

# Acute Administration of Cefepime Lowers L-Carnitine Concentrations in Early Lactation Stage Rat Milk<sup>1-3</sup>

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## Abstract

Our study investigated the potential for important in vivo drug-nutrient transport interactions at the lactating mammary gland using the L-carnitine transporter substrates, cefepime and L-carnitine, as proof-of-concept. On d 4 ( $n = 6$ /treatment) and d 10 ( $n = 6$ /treatment) of lactation, rats were administered cefepime (250 mg/h) or saline by continuous i.v. infusion (4 h). Serum and milk L-carnitine and cefepime concentrations were quantified by HPLC-UV. In whole mammary gland, organic cation/carnitine transporter (OCTN1, OCTN2, OCTN3, amino acid transporter B<sup>0+</sup> (ATB<sup>0+</sup>), and L-carnitine transporter 2 expression were determined by quantitative RT-PCR and by western blot and immunohistochemistry when possible. Cefepime caused a 56% decrease in milk L-carnitine concentrations on lactation d 4 ( $P = 0.0048$ ) but did not affect milk L-carnitine at lactation d 10 or serum L-carnitine concentrations at either time. The mean L-carnitine and cefepime milk:serum ratios (M/S) decreased from  $9.1 \pm 0.4$  to  $4.9 \pm 0.6$  ( $P < 0.0001$ ) and  $0.89 \pm 0.3$  to  $0.12 \pm 0.02$  ( $P = 0.0473$ ), respectively, between d 4 and d 10 of lactation. In both groups, OCTN2 ( $P < 0.0001$ ), OCTN3 ( $P = 0.0039$ ), and ATB<sup>0+</sup> ( $P = 0.004$ ) mRNA expression and OCTN2 protein ( $P < 0.0001$ ) were higher in mammary glands at d 4 of lactation compared with d 10. Immunohistochemistry revealed OCTN1 and OCTN2 localization in the mammary alveolar epithelium and OCTN3 expression in the interstitial space and blood vessel endothelium. In conclusion, cefepime significantly decreased milk L-carnitine concentrations only at d 4 of lactation. Relative to d 10, enhanced expression of OCTN2 and ATB<sup>0+</sup> in mammary glands at d 4 of lactation and higher M/S (L-carnitine and cefepime) suggests cefepime competes with L-carnitine for L-carnitine transporters expressed in the lactating mammary gland to adversely affect L-carnitine milk concentrations and these effects depend upon lactation stage. J. Nutr. 138: 1317-1322, 2008.

## Introduction

National and international programs advocate exclusive breast-feeding for the first 6 mo of life due to the considerable socio-economic and health benefits associated with breast-feeding. However, most mothers take 1 or more medications and supplements in the postpartum period and this has raised concerns about infant safety due to their transfer through the breast milk during lactation (1). Yet the recommendations on drug/supplement use during breast-feeding (2,3) are largely guided by experience of use, observational studies, epidemiological studies, case reports, and theoretical concerns. To provide additional data to improve the recommendations, most investigations in this area today aim to identify the extent and mechanism of transfer of such substances into milk and the subsequent relative infant dose following exposure via the breast milk (1,3,4). The relative infant

dose provides critical information regarding the possibility of adverse outcomes following infant exposure to compounds present in the breast milk (5). Although these efforts represent important achievements in the area, few studies have explored the potential negative outcomes of maternal medication use on mammary gland function (i.e. drug-nutrient interactions) and subsequent consequences on milk composition.

Drugs may disrupt milk volume and composition by altering the hormonal milieu necessary to support lactation, blood flow to the mammary gland, the amount of functional mammary tissue, or through direct or indirect interference with nutrient secretion by the mammary epithelium (6). In regards to the latter mechanism, recent studies have identified the expression of numerous solute carrier and ATP-binding cassette transporter families in the mammary gland during lactation (7,8). Their substrate profiles often include both nutrient and nonnutrient elements and various drugs (4). Consequently, the breast milk concentrations of many nutrients and some drugs are likely dependent upon the normal functioning of these transporters. Furthermore, a requirement for the same transport system makes possible the potential for reversible interactions between a drug and a nutrient. At other blood-epithelial barriers, drugs

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<sup>3</sup> Supplemental Table 1 is available with the online posting of this paper at [jn.nutrition.org](http://jn.nutrition.org).

