
ORIGINAL RESEARCH

Comparison of pharmacokinetics of L-carnitine, Acetyl-L-carnitine and Propionyl-L-carnitine after single oral administration of L-carnitine in healthy volunteers

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Manuscript submitted 17th October, 2008

Manuscript accepted 25th November, 2008

Clin Invest Med 2009; 32 (1): E13-E19.

Abstract

Purpose: To investigate the pharmacokinetics of L-carnitine (LC) and its analogues, acetyl-L-carnitine (ALC) and propionyl -L-carnitine (PLC) in healthy volunteers after single L-carnitine administration.

Methods: Liquid L-carnitine (2.0 g) was administered orally as a single dose in 12 healthy subjects. Plasma and urine concentrations of L-carnitine, ALC and PLC were detected by HPLC.

Results: The maximum plasma concentration (C_{\max}) and area under the curve ($AUC_{0-\infty}$) of L-carnitine was $84.7 \pm 25.2 \mu\text{mol}\cdot\text{L}^{-1}\cdot\text{h}$ and $2676.4 \pm 708.3 \mu\text{mol}\cdot\text{L}^{-1}\cdot\text{h}$, respectively. The elimination half-life of L-carnitine and the time required to reach the C_{\max} (T_{\max}) was 60.3 ± 15.0 and 3.4 ± 0.46 h, respectively. The C_{\max} of ALC ($12.9 \pm 5.5 \mu\text{mol}\cdot\text{L}^{-1}$) and PLC ($5.08 \pm 3.08 \mu\text{mol}\cdot\text{L}^{-1}$) was lower than L-carnitine ($P < 0.01$), so as the $AUC_{0-\infty}$ (166.2 ± 77.4 and $155.6 \pm 264.2 \mu\text{mol}\cdot\text{L}^{-1}\cdot\text{h}$, respectively, $P < 0.01$). The half-life of ALC (35.9 ± 28.9 h) and PLC (25.7 ± 30.3 h) was also shorter than L-carnitine ($P < 0.01$). The 24h accumulated urinary excretion of L-carnitine, ALC and PLC were 613.5 ± 161.7 , 368.3 ± 134.8 and $61.3 \pm 37.8 \mu\text{mol}$, respectively.

Conclusion: L-carnitine has a greater maximum plasma concentration than ALC and PLC. L-carnitine also has a

longer half-life than ALC and PLC. These data may have important implications in the designing of dosing regimens for L-carnitine or its analogues, such as ALC or PLC.

L-carnitine (3-hydroxy-4-N-trimethylammonium butyrate) is an endogenous compound mainly derived from food stuff, such as meat and dairy products.¹⁻³ It is also synthesized in the human, in the liver and kidneys, from the essential amino acids lysine and methionine.¹⁻³ In humans, the endogenous carnitine pool, which comprises free L-carnitine and a range of short-, medium- and long-chain esters, such as acetyl-L-carnitine (ALC) and propionyl-L-carnitine (PLC), is maintained by absorption of L-carnitine from dietary sources, biosynthesis within the body and extensive renal tubular reabsorption from glomerular filtrate.¹⁻³

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 and cardiac muscle.^{1,2} L-carnitine is an essen-

tial cofactor in the β -oxidation of long-chain fatty acids.⁴ It also acts as an acyl group acceptor in order to maintain sufficient cellular levels of free coenzyme A.^{5,6}

L-carnitine, ALC and PLC supplements have been used as adjuvant therapy in several clinical disorders. L-carnitine improves the clinical outcomes of heart failure, acute coronary syndrome or anemia in addition to the conventional pharmacotherapy.⁷⁻⁹ ALC has been used to manage patients with dementia, in particular Alzheimer's disease, with some success.¹⁰ PLC was found to stimulate energy production in ischemic muscles by increasing citric acid cycle flux and stimulating pyruvate dehydrogenase activity.¹¹ After 24 weeks of therapy, PLC improved mean maximum walking distance in patients with peripheral arterial obstructive disease.¹¹ Although the pharmacokinetics of L-carnitine have been studied before, little is known about the transformation from L-carnitine to ALC or PLC in human subjects.¹² The primary aim of the present study was to evaluate and compare the pharmacokinetic characteristics of L-carnitine, ALC and PLC after a single oral administration of L-carnitine in healthy volunteers. The results of this study may help with the future design of dosing regimens for L-carnitine, ALC and PLC.

