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PII: S0014-2999(10)00188-3
DOI: doi: 10.1016/j.ejphar.2010.02.045
Reference: EJP 66430
To appear in: European Journal of Pharmacology

Received date: 19 September 2009
Revised date: 4 February 2010
Accepted date: 24 February 2010

Please cite this article as: Schürch, Regula, Todesco, Liliane, Novakova, Katarina, Mevissen, Meike, Stieger, Bruno, Krähenbühl, Stephan, The plasma carnitine concentration regulates renal OCTN2 expression and carnitine transport in rats, European Journal of Pharmacology (2010), doi: 10.1016/j.ejphar.2010.02.045

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The plasma carnitine concentration regulates renal OCTN2 expression and carnitine transport in rats

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Abstract

Previous findings in rats and in human vegetarians suggest that the plasma carnitine concentration and/or carnitine ingestion may influence the renal reabsorption of carnitine. We tested this hypothesis in rats with secondary carnitine deficiency following treatment with N-trimethyl-hydrazine-3-propionate (THP) for 2 weeks and rats treated with excess L-carnitine for 2 weeks. Compared to untreated control rats, treatment with THP was associated with an approximately 70% decrease in plasma carnitine and with a 74% decrease in the skeletal muscle carnitine content. In contrast, treatment with L-carnitine increased plasma carnitine levels by 80% and the skeletal muscle carnitine content by 50%. Treatment with L-carnitine affected neither the activity of carnitine transport into isolated renal brush border membrane vesicles, nor renal mRNA expression of the carnitine transporter OCTN2. In contrast, in carnitine deficient rats, carnitine transport into isolated brush border membrane vesicles was increased 1.9-fold compared to untreated control rats. Similarly, renal mRNA expression of OCTN2 increased by a factor of 1.7 in carnitine deficient rats, whereas OCTN2 mRNA expression remained unchanged in gut, liver or skeletal muscle. Our study supports the hypothesis that a decrease in the carnitine plasma and/or glomerular filtrate concentration increases renal expression and activity of OCTN2.

Key words: renal carnitine reabsorption; OCTN2; secondary carnitine deficiency; N-trimethyl-hydrazine-3-propionate
1. Introduction

Carnitine is essential for the transport of activated long chain fatty acids into the mitochondrial matrix, where they can be metabolized by β-oxidation (Bremer, 1983; Fritz, 1955). Beside its role in fatty acid transport, carnitine is also important as a buffer of the CoA pool (Brass and Hoppel, 1980; Friolet et al., 1994) and for the export of potentially toxic acyl-groups from cells (Brass and Hoppel, 1980; Vernez et al., 2006). Loss of carnitine from the body is minimized by the Na\(^+\)-dependent high affinity carnitine transporter OCTN2 (SLC22A5), which is primarily responsible for reabsorption of filtered carnitine in the kidney (Nezu et al., 1999; Stieger et al., 1995; Tamai et al., 1998). In comparison to OCTN2, OCTN1 (SLC22A4), which is also expressed on the luminal side of proximal renal tubular cells (Koepsell et al., 2007), reveals lower transport activities for carnitine (Yabuuchi et al., 1999). In primary systemic carnitine deficiency (CDSP, OMIM 212140), caused by mutations in the SLC22A5 gene encoding OCTN2, proximal tubular reabsorption of carnitine is impaired, indicating that OCTN2 is the most important transporter for renal carnitine reabsorption (Nezu et al., 1999; Rahbeeni et al., 2002; Seth et al., 1999; Wang et al., 1999). Clinical findings in patients with primary carnitine deficiency, in particular myopathy, cardiomyopathy, hepatomegaly and failure to thrive, result from low tissue carnitine levels (Nezu et al., 1999; Treem et al., 1988).