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and nutrients competing for the same transporter have resulted in reduced nutrient transport (9–11), which have sometimes necessitated a nutritional intervention (9,10). However, drug-nutrient transport interactions at the lactating mammary gland and the consequences of these interactions have received very limited attention.

The conditionally essential nutrient, L-carnitine, has an important role in mitochondrial utilization of long chain fatty acids for energy production. This micronutrient has particular importance in the developing cardiovascular and neurological systems of the nursing neonate of all mammalian species (12). L-Carnitine biosynthesis is developmentally immature in the newborn and neonates depend critically on breast milk sources (13). As a cation at physiological pH, intracellular availability of L-carnitine and its transfer across blood-epithelial barriers requires specific transport systems. Several transporters mediate L-carnitine transfer across membranes and these include the organic cation/carnitine transporters (OCTN)<sup>4</sup>1, OCTN2, and OCTN3, amino acid transporter system B<sup>0,+</sup> (ATB<sup>0,+</sup>), and the L-carnitine transporter 2 (CT2) (14–17). Substrates of these transporters also include the cationic drugs such as verapamil, pyrilamine, valproate, and the  $\beta$ -lactam antibiotics, cephaloridine, cefoselis, cefepime, and cefluprenam (18–20). In vitro studies in cell culture confirm a significant interaction between such compounds and L-carnitine for transport across cellular membranes (18–20).

As a proof-of-concept, the purpose of our study was to show an important in vivo drug-nutrient transport interaction at the lactating mammary gland between L-carnitine and a known inhibitor of L-carnitine transport, the  $\beta$ -lactam antibiotic, cefepime. We measured both milk and serum L-carnitine to identify whether an interaction would be specific to the mammary gland or whether it had systemic effects on the lactating rat. We also performed our studies at early and mid-lactation stages to determine the significance of the interaction at different lactation stages. Finally, we measured mRNA and protein expression of L-carnitine transporters in the rat mammary gland and compared the expression with the magnitude of the in vivo L-carnitine-cefepime interaction. A thorough understanding of L-carnitine transporter expression in the lactating mammary gland is critically necessary to elucidate its role in the transport of L-carnitine into milk and the toxicological potential of drug-nutrient transport interactions during the breast-feeding period.

## Materials and Methods

**Animals, diet, and chemicals.** Female Sprague-Dawley rats ordered at gestation d 17 (for assessment of the cefepime-L-carnitine interaction at lactation d 4) ( $n = 6$  per treatment) and lactation d 3 (for assessment of the cefepime-L-carnitine interaction at lactation d 10) ( $n = 6$  per treatment) were obtained from Charles River Canada and were housed singly in a temperature- and humidity-controlled facility ( $22 \pm 2^\circ\text{C}$ ) on a 12-h-light/dark cycle (0700–1900). All rats had free access to food and water throughout the study and were allowed a 7-d acclimatization period. All procedures were conducted in accordance with the Canadian Council of Animal Care guidelines for the care and use of laboratory animals and were approved by the Animal Care and Supply Committee of the University of Saskatchewan.

Throughout the acclimatization period and during the study, rats consumed ad libitum a rat diet (Prolab RMH 3000, Purina) [% composition:

protein, 22.5; fat (ether extract), 4.5; fat (acid hydrolysate), 6.4; crude fiber, 4.0; ash, 6.1; calcium, 1.0; phosphorous, 0.75] that met the nutritional requirements for lactating Sprague-Dawley rats. No dietary manipulations were otherwise conducted and all dietary conditions were the same for the control and treated groups.

We purchased cefepime from the Royal University Hospital at the University of Saskatchewan (Saskatoon, SK). RNeasy Midi kits were obtained from Qiagen. We obtained all antibodies used in this study from Alpha Diagnostic International. The peroxidase substrate kit was purchased from Vector Laboratories. L-Carnitine and other chemicals not otherwise specified were obtained from Sigma-Aldrich.