Materials and methods

Drugs, reagents and apparatus

Standard preparations of L-carnitine (purity 99%) (Batch No.060708, 10 ml: 1 g) were obtained from Northeast Pharmaceutical Group Co., LTD, China. 1-Aminoanthracen (1-AA) and 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC-HCL) were supplied by Sigma, USA. Acetonitrile (HPLC grade reagent) was purchased from Honeywell international INC, USA. Other reagents (ammonium acetate, hydrochloric acid, acetone, glacial acetic acid, chloroform) were of analytical grade.

Waters 2690 HPLC equipment system and Waters 474 fluorescence detector (USA), Hypersil C₁₈ column

(4.6mm 200mm,5 μ m), Biochemistry analyser (SYNCHRON LX20,BECKMAN,USA), Haemodialyser (Haidylena, Egypt).

Study participants

This study was approved by the Institutional Review Board of Qingdao University. Informed written consent was obtained from all participants. Healthy volunteers were invited from the staff working at the hospital clinics and those who attended the clinics for annual health check-ups. All subjects were free from significant neurologic, pulmonary, gastrointestinal and hematologic diseases. Participants underwent a thorough medical examination, including blood cell counts, biochemistry profile, liver and renal function tests and electrocardiogram. The volunteers were not permitted to consume alcohol for 72 h before or during the study and were asked to abstain from any medications for at least 1 week before and during the study. Those who had a history of drug or alcohol abuse or allergy to the components of L-carnitine, and those who had concomitant drug therapy of any kind were excluded. Two weeks before the study, all subjects were prescribed a similar diet comprising mainly local rice, red meat and green vegetables.

Twelve healthy Chinese volunteers were recruited from 15 volunteers, three were excluded because one consumed alcohol before the study and two had a medical history. There were 6 men and 6 women with a mean age 27.7 \pm 4.7 yr. Their mean weight was 62.9 \pm 8.8 kg and the mean height 167.0 \pm 6.15 cm.

Study design

L-carnitine (2.0g) was administered orally as a solution. Immediately before (0 h) and at 0.5, 1.0, 1.5, 2, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 12.0, 24.0h after administration, venous blood was collected and transferred into a chilled tube, which was centrifuged within 10 min. The plasma was transferred into a polypropylene tube, which was kept at -20°C for

TABLE 1. Baseline plasma concentrations of L-Carnitine, ALC and PLC.

| | Concentrations ($\mu\text{mol} \cdot \text{L}^{-1}$) | | |
|--------------|--|---------|---------|
| | L-carnitine | ALC | PLC |
| Total (n=12) | 39.1±5.4 | 2.4±0.6 | 0.6±0.4 |
| Male (n=6) | 41.5±6.0 | 2.5±0.7 | 0.7±0.4 |
| Female (n=6) | 36.8±4.0 | 2.2±0.5 | 0.6±0.4 |

analysis. At the same time, urine from participants was collected before (0h) and at 0~2h, 2~4h, 4~8h, 8~12h, 12~24h after the drug administration: 5ml of urine was transferred into a polypropylene tube and kept at -20°C for analysis.