Considering the physiological roles of carnitine, it is not surprising that the regulation of OCTN2 expression is related to fatty acid metabolism. Activation of PPAR alpha is associated with an increase mRNA expression of OCTN2 in liver, skeletal muscle, gut and kidney from pigs (Ringseis et al., 2008), rats (Maeda et al., 2008; Ringseis et al., 2008),
2007) and mice (Hirai et al., 2007; Koch et al., 2008; van Vlies et al., 2007). In humans, treatment with insulin is associated with an increase in carnitine transport into and expression of OCTN2 in skeletal muscle (Stephens et al., 2006). In kidney and intestine, targeting of OCTN2 to the brush border membrane is dependent on proteins with PDZ domains such as PDZK1 (Kato et al., 2005; Koepsell et al., 2007), which also affects the activity of OCTN2.

Regarding renal transport of carnitine, the plasma carnitine concentration, which represents a surrogate of the carnitine concentration in the glomerular filtrate, may influence the activity of OCTN2. Rebouche and Mack reported that the sodium-dependent transport of L-carnitine into brush border membrane vesicles from rat kidney is decreased after treatment of the rats with L-carnitine (Rebouche and Mack, 1984). In vegetarians, the plasma carnitine concentrations are reduced and renal clearance of carnitine is decreased compared to omnivores (Lombard et al., 1989), suggesting that low carnitine plasma concentrations are associated with increased renal activity of OCTN2.

The aim of the current study was to find out whether the plasma carnitine concentration can influence activity and expression of the renal OCTN2 in rats. For this purpose, tissue mRNA expression and proximal tubular function of OCTN2 were studied in rats treated with excess carnitine, rats with secondary carnitine deficiency induced by treatment with N-trimethyl-hydrazine-3-propionate (THP) and untreated control rats.
2. Materials and Methods

2.1. Reagents

L-[\(^3\)H]-carnitine hydrochloride, D-[\(^3\)H]-glucose and L-[\(^3\)H]-alanine were obtained from Amersham-Pharmacia Biotech (Little Chalfont, Buckinghamshire, England). L-carnitine was from Fluka; all other chemicals were from Sigma.

2.2. Treatment of the animals and study protocol

The study had been reviewed and accepted by the Animal Ethics Committee of the Canton of Basel-Stadt. Male, 3 weeks old Sprague Dawley rats (45±4 g) were purchased from Harlan Teklad (Horst, The Netherlands) and housed in groups of two with free access to food and water. The specific treatments were started after an acclimation period of 14 days. Control rats (Control; n=6) were treated with ground-up, commercial rat food for 2 weeks. Rats treated with carnitine (Carnitine; n=6) received 50 mg L-carnitine per 100 g body weight per day for 2 weeks via the ground-up food. Rats treated with THP (THP rats; n=6) were treated with 20 mg per 100 g body weight THP per day via the ground-up food for 2 weeks (Spaniol et al., 2001).

During the last 24 hours of the respective treatment, rats were housed in metabolic cages for urine collection. The volume was registered and a sample frozen at −70°C. At the end of the treatment period, rats were killed by CO\(_2\) narcosis followed by decapitation. Blood was obtained from the trunk into a heparinized beaker, and pieces of liver, skeletal muscle (quadriceps femoris) and gut (close to stomach) were removed and quickly frozen in liquid nitrogen. Kidneys were removed, a small piece of one
kidney was frozen in liquid nitrogen and the remainder used for the preparation of proximal tubular brush border membrane vesicles as described below.

2.3. Isolation of proximal tubular brush border membrane vesicles

Rat kidney brush border membrane vesicles were prepared by an EGTA/magnesium precipitation method as described previously in detail (Biber et al., 1981; Stieger et al., 1995). After the final centrifugation step, membrane vesicles were taken up in 400 mM Hepes/Tris, pH 7.4, dispersed using a syringe with a fine needle and used immediately for transport experiments. Protein determinations were performed by the Lowry method after protein precipitation with trichloroacetic acid (Bensadoun and Weinstein, 1976).

