Decreased Tissue Distribution of L-Carnitine in Juvenile Visceral Steatosis Mice

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ABSTRACT

We kinetically analyzed the disposition of L-carnitine of juvenile visceral steatosis (JVS) mice compared with that of normal mice to elucidate the mechanism of the systemic L-carnitine deficiency of JVS mice. There were significant differences in the plasma concentration-time course of total radioactive carnitine (L-[3H]carnitine, [acetyl-3H]carnitine, and other [acyl-3H]carnitines) between normal and JVS mice after a single i.v. or p.o. administration of L-[3H]carnitine (250 ng/kg). The oral bioavailability of L-[3H]carnitine in JVS mice (0.341) was about half of that in normal mice (0.675). The cumulative urinary excretion of total radioactive carnitine in JVS mice was about 10-fold more than that in normal mice, and the total clearance of unchanged L-[3H]carnitine for JVS mice (6.70 ml/min) was significantly higher than that for normal mice (2.45 ml/min). The distribution volume at the steady state of unchanged L-[3H]carnitine in JVS mice (1.10 liters/kg) was significantly smaller than that in normal mice (8.16 liters/kg). At 4 h after an i.v. administration, the apparent tissue-to-plasma concentration ratios of unchanged L-[3H]carnitine for various tissues of JVS mice, except for brain, were about one half to one 20th of those in normal mice. In conclusion, this in vivo disposition kinetic study of L-carnitine supports the previous in vitro finding that the L-carnitine transporter is absent or functionally deficient in JVS mice because the renal reabsorption, the intestinal absorption, and the apparent tissue-to-plasma concentration ratios in JVS mice are significantly lower than those in normal mice. The JVS mouse should be a useful experimental model for studying carnitine deficiency diseases.

It is well known that L-carnitine plays an important role in the transport of long-chain fatty acids across the mitochondrial inner membrane for β-oxidation and energy metabolism (Bremer, 1962; Fritz and Yue, 1964). Primary chronic L-carnitine deficiency may cause encephalopathy through hypoketonemia and hyperammonemia, and cardiomyopathy or hepatic encephalopathy in combination with skeletal myopathy (Bremer, 1962; Fritz and Yue, 1964). It is well known that L-carnitine plays an important role in the transport of long-chain fatty acids across the mitochondrial inner membrane for β-oxidation and energy metabolism (Bremer, 1962; Fritz and Yue, 1964). Primary chronic L-carnitine deficiency may cause encephalopathy through hypoketonemia and hyperammonemia, and cardiomyopathy or hepatic encephalopathy in combination with skeletal myopathy (Bremer, 1962; Fritz and Yue, 1964). Furthermore, L-carnitine and acetylcarnitine may have a role in the clinical treatment of acute myocardial infarction (Iliceto et al., 1995) and Alzheimer’s disease (Parnetti, 1995).

In 1988, we found that homozygous mutant mice, named juvenile visceral steatosis (JVS) mice, have systemic L-carnitine deficiency and develop fatty liver, hyperammonemia, and hypoglycemia (Koizumi et al., 1988). There have been some in vitro studies on the mechanism of L-carnitine deficiency in JVS mice. Horiiuchi et al. (1994) examined kidney slice preparations and concluded that the primary deficiency of JVS mice is most probably related to a reduction in reabsorption of L-carnitine. Kuwajima et al. (1996) reported that at the endogenous L-carnitine concentration (50 μM), the L-carnitine transport activity of fibroblasts obtained from the heart of JVS mice was only 18% of that of normal mice. Our kinetic analysis using embryonic fibroblasts derived from normal and JVS mice suggested that JVS mice lack the high-affinity carnitine transporter, which has Na’ and temperature dependence (Hashimoto et al., 1998). These in vitro results suggest that the JVS mouse would be a useful animal model of primary L-carnitine transporter deficiency.

Therefore, the next step should be to elucidate the precise mechanism of the systemic L-carnitine deficiency by means of kinetic studies of the tissue distribution and elimination of L-carnitine in the whole animal. Such studies should also help to confirm the relationship between the development of disease and the effect of L-carnitine and to establish the role of transcellular transport in determining the characteristic tissue distribution of L-carnitine in JVS mice. In the present study, we investigated the disposition kinetics of L-carnitine

ABBREVIATIONS: JVS, juvenile visceral steatosis; TLC, thin-layer chromatography; Vd app, distribution volume at the steady state; CL tot, plasma total clearance; K p, app, apparent tissue-to-plasma concentration ratios.
after i.v. and p.o. administration in normal and JVS mice and confirmed the existence of an L-carnitine transporter in vivo.

