance with higher doses. A proportionate increase in the formation of the metabolites trimethylamine and trimethylamine-*N*-oxide was reinforced by a significant increase in mean plasma concentrations for these metabolites at the 2-g dose as compared to baseline. However, given the evidence for a decrease in the renal clearance of trimethylamine-*N*-oxide with increasing oral dose of L-carnitine, further studies investigating the exact mechanisms involved in the renal handling of this metabolite are warranted. The consequence of increased plasma concentrations of these metabolites is of interest, particularly considering their uncertain physiologic and toxicologic significance. The findings from this preliminary work cast doubt on the usefulness of L-carnitine at oral doses much greater than 1 - 2 g a day, at least in healthy adults.

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