2.4. Transport of L-carnitine into proximal tubular membrane vesicles

Transport experiments were performed with a rapid filtration technique using cellulose nitrate filters (0.65 mm pore size from Sartorius, Göttingen, Germany) as described previously (Stieger et al., 1983). Incubations contained (final concentrations, sodium gradient) 100 mM NaCl, L-carnitine as described in the Figures and Tables, 0.2 µCi L-[³H]-carnitine, 200 mM mannitol and 20 mM Hepes/Tris, pH 7.4. Incubations containing a choline gradient were composed of 100 mM choline chloride instead of NaCl, otherwise the composition was identical with the incubations containing the sodium gradient. Transport was started by adding 15 µl of membrane suspension (approximately 10 µg protein) to 30 µl of preheated incubation medium (37°C). Since pilot studies had shown that transport was linear up to 5 minutes, the kinetic experiments were performed after an incubation time of 2 minutes. At this time point, 30
µl of the incubation suspension were withdrawn, diluted with 5 ml ice-cold stop solution (100 mM choline chloride, 1 mM L-carnitine, 200 mM mannitol and 20 mM Hepes/Tris, pH 7.4), filtered as described above and the filter rewashed twice with ice-cold stop solution. The filters were solubilized with 2ml of 1% Triton X-100 (w/v) and the radioactivity was counted after addition of 5ml scintillation fluid (Ultima-gold™, Packard Bioscience, Zürich. Switzerland).

The Na\(^{+}\)-dependent L-carnitine uptake was obtained as the difference between total carnitine uptake (carnitine uptake determined in the presence of the sodium uptake buffer) and Na\(^{+}\)-independent carnitine uptake (carnitine uptake in the presence of the choline buffer).

In pilot experiments, the transport of D-glucose (final concentration 50 µM) and L-alanine (final concentration 50 µM) into brush border membrane vesicles was determined using the same conditions as described above for L-carnitine.

2.5. Carnitine and acylcarnitines in plasma, urine and tissues

The carnitine concentrations in plasma, skeletal muscle and urine were determined radioenzymatically using the method described by Brass and Hoppel (Brass and Hoppel, 1978). Plasma and muscle samples were first treated with perchloric acid (final concentration 3%), resulting in a supernatant and a pellet. Analysis of the supernatant yielded the free carnitine concentration, and, after alkaline hydrolysis, the total acid soluble carnitine concentration. The pellet was used to determine the long-chain acylcarnitine concentration (acyl group chain length ≥10 carbons) after alkaline hydrolysis. The short-chain acylcarnitine concentration (acyl group chain length <10
carbons) was calculated as the difference between the total acid soluble and the free carnitine concentration. The total carnitine concentration was calculated as the sum of the total acid soluble and long-chain acylcarnitine concentration. The creatinine concentrations in plasma and urine were determined by the hospital-based clinical chemistry laboratory using Jaffe’s method.

2.6. Expression of OCTN2 mRNA in rat tissues and in 293-EBNA cells

From frozen tissues, total RNA was obtained using QIAGEN mini-prep columns (Qiagen, Hombrechtikon, Switzerland) as described previously (Spaniol et al., 2003). 293-EBNA cells (ATCC, Molsheim, France) were grown to confluence and then exposed to 50 or 150 µmol/l L-carnitine or THP for 8 or 24 hours. Total RNA was isolated using RNeasy® as described by the manufacturer (Qiagen, Hombrechtikon, Switzerland). The quality of the total RNA samples was analysed using Agilent’s Pico-Chips (Agilent Technologies Schweiz, Basel, Switzerland). Superscript™ II together with Oligo (dT) and Random Hexamer primers (Invitrogen, Basel, Switzerland) were used for reverse transcription of 2 µg total RNA.

The real-time PCR was carried out in a total reaction volume of 10 µl containing the TaqMan mastermix, cDNA corresponding to 10 ng total RNA as described previously (Spaniol et al., 2001). The primers and probe were: forward primer 5’-C(A/C)TATGTGTGGCCTGGCTG-3’; reverse primer 5’-AACTTGCCCACCATCACCAG-3’; probe: 5’-FAM-CTCTTCTGGGCTGGCATCGTCCTCTCT-TAMRA-3’. The reactions were carried out on an ABI Prism 7900 sequence detection system (Applied Biosystems, Rotkreuz,
Switzerland). Thermocycling conditions used were: 95°C for 10 minutes followed by 40 cycles each of 95°C for 15 seconds and 60°C for 60 seconds.