Experimental Procedures

Materials. L-[methyl-3H]Carnitine hydrochloride (L-[3H]carnitine, 79 Ci/mmol, radiochemical purity, 99.6%) was purchased from Amersham International Ltd. (Buckinghamshire, UK). Acetyl-L-[methyl-3H]carnitine hydrochloride (L-acetyl-[3H]carnitine, 65 Ci/mmol, radiochemical purity, 97.5%) was purchased from Moravek Biochemicals Inc. (Brea, CA). All other chemicals were of reagent grade and were used without further purification. Endogenous unchanged L-carnitine was determined by using a commercial kit (Free Carnitine Kit; Kainos Co., Tokyo, Japan).

Animal Experiments. JVS mice were originally found among mice of the C3H.OH strain in our laboratory (Koizumi et al., 1988). The autosomal recessive mutant gene, jvs, was then backcrossed into C57BL/6 (CLEA, Tokyo, Japan), and this strain, C57BL/6- jvs, was used in this study.

Homozygous mutant mice (JVS) designated as jvs/jvs were identified as those having a swollen, fatty liver by observation through the abdominal wall at 2 to 5 days after birth. By mating heterozygous male mice with heterozygous female mice, we obtained three genetic litters. By mating heterozygous males with heterozygous females, the autosomal recessive mutant gene, jvs, was transmitted to the offspring. The autosomal recessive mutant gene, jvs, was then backcrossed into C57BL/6 (CLEA, Tokyo, Japan), and this strain, C57BL/6- jvs, was used in this study.

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Plasma Concentration-Time Course of Total Radioactive Carnitine. The plasma concentration-time courses of total radioactive carnitine after an i.v. or a p.o. administration of 250 ng/kg of 3H-caritine in normal and JVS mice are shown in Fig. 1. After i.v. administration, the behavior of total radioactive carnitine in plasma was biphasic, but there was a marked difference between the two types of mice. At
the distribution phase, the concentration in JVS mice was significantly higher than that in normal mice. The linear terminal elimination of JVS mice was remarkably faster than that of normal mice. On the other hand, although the plasma concentration of total radioactive carnitine gradually increased after p.o. administration, after 1 h, it was significantly lower in JVS mice than in normal mice.

As shown in Table 1, the value of the area under the plasma concentration-time curve (AUC$_{0-\infty}$) from time zero to 4 h after i.v. administration of L-[3H]carnitine to JVS mice was significantly larger than that for normal mice ($p < .001$), whereas its value after p.o. administration was significantly smaller ($p < .001$). The value of the bioavailability in JVS mice was about half of that in normal mice.

**Tissue Concentration of Total Radioactive Carnitine.** The concentrations of total radioactive carnitine in various tissues at 4 h after a single i.v. administration of L-[3H]carnitine are shown in Fig. 2. The tissue concentrations of total radioactive carnitine in normal mice were spleen > kidney > liver > heart > lung > gut > muscle > brain. The concentrations in the tissues, except for the brain, of JVS mice were significantly lower than those of normal mice. The concentration in the brain was very low, and there was no significant difference between the two types of mice.

**Metabolism of L-[3H]Carnitine.** Figure 3A shows the TLC of authentic unchanged L-[3H]carnitine and [acetyl-3H]carnitine. The peaks of the two drugs were well separated, and the $R_T$ values for unchanged and [acetyl-3H]carnitine were 0.32 and 0.5, respectively. Figure 3, B and C, shows the chromatograms of plasma samples at 4 h after i.v. injection of 250 ng/kg L-[3H]carnitine in normal and JVS mice, respectively. The first and second peaks were identified as unchanged carnitine and acetylcarnitine, respectively.

Figure 4 shows the time courses of unchanged L-[3H]carnitine, [acetyl-3H]carnitine, and other [acyl-3H]carnitines in plasma as percentages of total radioactive carnitine. In normal mice, unchanged L-[3H]carnitine rapidly decreased and [acetyl-3H]carnitine was concomitantly produced, and a steady state was reached at about 1 h after the administration. In JVS mice, the conversion of carnitine to acetylcarnitine was slow and reached at a steady state after about 2 h. The ratios of L-[3H]carnitine to total radioactive carnitine in normal and JVS mice were about 0.3 and 0.5, respectively, at the steady state. The levels of other [acyl-3H]carnitines were negligible (below 10%).