Measurement of L-carnitine, ALC and PLC

The samples of 100 μl of plasma and urine, containing L-carnitine, ALC and PLC, were extracted by protein precipitation with 500 μl acetonitrile. The supernate (500 μl) was added in 400 μl phosphate buffer (pH 3.5). 20 μl of 1M HCL, 100 μl of 1-aminoanthracene (1AA) solution (16mg/ml, 1-AA was dissolved in acetone) and 100 μl of the EDC solution (160mg/ml, EDC in 0.01M $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ pH 3.5) was sequentially added to the samples. The mixture was incubated at room temperature for 20 min and the excess reagent was removed by washing the sample twice with 3 ml of ethylether. All of the aqueous phase was then transferred to a plastic tube, to which 800 μl of 0.01 M $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ (pH 9.1) was added to adjust the pH of the samples. The samples were washed twice with 3 ml chloroform to eliminate the interference of amino acids. At this pH value the amides resulting from the reaction of amino acids with 1AA are highly soluble in chloroform, whereas the solubility of the derivatives of carnitines in chloroform is negligible.

The final samples (500 μl) were diluted with 500 μl of 0.01 M $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ (pH 3.5). 50 μl of the final samples were injected into high-performance liquid chromatography (HPLC). The HPLC mobile phase was prepared by mixing 700 ml of 0.1 M ammonium acetate in water (pH 3.5 adjusted with acetic acid) with 300 mL of acetonitrile. The peak area ratios of L-

carnitine and internal standard (2 $\mu\text{g}/\text{mL}$ γ -butyrobetaine hydrochloride) were used to measure the concentrations of L-carnitine, ALC and PLC. The excitation and emission wavelengths of the spectrofluorimeter were 248 and 418 nm, respectively.

Determination of accumulated urinary excretion and accumulated excretion rate

The accumulated urinary excretion was a summation of excretion of 0~24h through multiplying the urine concentration and volume in 0~2h, 2~4h, 4~8h, 8~12h, 12~24h, respectively. The accumulated urinary excretion rate of L-carnitine was calculated by dividing accumulated excretion of L-carnitine with oral dose (2.0 g).

Data Analysis

The pharmacokinetic parameters of L-carnitine, ALC and PLC were calculated by Drug and Statistic program (DAS, version 2.0, Statistical Solutions, Sun Ru Yuan, China). The pharmacokinetic parameters derived from these calculations were maximum plasma concentration (C_{max}), half-life ($t_{1/2}$), area under the curve (AUC), time to reach the C_{max} (T_{max}).

Data were expressed as means \pm SD. SPSS15.1 software was used for data analysis. Numerical data were analyzed with one-way ANOVA. Categorical data were analyzed with Chi-square test. $P < 0.05$ was considered statistically significant.

Results

Baseline plasma concentrations

The baseline plasma concentrations of L-carnitine, ALC and PLC are shown in Table 1. There was no significant difference in these baseline concentrations between male and female subjects ($P > 0.05$).

Mean plasma concentration-time curve

The mean plasma concentration-time curve and the comparison between males and females are shown in

Fig 1 and Fig 2, respectively. There was no difference in the mean concentrations of L-carnitine, ALC and PLC between male and female groups in any time-point following the administration of L-carnitine ($P>0.05$).

Pharmacokinetic parameters

Pharmacokinetic parameters of L-carnitine, ALC and PLC after single oral administration of L-carnitine are shown in Table 2. The elimination half life of L-carnitine was longer than that of ALC or PLC ($P<0.01$). The maximum plasma concentration and the area under the curve of L-carnitine was greater than that of ALC or PLC ($P<0.01$).

Correlation analysis

There was a correlation in plasma concentrations between L-carnitine and ALC ($r=0.8626$, $P<0.01$), L-carnitine and PLC ($r=0.8368$, $P<0.01$), and between ALC and PLC ($r=0.5361$, $P<0.01$).

Accumulated urinary excretion and urinary excretion rate

The 24 h accumulated urinary excretion of L-carnitine ($613.5\pm 161.7\mu\text{mol}$) was greater than that of ALC ($368.3\pm 134.77\mu\text{mol}$, $P<0.01$) and PLC ($61.3\pm 37.8\mu\text{mol}$, $P<0.01$) (Fig 3). The accumulated urinary excretion rate of L-carnitine was 6.1% within 24h after its administration.