Quantification of OCTN2 tissue and cellular mRNA levels was performed with GAPDH or 18S rRNA as an endogenous control as described previously (Spaniol et al., 2001). The results are expressed relative to the values obtained in control rats or untreated cells, which were set at 100%.

2.7. Curve fitting and statistics

Kinetic parameters of the sodium-dependent transport of L-carnitine into brush boarder membrane vesicles were calculated using SigmaPlot Version 11 (Scientific Solutions, Pully Lausanne, Switzerland). The sodium-dependent part of the transport activity (v) was fitted using Michaelis-Menten kinetics

\[ v = \frac{V_{\text{max}} \times S}{K_m + S} \]

where \( V_{\text{max}} \) is the maximal velocity, \( S \) the L-carnitine concentration and \( K_m \) the Michaelis-Menten constant. Every animal was analyzed individually and the statistics were performed using the constants (\( V_{\text{max}}, K_m \)) generated from each animal. Means were compared by one-way ANOVA followed by the Student-Newman-Keuls test in case of a significant finding. The SigmaStat version 3.5 software (Scientific Solutions, Pully Lausanne, Switzerland) was used for the statistical calculations.

The renal clearances of carnitine and creatinine were determined by dividing the renal excretion of carnitine or creatinine over 24 hours by the respective plasma
concentrations. The excretion fraction of free and total carnitine was determined by dividing the respective renal clearance by the renal clearance of creatinine. Data are presented as mean±standard deviation (S.D.) if not stated otherwise.

3. Results

3.1. Characterization of the animals

At the end of the respective treatment periods, body weights were 198±19 g for control rats, 195±19 g for rats treated with L-carnitine and 185±30 g for rats treated with THP. The average food intake per rat and day was 22 g for control rats, 19 g for rats treated with L-carnitine and 18 g for rats treated with THP.

Treatment with L-carnitine was associated with an increase in the plasma concentration (reaching 82% for total carnitine) and urinary excretion of free and short-chain acylcarnitines compared to control rats (Tables 1 and 2). In comparison, treatment with THP was associated with a significant decrease of plasma free, short-chain acylcarnitine and total carnitine (reaching 70% for total carnitine) and with an increase in renal carnitine excretion. Accordingly, compared to control rats, renal clearance of carnitine was significantly increased in rats treated with THP, but not in rats treated with L-carnitine.

Similar to plasma, treatment with L-carnitine was associated with an increased skeletal muscle carnitine content (50% increase for total carnitine), whereas treatment with THP decreased the total carnitine content in skeletal muscle relative to control rats by 74% (Table 3).
3.2. Carnitine transport into rat kidney brush border membrane vesicles

As shown Fig. 1 and Table 4, treatment with L-carnitine did not affect the sodium-dependent transport of L-carnitine into brush border membrane vesicles. In contrast, treatment with THP was associated with a 1.9-fold increase in $V_{\text{max}}$, but not in the $K_m$, of L-carnitine transport into brush border membrane vesicles.

In contrast to the transport of L-carnitine, treatment with L-carnitine or THP did not affect the transport of D-glucose or L-alanine (data not shown).

3.3. OCTN2 mRNA expression in different tissues

As shown in Fig. 2, treatment with L-carnitine was not associated with a change in OCTN2 mRNA expression in gut, liver, kidney or skeletal muscle compared to control rats. In contrast, treatment with THP was associated with a 74% increase in OCTN2 mRNA expression in the kidney compared to control rats. However, treatment with THP did not affect OCTN2 mRNA expression in gut, liver or skeletal muscle compared to control rats.