**Cefepime i.v. infusion study.** A pilot study (total of 4 rats used) was conducted to optimize the time between pup removal from the dam and initiation and duration of cefepime infusion. This was necessary to ensure sufficient milk volume collection (particularly at 0 time) for analysis but to avoid dilution effects due to excessive milk accumulation in the mammary gland and intraday variation in L-carnitine concentrations. One day prior to the infusion study, the right jugular vein of each lactating dam was surgically cannulated (silastic tubing, 0.64-mm i.d.  $\times$  1.19-mm o.d., Dow Corning) under isoflurane anesthesia. On lactation d 4 or 10, rat pups were removed from the dams 2 h before dosing. Cefepime (250 mg/h) or saline (control) was administered by continuous i.v. infusion (4 h) via the jugular catheter following an i.v. loading dose (50 mg). We drew blood samples just prior to initiation and termination of the infusion. Milk samples were collected by manual expression of the mammary gland under light isoflurane anesthesia. All samples were stored frozen ( $-20^\circ\text{C}$ ) until analysis. Following termination of the infusion, dams were humanely killed (by isoflurane overdose and thoracic cavity/cardiac severance) and mammary glands were rapidly excised. Representative sections of whole mammary gland were stored in RNAlater (Ambion), 10% paraformaldehyde, or flash frozen in liquid nitrogen and stored at  $-80^\circ\text{C}$  until further processing.

**L-Carnitine analysis.** Milk and serum L-carnitine were quantified by HPLC-UV with precolumn derivatization according to Feng et al. (21) but modified to allow a 20- $\mu\text{L}$  milk or serum sample (instead of 100  $\mu\text{L}$ ). The HPLC system was the same as described below for cefepime analysis. The standard curve range was 2.5–40  $\mu\text{mol/L}$  and the limit of detection was 0.16  $\mu\text{mol/L}$ . Intra- and interassay accuracy and precision ranged from 6 to 14%.

**Cefepime analysis.** Milk and serum cefepime concentrations were measured with HPLC-UV. In screw-top glass culture tubes, 20  $\mu\text{L}$  of rat plasma or milk was added with 180  $\mu\text{L}$  acetonitrile:methanol (9:1) and mixed well. The mixtures were vortex-mixed (5 min) and centrifuged at 10,000  $\times$  g; 20 min. The supernatant was transferred to HPLC vials and 10  $\mu\text{L}$  was injected onto the cyano column (HyperClone 5  $\mu\text{m}$ , 250  $\times$  4.6 mm, Phenomenex) with methanol:double distilled water (1:1) as the mobile phase delivered at 1 mL/min. The UV wavelength used for detection was 260 nm. The standards were prepared in male rat serum as described above. The standard curve ranged from 3.1 to 100 mg/L and the limit of detection was 0.20 mg/L. Intra- and interassay accuracy and precision ranged from 5 to 14%. Quality control samples at 3 different concentrations performed in duplicate were assessed as acceptance criteria for individual HPLC analyses.

**Observed milk:serum ratios.** We determined observed milk:serum ratios (M/S) of L-carnitine and cefepime from the ratio of the serum and milk concentrations (C) as follows:

$$\frac{M}{S} = \frac{C_{\text{Milk}}}{C_{\text{Serum}}} \quad (\text{Eq. 1})$$

The mean M/S for L-carnitine was calculated for each lactation day by using the serum and milk concentrations determined in both control and treated groups before the start of the saline (control) or cefepime (treated) infusion (i.e. the 0 h milk and serum concentrations such that  $n = 12$  for L-carnitine M/S determination for each lactation day). For cefepime, the M/S was calculated in treated rats using the ratio of the milk and serum concentrations of cefepime determined at the end of the 4-h cefepime infusion ( $n = 6$ ).