Discussion

The major findings of this study are: 1) After oral administration of L-carnitine, the maximum plasma concentration and the area under the curve of L-carnitine were greater than those of ALC or PLC; 2). There was no difference in the mean concentrations of L-carnitine, ALC and PLC between male and female groups in any time following the administration of L-carnitine; 3) The half-life of L-carnitine was longer than that of ALC or PLC; 4) The 24h accumulated

urine excretion of L-carnitine was higher than that of ALC and PLC.

This is the first study to investigate the pharmacokinetics of L-carnitine, ALC and PLC after single oral administration of L-carnitine in healthy Chinese volunteers. Before L-carnitine administration, the mean plasma concentration of L-carnitine was significantly higher than ALC or PLC. The levels of baseline plasma L-carnitine, ALC and PLC in the Chinese subjects seem lower than in the other population.^{13,14}

The small intestine is the main site of L-carnitine absorption.^{15,16} Absorption of L-carnitine is characterized by slow mucosal uptake, prolonged mucosal retention and slow mucosal exit into the blood.^{17,18} In humans, the time to achieve maximum plasma concentrations after oral administration of L-carnitine can be up to 4–6 hours.¹⁹ In this study we found that the absorption half-life of L-carnitine was about 1.6 h and the time to reach maximum plasma concentration was approximately 3.4 h, which is consistent with the pre-

TABLE 2. The main pharmacokinetic parameters after single oral administration of L-carnitine.

| | L-carnitine | ALC | PLC |
|--|--------------------|-------------------|---------------------|
| $t_{1/2\alpha}$ (h) | 1.6 \pm 1.2 | ———— | ———— |
| $t_{1/2}$ (h) | 60.3 \pm 14.9 | 35.9 \pm 28.9 | 25.7 \pm 30.3 |
| V1/F(L) | 116.5 \pm 38.3 | 615.4 \pm 261.9 | 1163.5 \pm 1707.6 |
| CL/F(L·h ⁻¹) | 4.03 \pm 1.10 | 74.85 \pm 69.80 | 332.24 \pm 444.09 |
| AUC(0-t) (μmol·L ⁻¹ ·h) | 1354.4 \pm 325.0 | 119.5 \pm 55.8 | 57.9 \pm 48.5 |
| AUC(0-∞)(μmol·L ⁻¹ ·h) | 2676.4 \pm 708.3 | 166.2 \pm 77.4 | 155.6 \pm 264.2 |
| Ka(h ⁻¹) | 0.8 \pm 0.9 | 18.5 \pm 19.7 | 0.9 \pm 0.7 |
| $t_{1/2Ka}$ (h) | 1.0 \pm 0.8 | 0.6 \pm 0.6 | 1.2 \pm 0.9 |
| T _{max} (h) | 3.4 \pm 0.5 | 2.4 \pm 0.7 | 3.8 \pm 0.8 |
| C _{max} (μmol·L ⁻¹) | 84.7 \pm 25.2 | 12.9 \pm 5.5 | 5.1 \pm 3.1 |

$t_{1/2\alpha}$ = Distribution half-times; $t_{1/2}$ = biological half-life; V1/F= oral volume of distribution of the central compartment; CL/F= oral clearance from central compartment; AUC=Area under curve; Ka= absorption rate constant; $t_{1/2Ka}$ = absorption half-time; T_{max}=Time of maximum concentration; C_{max}=Maximum concentration

AUC(0-t)= The AUC from the time of dosing to the time of the last measurable concentration; AUC(0-∞)= The AUC from the time of dosing to the time of the last measurable concentration was extrapolated to infinity.