**Plasma Concentration-Time Course of Unchanged L-[3H]Carnitine.** Figure 5 shows the plasma concentration-time course of unchanged L-[3H]carnitine after an i.v. dose of 250 ng/kg in normal and JVS mice. The ratios of unchanged L-[3H]carnitine to the total radioactive carnitine gradually decreased until 60 min in both cases, and thereafter the ratios were fairly constant at 0.27 for normal mice and 0.46 for JVS mice.

The kinetic parameters of unchanged L-[3H]carnitine for normal and JVS mice are listed in Table 2. The $AUC_{0-\infty}$ value from zero to infinite time for JVS mice was significantly smaller than that for normal mice. The value of the distribution volume at the steady state (Vd$_{ss}$) in JVS mice was about one eighth of that in normal mice, whereas the value of the plasma total clearance (CL$_{tot}$) was about three times larger. The linear terminal elimination rate ($k_e$) in normal mice was significantly smaller than that in JVS mice. The unbound fraction of unchanged L-[3H]carnitine in plasma was close to 1.0 in both cases.

**Tissue Distribution of Unchanged L-[3H]Carnitine.** Table 3 shows the percentage of unchanged L-[3H]carnitine and [acetyl-3H]carnitine with respect to total radioactive carnitine for each tissue at 4 h after the i.v. dose of 250 ng/kg in normal and JVS mice. Unchanged carnitine in every tissue except for muscle accounted for more than 80% of total radioactivity, whereas in muscle, exogenously administered carnitine was present to the extent of about 50 to 60% as the unchanged form and about 40% as acetylcarnitine in both types of mice.

The $K_{p, app}$ values of total radioactive carnitine for tissues except for brain in JVS mice were smaller than those in normal mice. This tendency was more marked for the $K_{p, app}$

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**TABLE 1**

Bioavailability of L-[3H]carnitine in mice

Each value represents mean ± S.D. of five mice.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Mice</th>
<th>JVS Mice</th>
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<tbody>
<tr>
<td></td>
<td>i.v.</td>
<td>p.o.</td>
</tr>
<tr>
<td>AUC$_{0-\infty}$ (ng · min/ml)$^a$</td>
<td>29.2 ± 1.3</td>
<td>19.7 ± 1.0</td>
</tr>
<tr>
<td>Bioavailability</td>
<td>0.675</td>
<td>0.341</td>
</tr>
</tbody>
</table>

$^a$ AUC$_{0-\infty}$ area under plasma concentration-time curve from 0 to 4 h of period was calculated from data on total radioactive carnitine, i.e., unchanged L-[3H]carnitine, [acetyl-3H]carnitine, and other [acyl-3H]carnitines after a single i.v. or p.o. administration of L-[3H]carnitine (250 ng/kg) in mice.
value of unchanged L-[^3]H]carnitine, indicating that exogenously administered carnitine is less well distributed to these tissues of JVS mice compared with the case of normal mice.

There was no significant difference in the $K_{p,\text{app}}$ value of unchanged L-[^3]H]carnitine in the brain (about 1) between the two types of mice. On the other hand, the $K_{p,\text{app}}$ values of [acetyl-[^3]H]carnitine were below 1 for most tissues in both types of mice, indicating that acetylcarnitine was not accumulated in most tissues.

**Urinary Excretion of L-[^3]H]Carnitine.** Figure 6 shows the urinary excretion of total radioactive carnitine for 4 h after a single i.v. dose of 250 ng/kg L-[^3]H]carnitine in normal and JVS mice. The cumulative amounts of total radioactive carnitine for 4 h in normal and JVS mice were 0.22 and 2.25 ng, respectively. The values of urinary recovery of total radioactive carnitine were about 4% and 45% of the administered L-[^3]H]carnitine in normal and JVS mice, respectively.

Figure 7 shows the result of TLC of the urine sampled for 4 h after the i.v. dose of 250 ng/kg L-[^3]H]carnitine in normal (Fig. 7A) and JVS (Fig. 7B) mice. The urine of normal mice contained only a small amount of radioactivity with high $R_f$.
values, possibly due to long-chain acylcarnitines, whereas in the urine of JVS mice, the total radioactive carnitine consisted almost entirely of unchanged carnitine and acetyl-

Discussion
The weanlings of JVS mice cannot live unless L-carnitine is supplied to maintain a plasma level 3 to 5 μg/ml L-carnitine. However, the chronic fatty liver is not improved by this L-carnitine supplement. The JVS mice seem to be congenitally carnitine deficient.