<sup>4</sup> Abbreviations used: ATB<sup>0,+</sup>, amino acid transporter B<sup>0,+</sup>; BSA, bovine serum albumin; CT2, L-carnitine transporter 2; M/S, milk:serum concentration ratio; OCTN, organic cation/carnitine transporter; QRT-PCR, quantitative RT-PCR.

**Total mRNA isolation and quantitative RT-PCR analysis.** Total mRNA was extracted from mammary gland stored in RNeasy lysis solution using RNeasy Midi kits according to the manufacturer's instructions. RNA purity and quantity were determined spectrophotometrically by measurement at 260 nm and the OD<sub>260</sub>/OD<sub>280</sub>, respectively. Specific primers (Supplemental Table 1) for all OCTN, ATB<sup>0,+</sup>, and CT2 were designed using Primer3 software (22). We conducted quantitative RT-PCR (QRT-PCR) analysis using a QuantiTect SYBR Green RT-PCR kit (Applied Biosystems) and an Applied Biosystems 7300 Real-Time PCR system. Real-time PCR assays were optimized to give PCR efficiency between 1.9 and 2.1 and a single melt-peak corresponding to the appropriate PCR product as verified by 2% agarose gel electrophoresis. Fold differences in mRNA expression were calculated using the standard curve method for relative quantitation with expression normalized to  $\beta$ -actin. Control tissues were obtained from male Sprague-Dawley rats at a similar age as the lactating dams.

**Western blot analysis.** Proteins were extracted from 300 mg of mammary gland tissue and reference tissues (kidney and testis from male Sprague-Dawley rats). Briefly, samples were homogenized in Radio-Immuno precipitation assay buffer (50 mmol/L Tris, 150 mmol/L NaCl, 10 mmol/L EDTA, 1.0% NP-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, pH 7.4) plus Protease Inhibitor Cocktail (Sigma-Aldrich) and 1 mmol/L phenylmethanesulphonyl fluoride. The homogenates were incubated in ice for 1 h and centrifuged at 12,000  $\times$  g; 20 min at 4°C. The supernatants were collected in new glass culture tubes. Protein concentrations were estimated using Bradford Reagent (Sigma-Aldrich). Forty micrograms of protein was separated on a sodium dodecyl sulfate gel containing 10% polyacrylamide (SDS-PAGE) by standard methods and transferred onto 0.2  $\mu$ m polyvinylidene difluoride membranes with a Bio-Rad Trans-Blot (Bio-Rad Laboratories) at 25 V for 80 min. Membranes were incubated with 3% bovine serum albumin (BSA) in 1 $\times$  TBST (Tris-buffered saline with 0.1% Tween-20) for 1 h at room temperature. Primary antibody (rabbit anti-mouse OCTN IgG Affinity-pure for OCTN1, OCTN2, and OCTN3; Alpha Diagnostic International) was diluted as recommended by the manufacturer (1:1000) in 3% BSA in 1 $\times$  TBST. Membranes were blotted in the primary antibody solution overnight at 4°C with constant shaking. The membrane was then washed in 1 $\times$  TBST for 20 min 4 times at room temperature. Membranes were incubated with product labeled as (H+L)-peroxidase-conjugated affinity-pure goat anti-rabbit IgG (1:5000 in 3% BSA in 1 $\times$  TBST; Alpha Diagnostic International) for 1 h at room temperature followed by washing in 3% BSA in 1 $\times$  TBST for 1 h. After 4 washings with 1 $\times$  TBST for 20 min at room temperature, the membrane was then transferred to the ECL Western Blotting Detection Reagents and Analysis system (Amersham Biosciences) for 1 min. The solution was removed and the membrane was then exposed to film (Kodak BioMax XAR) for 5 min. The density of each band was measured by using the ImageJ program (23). The specificity of each antibody was determined by overnight incubation of primary antibody with control/blocking peptide (Alpha Diagnostic International) before application to the membrane.