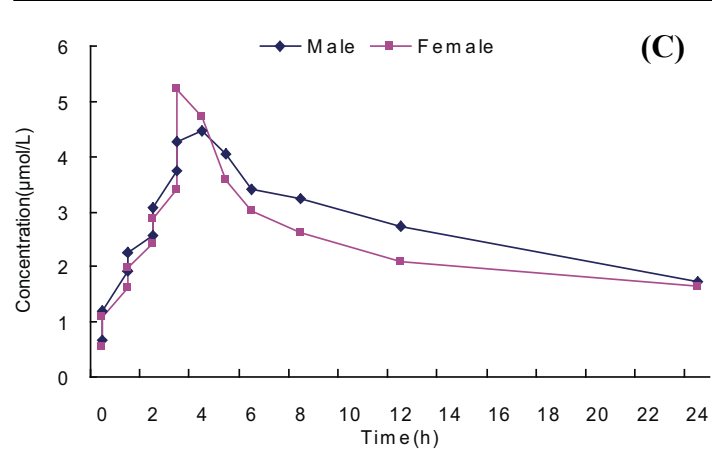
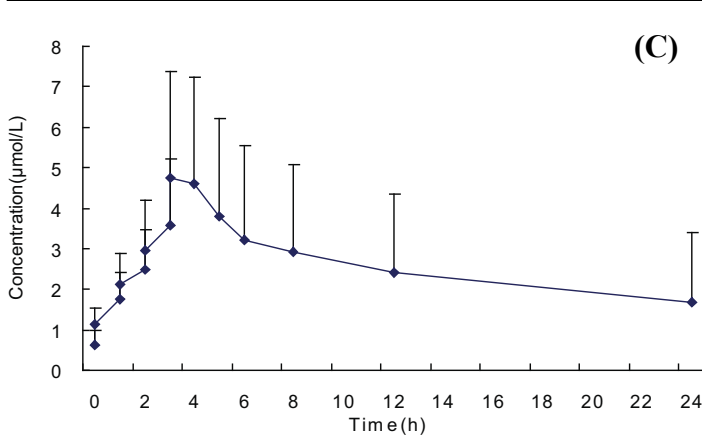
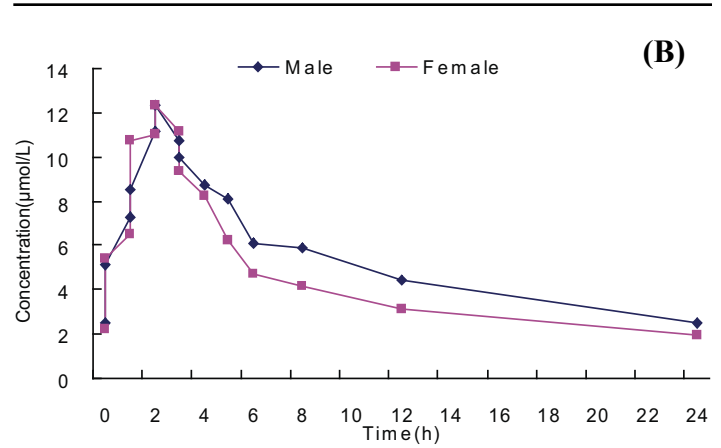
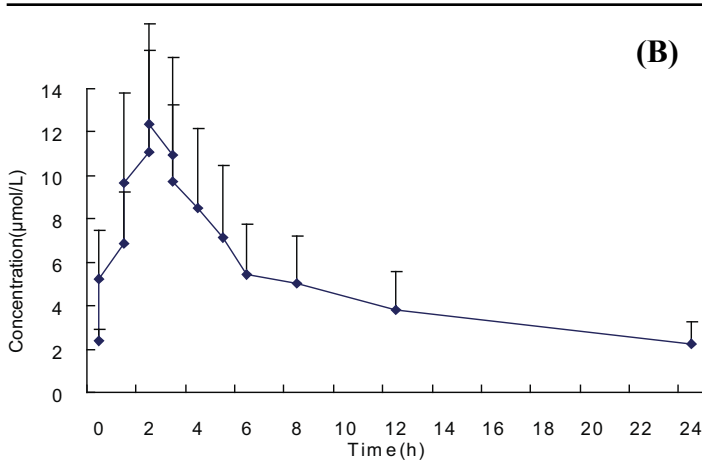
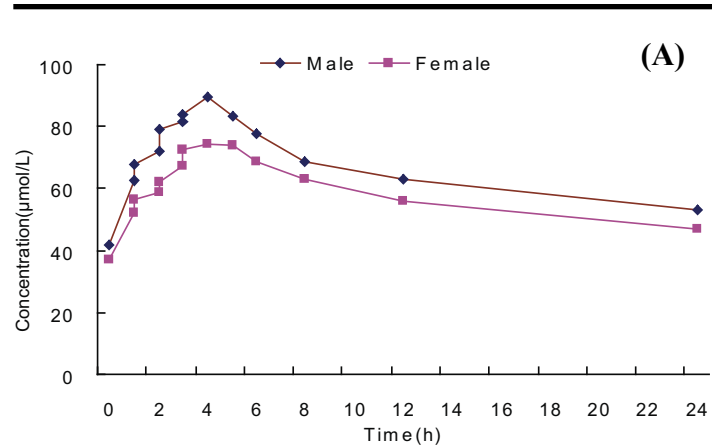
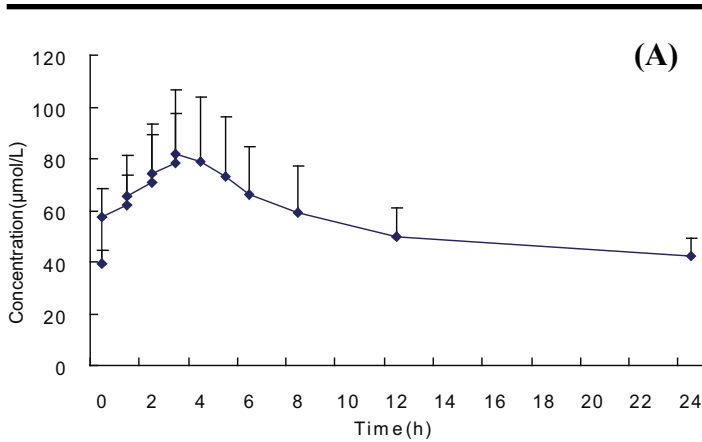