3.4. OCTN2 mRNA expression in 293-EBNA cells

To investigate a possible direct effect of THP on OCTN2 mRNA expression in mammalian cells, the effect of L-carnitine and THP on OCTN2 mRNA expression was studied in cultured 293-EBNA cells, a human, proximal tubular cell line. Neither THP nor L-carnitine (both tested at 50 and 150 µmol/l for 8 and 24 hours) affected the mRNA expression of OCTN2 (data not shown).
4. Discussion

The study was designed to test the hypothesis that the plasma carnitine concentration (a surrogate of the carnitine concentration in the glomerular filtrate) can influence the activity and expression of the renal OCTN2. Our results indicate that this is indeed the case. Rats with decreased plasma carnitine concentrations due to treatment with THP revealed a higher transport activity for L-carnitine into renal brush border membrane vesicles and a higher renal OCTN2 mRNA expression than control rats. Studies in vegetarians suggest that the plasma carnitine concentration is related to renal reabsorption of carnitine. Vegetarians show a decrease in the plasma carnitine concentration compared to healthy omnivores (Lombard et al., 1989; Rebouche et al., 1993) and a decrease in renal clearance of carnitine, suggesting an increased activity of OCTN2 (Lombard et al., 1989), the renal transporter of carnitine (Nezu et al., 1999). In support of this interpretation, a study in patients treated with valproic acid has shown that the kidney increases the reabsorption of free carnitine during long-term treatment, counteracting carnitine deficiency associated with this drug (Stadler et al., 1999). Since endogenous biosynthesis of carnitine is limited (Bremer, 1983), this mechanism can be considered as a way to conserve the carnitine body stores in situations with limited exogenous supply of carnitine. Our studies are in agreement with the findings in vegetarians and indicate that in rats, renal mRNA expression and activity of OCTN2 are regulated by the carnitine concentration in plasma. Our study reveals also the possibility
that the carnitine concentration in the glomerular filtrate, which corresponds to the carnitine plasma concentration, regulates renal OCTN2 expression and activity.

Caloric restriction has recently been proposed as one of the factors responsible for increased expression of OCTN2 mRNA in rat kidney (Luci et al., 2008). In our study, all rats had free access to food, ingested approximately the same amount of food and had a similar weight gain. These findings exclude the possibility that the observed increase in renal OCTN2 expression and activity in our study is due caloric restriction. Nevertheless, beside the carnitine plasma and/or glomerular filtrate concentration, caloric restriction represents an additional mechanism for the regulation of renal OCTN2 expression.

Interestingly, mRNA expression of OCTN2 was affected by carnitine deficiency only in the kidney, but not in the other organs investigated. While the exposure to carnitine of gut and possibly also liver was most probably not different between THP treated and control rats, skeletal muscle and kidney of THP treated rats had clearly a lower carnitine exposure than control rats. These findings indicate that the regulation of OCTN2 mRNA expression is different in skeletal muscle and in kidney. In support of this statement, glucose and insulin have been shown to increase OCTN2 mRNA expression and OCTN2 activity in skeletal muscle of humans (Stephens et al., 2006), but not in human kidney cell lines (L. Todesco and S. Krähenbühl, unpublished results).

Studies by the group of Rebouche have previously suggested that renal transport of carnitine is decreased in rats (Rebouche and Mack, 1984) or human vegetarians (Rebouche et al., 1993) supplied with exogenous carnitine. In rats, Rebouche and Mack have shown that carnitine supplements decreases the transport activity for L-carnitine
into isolated proximal tubular brush border membrane vesicles (Rebouche and Mack, 1984). Our data did not confirm these findings; exogenous carnitine affected neither transport of L-carnitine nor renal expression of OCTN2. This discrepancy may be explained by methodological differences between the studies. Rebouche and Mack measured the L-carnitine transport at higher concentrations than we did (up to 500 μM compared to 50 μM in our study) and found a $K_m$ value for carnitine of 55 μM (Rebouche and Mack, 1984). This value is considerably higher than the values obtained in the current and in other investigations, which are in the range of 10 μM (Stieger et al., 1995; Tamai et al., 1998). These discrepancies make a comparison of the two studies difficult.