**Immunohistochemistry.** Kidneys and mammary glands from lactating dams and testis from male rats were fixed with 4% paraformaldehyde and paraffin-embedded sections (5  $\mu$ m) prepared using standard procedures. Sections were incubated with OCTN antibodies (1:100) (Alpha Diagnostic International) for 1 h and then (H+L)-peroxidase-conjugated affinity-pure goat anti-rabbit IgG (1:500) for 30 min. Immunoreactions were visualized by using VIP Substrate kit for peroxidase (Vector Laboratories) according to the manufacturer's instructions. We tested nonspecific binding by using premixed control peptide with the respective OCTN antibodies. For negative controls, only secondary antibody was applied to the slides.

**Statistical analysis.** All data are means  $\pm$  SEM. Two-way ANOVA (day  $\times$  treatment) was used to assess differences in milk and serum L-carnitine concentrations. When a main effect or the interaction was significant, a Tukey's studentized range test (honestly significant difference) was used for post hoc comparisons. Differences in L-carnitine M/S at d 4 and 10 of lactation were analyzed with an unpaired *t* test and

cefepime M/S was analyzed using an unpaired *t* test with Welch's correction. Differences in individual L-carnitine transporter expression between d 4 and 10 of lactation were analyzed with an unpaired *t* test. We used a paired *t* test to assess differences in the milk or serum concentrations of L-carnitine at the initiation and termination of the 4-h saline infusion (control rats) within a specific lactation stage. The significance level was  $\alpha = 0.05$ .

## Results

**In vivo cefepime-L-carnitine interaction study.** In control rats, milk L-carnitine concentrations were  $\sim$ 100% higher ( $P = 0.0083$ ) at lactation d 4 than at d 10 and serum L-carnitine concentrations did not differ between the 2 lactation stages (Table 1). Milk and serum L-carnitine concentrations in control rats did not change between the initiation and the termination of the 4-h infusion (data not shown). Cefepime caused a 56% decrease in L-carnitine milk concentrations at d 4 of lactation ( $P = 0.0048$ ) but no change at d 10 (Table 1). At both d 4 and 10 of lactation cefepime administration did not alter serum L-carnitine concentrations (Table 1). In control rats, L-carnitine M/S at d 4 of lactation was  $9.1 \pm 0.4$ , which was higher than the M/S of  $4.9 \pm 0.6$  at d 10 of lactation ( $P < 0.0001$ ). Cefepime M/S was also higher in treated rats at d 4 of lactation with an M/S of  $0.89 \pm 0.3$  compared with  $0.12 \pm 0.02$  at d 10 of lactation ( $P = 0.0473$ ).

**Expression of L-carnitine transporters.** QRT-PCR analysis of OCTN1, OCTN2, OCTN3, ATB<sup>0,+</sup>, and CT2 in whole mammary gland of control and treated rats at lactation d 4 and 10 revealed that OCTN1 and CT2 expression were similar at both lactation stages (Table 2). However, expression of OCTN2, OCTN3, and ATB<sup>0,+</sup> in whole mammary gland was significantly higher at lactation d 4 than at d 10 ( $P < 0.05$ ) (Table 2).

Western blot analysis of the OCTN transporters in control and treated rat whole mammary gland revealed no changes in OCTN1 and OCTN3 expression between lactation d 4 and 10 (Fig. 1). However, OCTN2 protein in whole mammary gland was  $\sim$ 100% higher at lactation d 4 compared with d 10 (Fig. 1).

Immunohistochemical analysis showed that OCTN1 was mainly localized on the blood vessel endothelium as well as the secretory alveolar apical membranes in the mammary gland (Fig. 2). However, slight staining for OCTN1 was also observed on the secretory alveolar basolateral membranes in the mammary gland. OCTN2 expression was observed in the lactating mammary gland, but we could not definitively determine the localization of OCTN2 on the apical or basolateral membranes of the secretory alveoli of the mammary gland (Fig. 2). OCTN3

**TABLE 1** Serum and milk free L-carnitine concentrations in rats administered saline (control) or cefepime (treated) (250 mg/h with a 50-mg loading dose) i.v. for 4 h at d 4 or 10 of lactation<sup>1</sup>