We demonstrated that the bioavailability of L-[3H]carnitine in JVS mice was about half of that in normal mice (Fig. 1, Table 1). Gudjonsson et al. (1985) examined the small intestinal absorption of carnitine using single-pass perfusion techniques in rats and found a partially saturable absorption process. McCloud et al. (1996) reported that the uptake of L-carnitine in Caco-2 cells involves a carrier-mediated system that is dependent on temperature, Na⁺, and energy but independent of pH. Therefore, JVS mice seem to lack the carrier-mediated membrane transport system for L-carnitine in the gastrointestinal tract. Moreover, we observed that the distribution of total radioactive carnitine into several tissues, except for the brain, after a single i.v. injection of L-[3H]carnitine (250 ng/kg) was reduced in JVS mice (Fig. 2 and Table 4). Because the concentrations of radiolabeled L-carnitine in the body after the injection of L-[3H]carnitine were estimated to be 3 log orders of magnitude lower than those of endogenous carnitine, the disposition kinetic data obtained in this study should reflect the behavior of endogenous carnitine.

L-Carnitine is known to be converted to acylcarnitines in tissues (Rebouche and Paulson, 1986; Hokland, 1988). After an i.v. injection of L-[3H]carnitine, the fraction of unchanged

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Mice</th>
<th>JVS Mice</th>
</tr>
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<tbody>
<tr>
<td>AUC infinity (ng·min/ml)</td>
<td>101.8 ± 13.1</td>
<td>37.3 ± 1.2²</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>3220 ± 830</td>
<td>164 ± 15²</td>
</tr>
<tr>
<td>Vdss (liter/kg)</td>
<td>8.16 ± 2.73</td>
<td>1.10 ± 0.12²</td>
</tr>
<tr>
<td>CLss (ml/min/kg)</td>
<td>2.45 ± 0.32</td>
<td>6.70 ± 0.21²</td>
</tr>
<tr>
<td>kₑ (h⁻¹)</td>
<td>0.017</td>
<td>0.198</td>
</tr>
<tr>
<td>fₑ</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Abbreviations: AUC infinity, area under plasma concentration-time curve from 0 to infinity; MRT, mean residence time from 0 to infinity; Vdss, distribution volume at steady state; CLss, plasma total clearance; kₑ, linear terminal elimination rate; fₑ, unbound fraction in plasma.

Significantly different from normal mice at ² p < .01 and ³ p < .001.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Normal Mice</th>
<th>JVS Mice</th>
</tr>
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<tbody>
<tr>
<td>Brain</td>
<td>87.2 ± 2.7</td>
<td>80.2 ± 4.2</td>
</tr>
<tr>
<td>Lung</td>
<td>87.7 ± 2.3</td>
<td>87.1 ± 3.7</td>
</tr>
<tr>
<td>Heart</td>
<td>90.6 ± 2.6</td>
<td>86.8 ± 1.3</td>
</tr>
<tr>
<td>Liver</td>
<td>99.5 ± 0.6</td>
<td>100</td>
</tr>
<tr>
<td>Kidney</td>
<td>94.2 ± 2.4</td>
<td>92.0 ± 1.4</td>
</tr>
<tr>
<td>Gut</td>
<td>93.0 ± 1.5</td>
<td>93.8 ± 0.7</td>
</tr>
<tr>
<td>Spleen</td>
<td>88.9 ± 3.4</td>
<td>89.5 ± 3.4</td>
</tr>
<tr>
<td>Muscle</td>
<td>60.2 ± 4.5</td>
<td>52.8 ± 5.7</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Normal Mice</th>
<th>JVS Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Radioactive Carnitine</td>
<td>0.38 ± 0.05</td>
<td>0.14 ± 0.15</td>
</tr>
<tr>
<td>Unchanged L-[3H]Carnitine</td>
<td>1.14 ± 0.15</td>
<td>0.211</td>
</tr>
<tr>
<td>[acetyl-3H]Carnitine</td>
<td>8.71 ± 0.60</td>
<td>29.5 ± 2.0</td>
</tr>
<tr>
<td>Kidney</td>
<td>9.82 ± 2.10</td>
<td>31.6 ± 6.74</td>
</tr>
<tr>
<td>Gut</td>
<td>4.98 ± 0.19</td>
<td>15.8 ± 0.6</td>
</tr>
<tr>
<td>Spleen</td>
<td>10.4 ± 3.5</td>
<td>31.6 ± 6.7</td>
</tr>
<tr>
<td>Muscle</td>
<td>1.05 ± 0.20</td>
<td>2.16 ± 0.41</td>
</tr>
</tbody>
</table>