In human vegetarians, Rebouche et al. supplied only a limited amount of exogenous carnitine, which did not significantly affect the plasma carnitine concentration (Rebouche et al., 1993). Nevertheless, this small amount of exogenous carnitine was associated with an increase in renal carnitine clearance, suggesting that small amounts of exogenous carnitine can decrease the activity of the renal OCTN2. In the current study, carnitine supplementation was associated with an increase in the plasma carnitine concentration and renal excretion, but renal clearance of carnitine as well as renal OCTN2 mRNA expression and activity remained unchanged. The discrepancy between the studies may be explained by the amount of carnitine administered and/or by species differences.

In conclusion, we provide evidence that a decrease in the carnitine plasma concentration is associated with increased renal mRNA expression and activity of
OCTN2, whereas OCTN2 expression is not affected in other organs. Further studies are needed to find out the precise molecular mechanism responsible for these findings.

**Acknowledgements**

We thank Ms. Beatrice Vetter for performing the carnitine assays. The project was supported by a grant from the Swiss National Science Foundation to SK (310000-112483).
References


Table 1

| Plasma carnitine concentrations. Rats were treated with L-carnitine (50 mg/kg body weight for 2 weeks; Carnitine) or THP (20 mg/kg body weight for 2 weeks; THP), or were age-matched control rats (Control). At the end of the treatment period, rats were |
killed and the carnitine concentration in plasma was determined as described in Methods. Data are presented as mean±S.D., units are µmol/l.

<table>
<thead>
<tr>
<th></th>
<th>Control (n=6)</th>
<th>Carnitine (n=6)</th>
<th>THP (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free carnitine</td>
<td>22.2±4.9</td>
<td>42.0±4.3*</td>
<td>5.9±1.6&lt;sup&gt;a&lt;/sup&gt;,&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Short-chain acylcarnitine</td>
<td>39.3±17.9</td>
<td>71.0±9.0*</td>
<td>10.9±1.9&lt;sup&gt;a&lt;/sup&gt;,&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Long-chain acylcarnitine</td>
<td>2.8±0.6</td>
<td>3.7±0.8</td>
<td>2.4±0.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total carnitine</td>
<td>64.2±17.7</td>
<td>117±10*</td>
<td>19.2±3.1&lt;sup&gt;a&lt;/sup&gt;,&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
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</table>

<sup>a</sup>P<0.05 vs. control

<sup>b</sup>P<0.05 vs. carnitine
Table 2

*Urinary carnitine excretion.* Rats were treated with L-carnitine (50 mg/kg body weight for 2 weeks; Carnitine) or THP (20 mg/kg body weight for 2 weeks; THP), or were age-matched control rats. At the end of the treatment period, rats were killed and the carnitine concentration in urine as well as the creatinine concentrations in urine and plasma were determined as described in Methods. Calculations were performed as described in Methods. Data are presented as mean±S.D..

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Carnitine</th>
<th>THP</th>
</tr>
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<tbody>
<tr>
<td>Free carnitine (µmol/24 h)</td>
<td>0.36±0.21</td>
<td>0.85±0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.54±0.25</td>
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<tr>
<td>Short-chain acylcarnitine (µmol/24 h)</td>
<td>0.12±0.10</td>
<td>0.67±0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.15±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Total carnitine (µmol/24 h)</td>
<td>0.48±0.16</td>
<td>1.52±0.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.69±0.28&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Free carnitine clearance (ml/24h)</td>
<td>17.0±11.8</td>
<td>20.5±6.4</td>
<td>96.0±46.8&lt;sup&gt;a,b&lt;/sup&gt;</td>
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<tr>
<td>Total carnitine clearance (ml/24h)</td>
<td>8.6±4.2</td>
<td>13.7±5.1</td>
<td>41.6±16.6&lt;sup&gt;a,b&lt;/sup&gt;</td>
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<td>Excretion fraction free carnitine (% of filtered load)</td>
<td>0.48±0.30</td>
<td>0.61±0.20</td>
<td>2.73±1.37&lt;sup&gt;a,b&lt;/sup&gt;</td>
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<td>Excretion fraction total carnitine (% of filtered load)</td>
<td>0.25±0.11</td>
<td>0.41±0.15</td>
<td>1.18±0.46&lt;sup&gt;a,b&lt;/sup&gt;</td>
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<sup>a</sup>P<0.05 vs. control