Lactation day	Serum		Milk	
	Control	Treated	Control	Treated
	$\mu\text{mol/L}$			
4	30.5 $\pm$ 1.7	23.8 $\pm$ 2.6	211 $\pm$ 29	91.6 $\pm$ 16*
10	27.4 $\pm$ 3.1	24.1 $\pm$ 2.2	111 $\pm$ 9.4 <sup>#</sup>	107 $\pm$ 10

<sup>1</sup> Values are means  $\pm$  SEM,  $n = 6$ . \*Different from control,  $P = 0.0048$ ; <sup>#</sup>different from d 4,  $P = 0.0083$ . For serum data, time, treatment, and their interaction were not significant.

**TABLE 2** mRNA expression of L-carnitine transporters (OCTN1, OCTN2, OCTN3, ATB<sup>0,+</sup>, and CT2) in Sprague-Dawley rat whole mammary gland at d 4 and 10 of lactation<sup>1</sup>

Lactation day	OCTN1	OCTN2	OCTN3	ATB <sup>0,+</sup>	CT2
4	7.0 ± 1.0	8.1 ± 0.9	4.3 ± 0.5	16 ± 3.5	0.21 ± 0.04
10	8.5 ± 2.2	3.4 ± 0.3*	2.5 ± 0.3*	1.2 ± 0.2*	0.30 ± 0.05

<sup>1</sup> Values are means ± SEM, *n* = 12 control and treated rats. \*Different from d 4, *P* < 0.004.

was highly expressed in the interstitial space as well as in the blood vessel endothelium (Fig. 2).

## Discussion

The risk of nutrient deficiencies in the infant following drug- or toxicant-induced changes in breast milk composition has received limited attention (24–26). Yet, breast milk concentrations of many solutes (i.e. nutrients, drugs) (27) depend upon the activity of a variety of solute carrier and ATP-binding cassette transporters expressed at the lactating mammary epithelium (7,28). Although adverse drug-nutrient transport interactions are known to occur at other epithelial barriers (i.e. gastrointestinal tract, kidney, liver, blood-brain barrier) (10,11), our unawareness of the potential for such interactions at the lactating mammary epithelial barrier represents a fundamental knowledge gap. Animal studies have substantiated the impact of unbalanced nutrition on epigenetic and metabolic programming of biochemical and physiological pathways of the infant, particularly during critical developmental windows (29–32). Any maternal factor (i.e. disease, diet, exposure to xenobiotics) (25,26,33,34) that adversely alters breast milk volume and/or composition could have serious consequences on infant development because of essential nutrient deficiencies in the mother's milk (31,35–37).

In this study, we focused on the conditionally essential nutrient, L-carnitine, because newborns depend almost exclusively on maternal milk for their L-carnitine requirements (38–40). Also, L-carnitine is actively transported into breast milk (28,41), resulting in high milk concentrations relative to maternal blood concentrations, particularly in the first few months of lactation (7,13,28,41–44).

In our study, L-carnitine and cefepime M/S were higher at d 4 of lactation, which is consistent with data reported in the literature (13,28,41–44). Furthermore, cefepime significantly decreased the milk concentration of L-carnitine at d 4, but not d 10, of lactation. The higher M/S and the ability of cefepime to affect L-carnitine milk concentrations at d 4 of lactation may relate to the observed changes in the expression levels of the major L-carnitine transporters in the mammary gland with stage