N.D., not determined.
Significantly different from normal mice at ² p < .05, ³ p < .01, and ⁴ p < .001.
L-[3H]carnitine in plasma from normal mice was decreased rapidly, concomitantly with the appearance of [acetyl-3H]carnitine. The plasma concentration ratio of L-[3H]carnitine and [acetyl-3H]carnitine was about 1:2 at 1 h. The plasma concentration of unchanged L-[3H]carnitine in JVS mice decreased more slowly than that in normal mice, and the concentration ratio of unchanged L-[3H]carnitine and [acetyl-3H]carnitine was about 1:1 at 2 h after administration. Exogenously administered L-carnitine was present to the erologous gene expression technology (Berardi et al., 1995), it

1977; Martinuzzi et al., 1991; Stieger et al., 1995) and het-

erologous gene expression technology (Berardi et al., 1995), it

has been suggested that transcellular transport of L-carni-
tine involves a carrier-mediated transport mechanism. Our

findings on the specific characteristics of tissue distribution

carnitine in normal and JVS mice support the idea that a
carnitine transporter contributes to the tissue distribution

carnitine. This in vivo disposition kinetic study indicated

that JVS mice lack or have a decreased transporter function

in the whole body. However, there was only a slight differ-

ece in the distribution of unchanged L-[3H]carnitine into the

brain between the two types of mice, and the Kp, app value

was about 1. Mroczkowska et al. (1996) reported that the

uptake of carnitine by brain capillary endothelial cells is not

related to any Na\(^+\)-dependent transport process. This seems
to support our in vivo results, suggesting that no carnitine

transporter is present at the blood-brain barrier.

The higher k\textsubscript{s} value of unchanged L-[3H]carnitine in JVS mice than in normal mice was reflected by the larger CL\textsubscript{tot} and lower Vd\textsubscript{ss} values in JVS mice (Fig. 5 and Table 2). The urinary excretion of total radioactive carnitine in JVS mice was about 10-fold higher than that in normal mice (Fig. 6). In urine from JVS mice, unchanged L-[3H]carnitine and [acetyl-3H]carnitine were detected as major peaks on TLC. In urine from normal mice, radioactivity was detected at high R\textsubscript{f} values, presumably due to long-chain [acetyl-3H]carnitines but not unchanged carnitine or acetyl carnitine (Fig. 7). This indicates that normal mice reabsorbed both unchanged L-carnitine and acetyl carnitine, whereas JVS mice did not, suggesting a defect of the carnitine transporter in the kidney, as reported by Horiuichi et al. (1994).

Various values of the Michaelis constant (K\textsubscript{m}) of the high-

affinity site on the carnitine transporter have been reported in

isolated, perfused rat heart (24 \(\mu\)M) (Vary and Neely, 1982), human heart cultured cells (4.8 \(\mu\)M) (Bohmer et al., 1997), human muscle cultured cells (0.5–10 \(\mu\)M) (Martinuzzi et al., 1991), and rat kidney brush-border membrane vesicles (17.4 \(\mu\)M) (Stieger et al., 1995). In this study, the plasma concentration of endogenous L-carnitine after overnight starvation was about 17 and 16 \(\mu\)M in normal mice and JVS mice, respectively. These values are similar to the reported K\textsubscript{m} values of carnitine, implying that the operation of the carnitine transport system could be properly evaluated in our in vivo experiments.

There is an increasing number of reports dealing with
carnitine deficiency in humans. It has been suggested that

symptoms can be a consequence of acceleration of

urinary excretion, decrease in tissue uptake, and abnormality of mitochondrial function (Stanley et al., 1990; Bennett et al., 1996; Charmers et al., 1997; Rinaldo et al., 1997). Our present study on the disposition kinetics of carnitine in normal and JVS mice supports the idea that a lack or functional deficiency of the transcellular carnitine transporter system...
exists in JVS mice, as suggested previously on the basis of in vitro kinetic studies using embryonic fibroblasts derived from normal and JVS mice.

In conclusion, our results suggest that the JVS mice will be a useful experimental model in which to investigate the relationship between carnitine deficiency diseases and carnitine transport characteristics.

References


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