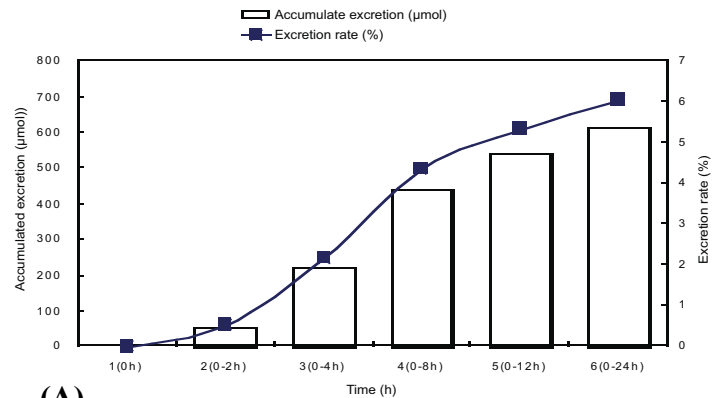
FIGURE 1. Mean plasma concentration-time curve of L-carnitine (Fig 1A), ALC (Fig 1B) and PLC (Fig 1C) after single oral administration.

FIGURE 2. Comparison of plasma concentration-time curve of L-carnitine (Fig 2A), ALC (Fig 2B) and PLC (Fig 2C) in male and female subjects.