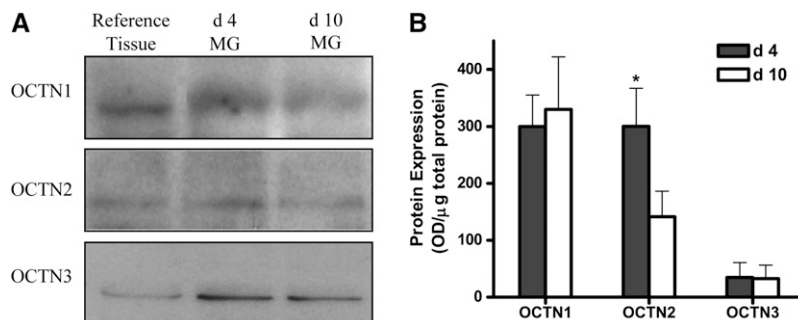
of lactation (28,41,44). Although OCTN1 and CT2 mRNA expression remained constant, OCTN2, OCTN3, and ATB<sup>0,+</sup> expression in the mammary gland was greater at d 4 of lactation than at d 10. In human mammary gland epithelia cell cultures (MCF12A), hOCTN2 and ATB<sup>0,+</sup> principally mediate the active uptake of L-carnitine into cells (44). Therefore, the diminished expression of OCTN2 and ATB<sup>0,+</sup> in lactation d 10 mammary glands may explain the reductions in L-carnitine and cefepime M/S and the inability of cefepime to alter L-carnitine milk concentrations at d 10 of lactation. These conclusions are supported by the immunohistochemical localization of OCTN1 and OCTN2 in the rat mammary alveolar epithelium (Fig. 2), which is consistent with expression reported in human mammary gland (44).

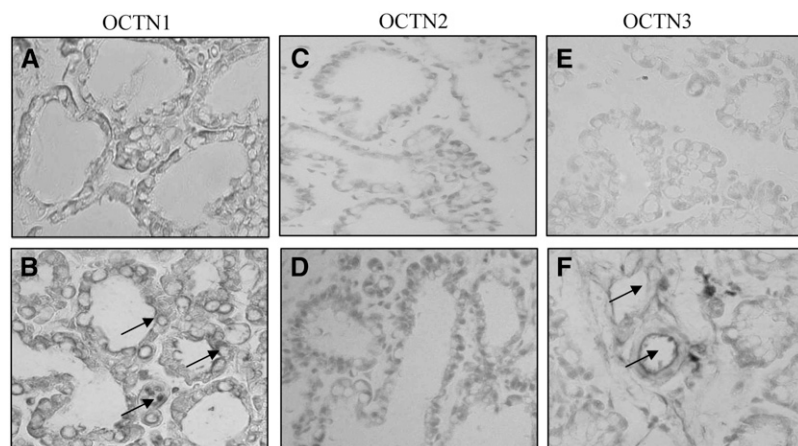
Interestingly, we observed strong expression of OCTN3, a high affinity L-carnitine transporter with known limited tissue distribution (i.e. kidney, intestine, and testis) (45,16,46), in blood vessel endothelium and in the interstitium of the mammary gland. OCTN3 has expression in peroxisomes (46,47), where it participates in peroxisomal  $\beta$ -oxidation by transporting short-chain fatty acids out of the peroxisome (48). OCTN3's role in the rat mammary gland is not clear but may have importance in overall fatty acid oxidation and cellular energy production (49,50).

Although the short cefepime infusion (4 h) caused significant changes in milk L-carnitine concentrations at d 4 of lactation, maternal systemic (i.e. blood) concentrations remained unchanged. Inhibition of active renal reabsorption of L-carnitine causes excessive L-carnitine loss (32) and long-term treatment with L-carnitine transporter inhibitors (i.e. valproic acid) may lead to secondary L-carnitine deficiencies (51). Our data suggest the short-term exposure to cefepime did not cause a significant acute interaction with the renal tubular L-carnitine transporters. However, with chronic administration (the typical therapeutic situation), sustained cefepime-L-carnitine transport interactions at both the lactating mammary and renal tubular epithelia could result in more pronounced reductions in L-carnitine concentrations in breast milk, which would further compromise the nursing infant's L-carnitine status.