<sup>b</sup>P<0.05 vs. carnitine
Table 3

*Skeletal muscle carnitine content.* Rats were treated with L-carnitine (50 mg/kg body weight for 2 weeks; Carnitine) or THP (20 mg/kg body weight for 2 weeks; THP), or were age-matched control rats. At the end of the treatment period, rats were killed and the carnitine content in skeletal muscle was determined as described in Methods. Data are presented as mean±S.D., units are µmol/g dry weight.

<table>
<thead>
<tr>
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<th>Control</th>
<th>Carnitine</th>
<th>THP</th>
</tr>
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<tbody>
<tr>
<td>Free carnitine</td>
<td>5.64±0.42</td>
<td>7.74±2.69</td>
<td>1.26±0.39</td>
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<tr>
<td>Short-chain acylcarnitine</td>
<td>1.80±1.10</td>
<td>4.10±2.84</td>
<td>0.52±0.20</td>
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<tr>
<td>Long-chain acylcarnitine</td>
<td>0.81±0.23</td>
<td>0.57±0.13</td>
<td>0.39±0.12</td>
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<tr>
<td>Total carnitine</td>
<td>8.25±0.83</td>
<td>12.4±3.0</td>
<td>2.17±0.23</td>
</tr>
</tbody>
</table>

*aP<0.05 vs. control
*bP<0.05 vs. carnitine*
Table 4

Kinetic constants of the sodium-dependent transport of L-carnitine into rat brush border membrane vesicles. Rats were treated with L-carnitine (50 mg/kg body weight for 2 weeks; Carnitine) or THP (2 weeks with 20 mg/kg body weight; THP) or were age-matched control rats. The sodium-dependent part of the transport activity was calculated as the difference between the transport activities in the presence of a sodium minus the activity in the presence of a choline gradient. The resulting transport activities could be described by Michaelis-Menten kinetics and were analyzed by linear regression as described in Methods. Results are presented as mean±S.D. from n=6 independent experiments.

<table>
<thead>
<tr>
<th></th>
<th>( V_{\text{max}} ) (pmol x min(^{-1}) x mg protein(^{-1}))</th>
<th>( K_m ) (µmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>69.6±20.0</td>
<td>17.8±4.5</td>
</tr>
<tr>
<td>Carnitine-treated</td>
<td>63.5±19.4</td>
<td>15.4±6.6</td>
</tr>
<tr>
<td>THP-treated</td>
<td>131±25(^{\text{a},\text{b}})</td>
<td>22.2±10.9</td>
</tr>
</tbody>
</table>

\(^{\text{a}}\)P<0.05 vs. control

\(^{\text{b}}\)P<0.05 vs. carnitine
Figure legends

Fig. 1

_Transport of L-carnitine into rat kidney brush border membrane vesicles._ Isolation of vesicles and transport experiments are described in the Method section. Activities were determined in the presence of a sodium gradient (sodium) or of a choline gradient (choline). The sodium-dependent portion of L-carnitine transport was obtained by subtraction of the transport activity in the presence of the sodium minus the choline gradient (sodium-choline). Rats treated with THP have higher sodium-dependent transport activities compared to control rats (control) or rats treated with carnitine (carnitine substitution). Numerical values are given in Table 4. Data are means±S.D. from n=6 independent measurements.

Fig. 2

OCTN2 mRNA expression in different organs. Rats were untreated controls (Control) or treated with L-carnitine (Carnitine) or with THP (THP) as described in Methods. OCTN2 mRNA expression was determined by rt PCR as described in Methods using GAPDH mRNA as a reference. With the exception of an increase in the renal OCTN2 mRNA expression in rats treated with THP, there were no differences in the organ-specific OCTN2 mRNA expression.