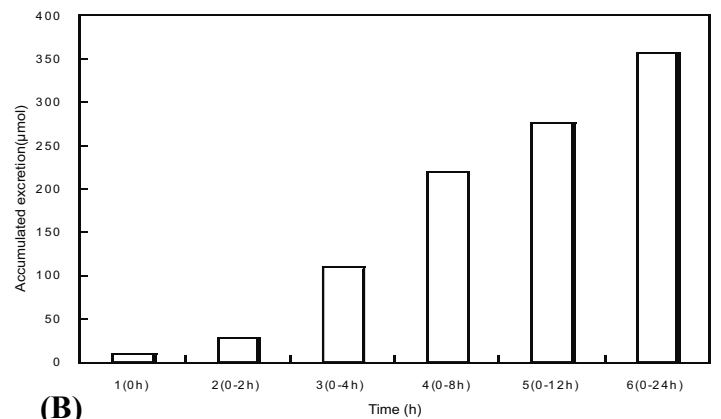
vious report.¹⁹ Intravenous injection of PLC not only increases the plasma concentrations of PLC, it also elevates plasma concentrations and urinary excretion of L-carnitine and ALC.²⁰ In our study there was also an increase in the plasma levels, and the area under the curve of ALC and PLC within two hours after the oral administration of L-carnitine. We also found a close correlation in the plasma concentrations between L-carnitine, ALC and PLC, suggesting a reciprocal transformation among the three carnitine analogues. It is unclear in this study where the transformation was taking place but earlier studies showed that acetylation of L-carnitine and the transformation to ALC or PLC can take place during the absorption process.^{16,17}

In 1991, Rebouche²¹ provided a quantitative estimation of the fate of an oral tracer dose of L-[methyl-3H]-carnitine in five men who were receiving a high-carnitine diet and L-carnitine supplementation. It was found that the absorption of oral L-[3H]-carnitine was slow and incomplete, with t_{max} values of 2–4.5 hours. This suggests prolonged retention of that fraction of the dose that had been incorporated into the body's carnitine pool. In their study, only 6.3% of the oral L-carnitine dose was recovered unchanged in the urine, with a further 34% recovered in urine as metabolites, mostly [3H]-trimethylamine-N-oxide.²¹ About 22% of the dose was recovered in faeces, mostly as labeled γ -butyrobetaine.²¹ Our experiment found that after single oral administration of L-carnitine, the accumulated excretion of L-carnitine, ALC and PLC varied from 613.5 to 61.3 μmol , and the excretion rate of L-carnitine was only 6.1%, which was consistent with Rebouche's report.²¹

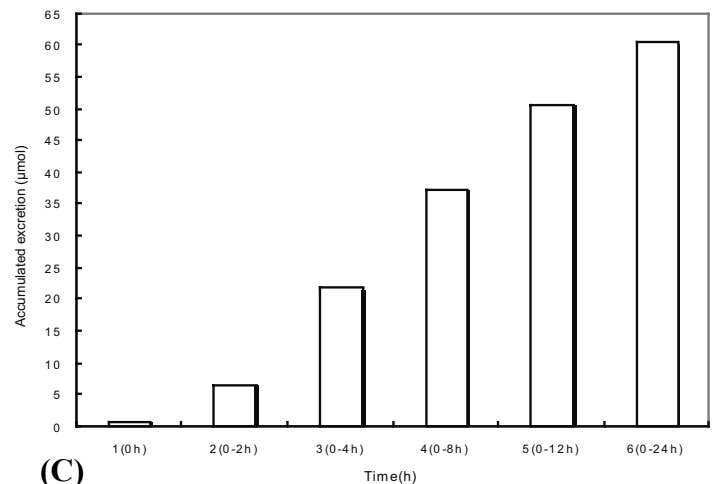
In summary, this study in healthy Chinese subjects has revealed some valuable pharmacokinetic characteristics of L-carnitine, ALC and PLC after single oral administration of L-carnitine. It showed a close correlation in plasma concentrations between L-carnitine, ALC, and PLC. It also revealed the half-lives, maximum plasma concentration and urinary excretion of the three carnitine analogues. These data may be useful in the designing of therapeutic regi-



(A)



(B)



(C)

FIGURE 3. The accumulated urinary excretion of L-carnitine (Fig 3A), ALC (Fig 3B), PLC (Fig 3C) and the urinary excretion rate of L-carnitine (A) after single oral administration of L-carnitine.

mens of L-carnitine or its analogues. Given the relatively long elimination half-lives of L-carnitine, ALC and PLC, once daily dosing is probably adequate to achieve an optimal plasma concentration. The similar pharmacokinetic properties between male and female subjects indicate that there should be no dosing differences between the two sexes.

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