When placed within the context of infant exposure risk during breast-feeding, cefepime administration to the mother during early lactation may pose a greater risk to the nursing infant than cefepime use later in lactation. According to our data, cefepime would affect milk L-carnitine concentrations early in lactation to reduce L-carnitine availability to the nursing infant (nutritional deficiency). At the same time, higher milk concentrations of cefepime in early lactation would enhance cefepime exposures in the nursing infant (pharmacological toxicity). This exposure is further compounded by the immaturity of the infant's drug elimination mechanisms in early postnatal life (52). Inefficient elimination of cefepime in the developing infant can result in

**FIGURE 1** (A) Western blot analysis of OCTN transporters isolated from reference tissue and mammary gland (MG) at lactation d 4 and 10 combining both control and cefepime-treated (250 mg/h for 4 h with a 50-mg loading dose) dams. (B) Western blot analysis of OCTN transporter expression (OD/ $\mu$ g total protein) in mammary gland at lactation d 4 and 10. Values are means ± SEM, *n* = 12. \*Different from lactation d 10, *P* < 0.05.





**FIGURE 2** Localization of OCTN1, OCTN2, and OCTN3 proteins in the lactating mammary gland of a lactation d 4 control rat by immunohistochemistry. (A, C, E) OCTN antibody (dilution 1:100) preincubated with the appropriate OCTN control peptide (100 mg/L). (B, D, F) OCTN antibody (dilution 1:100); magnification 400 $\times$ . OCTN1 (A, B) was mainly localized on the blood vessel endothelial cell membrane as well as the secretory alveoli apical membrane in the mammary gland (as shown by the arrow in B). However, slight staining for OCTN1 was also found in the secretory alveoli basolateral membranes. OCTN2 (C, D) was localized to the secretory alveolar membranes of the mammary gland. However, the tissue sections failed to distinctively identify whether OCTN2 had apical or basolateral expression. OCTN3 (E, F) was highly expressed in the interstitial space as well as on blood vessel endothelium (as indicated by the arrow in F).

cefepime accumulation to potentially toxic levels and interference with endogenous L-carnitine transport function in the nursing infant. Lower L-carnitine coupled with higher cefepime milk concentrations in early lactation may have a considerable influence on the nursing infant's L-carnitine status. Hence, lactation stage can be a critical factor when evaluating infant exposure risks after maternal medication use during breast-feeding.

How cefepime-mediated reductions in L-carnitine milk concentrations influence infant development remains unknown and warrants investigation. Such investigations are particularly relevant, because research draws an ever-compelling association between early life nutrition and the origin of diseases like cardiovascular disease, obesity, and type II diabetes (31,32,53). Through regulation of gene expression, dietary factors can alter disease initiation or progression. Even short-lived nutritional imbalances have lasting influences on health when such stimuli occur at critical developmental windows (54). Studies have shown that reduced L-carnitine availability can alter gene expression and activity of key proteins involved in long-chain fatty acid metabolism in rats (55). In human infants < 4 mo of age, lack of dietary L-carnitine affects lipid metabolism and FFA concentrations (56). However, dietary supplementation with L-carnitine corrects impairment in fatty acid oxidation in patients with L-carnitine deficiency due to defects in OCTN2 function (57). Interestingly, individuals with L-carnitine deficiency can remain asymptomatic for several years before overt clinical signs occur (58). Yet, early identification of L-carnitine deficiency may be critical, because infantile-onset cardiomyopathy, as a result of L-carnitine deficiency due to an OCTN2 transporter defect, is preventable by dietary L-carnitine supplementation (58). Because the postnatal period represents a vulnerable stage of development, any factor affecting milk concentrations of L-carnitine and subsequent L-carnitine status of the infant could have important health consequences on the developing individual (31,36,37).

In summary, our study provided proof-of-concept for the ability of a drug to adversely affect the micronutrient composition of breast milk through possible competitive interactions with nutrient transporters in the lactating mammary gland. Cefepime's ability to reduce milk concentrations of L-carnitine at one stage of lactation (i.e. d 4) but not at another (i.e. d 10) emphasizes the importance of examining competitive drug-nutrient transporter interactions at different lactation stages. Future studies should investigate other relevant drug-nutrient transport interactions at the lactating mammary gland and the health consequences of such interactions on the breast-feeding

mother-infant dyad. Eventually, such research may identify occasions when nutritional interventions are required to overcome nutrient deficiencies in the breast-feeding mother-infant dyad imposed by an interacting maternal medication.

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