The Correlation between Plasma L-carnitine and Growth Performance in Suckling Pigs in Respect of mRNA Expression of a Carnitine Transporter, OCTN2, in Lactating Porcine Mammary Tissues

Boonrit Thongsong[1*]   Sarinee Kalandakanond-Thongsong[2]

Abstract

Two objectives of this study were to investigate the weekly change in plasma L-carnitine concentration during the 28 day suckling period and to determine the relative mRNA expression level of a L-carnitine transporter, OCTN2, in porcine mammary tissue at early and peak lactation. The results showed that the L-carnitine concentration of piglets after giving birth was 3.94±0.13 mg/L. L-carnitine levels on day 7 were significantly decreased ($p<0.05$) compared to those on the 1st day. On day 14, L-carnitine level increased significantly ($p<0.05$) to the concentration of 3.99±0.55 mg/L and maintained this level until weaning day. As with the increase in plasma L-carnitine concentration 1 week following suckling age, the body weight also increased significantly ($p<0.05$). The linear correlation of suckling ages and L-carnitine levels has a significant and positive correlation ($R^2_{Adj}=0.1328$, $p<0.05$). The mRNA expression of OCTN2 found in these lactating sow mammary tissues showed the relative abundance of this carnitine transporter. It tended to be decreased in lactating mammary tissue of sows at their peak compared to those at early lactation ($p=0.065$). Therefore, the significant change or maintenance in plasma L-carnitine concentration and body weight gain during suckling period provided an insight into the effects of L-carnitine in suckling piglets. Additionally, the mRNA expression of OCTN2 in the lactating mammary cells will be further investigated in cellular and molecular regulatory mechanisms to transport L-carnitine from dams to their piglets via the mammary glands.

[1]Department of Animal Husbandry, [2]Department of Veterinary Physiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, THAILAND 10330.

[*]Corresponding author; E-mail : Boonrit.T@chula.ac.th

บทคัดย่อ

สหสัมพันธ์ระหว่างแอล-คาร์นิทีนในพลาสม่าและสมรรถนะการเติบโตในลูกสุกรระยะกินนม สัมพันธ์กับการแสดงออกของจีนตัวขนส่งแอล-คาร์นิทีน (โอซีทีเอ็น-สอง) ในเนื้อเยื่อเต้านมสุกรระยะให้นม

บุญฤทธิ์ ทองทรง[1*] สฤณี กลันทกานนท์-ทองทรง[2]

    การทดลองครั้งนี้มี 2 วัตถุประสงค์ ได้แก่ เพื่อศึกษาการเปลี่ยนแปลงระดับความเข้มข้นของแอล-คาร์นิทีน ในพลาสม่าทุกสัปดาห์ในช่วงอายุของลูกสุกรระยะกินนมจากแม่สุกร (28 วัน) และเปรียบเทียบปริมาณการแสดงออกของจีนตัวขนส่งแอล-คาร์นิทีน ที่มีชื่อว่าโอซีทีเอ็น-สอง ในเนื้อเยื่อเต้านมของแม่สุกรระยะให้นมช่วงต้นและช่วงสูงสุดของการให้น้ำนม ผลการศึกษาพบ ค่าความเข้มข้นของแอล-คาร์นิทีนในลูกสุกรหลังคลอด (อายุ 1 วัน) เท่ากับ 3.94±0.13 มก.ต่อล. ซึ่งลดลงอย่างมีนัยสำคัญทางสถิติเมื่อลูกสุกรอายุ 7 วัน (1.75±0.61 มก.ต่อล.) ส่วนหลังจากที่ลูกสุกรอายุ 14 วัน (3.99±0.55) 21 วัน (4.44±0.50) และ 28 วัน (3.78±0.86 มก.ต่อล.) ค่าความเข้มข้นของแอล-คาร์นิทีน เพิ่มสูงขึ้นอย่างมีนัยสำคัญทางสถิติและคงระดับความเข้มข้นไว้ เมื่อเปรียบเทียบกับที่อายุ 7 วัน ซึ่งการเพิ่มขึ้นของระดับแอล-คาร์นิทีนหลังจากอายุ 7 วัน มีความสัมพันธ์กับน้ำหนักตัวที่เพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ เกิดความสัมพันธ์เชิงเส้นในทางบวกและมีค่านัยสำคัญทางสถิติระหว่างอายุกับค่าความเข้มข้นของแอล-คาร์นิทีนของลูกสุกร ส่วนผลการแสดงออกของจีนตัวขนส่งแอล-คาร์นิทีน (โอซีทีเอ็น-สอง) สามารถพบได้ในเนื้อเยื่อเต้านมของแม่สุกรระยะให้นม ซึ่งเมื่อเปรียบเทียบปริมาณการแสดงออกของจีนดังกล่าวในระยะให้นมช่วงต้นกับช่วงสูงสุดของการให้น้ำนม พบว่ามีแนวโน้มลดลง ดังนั้นการเปลี่ยนแปลงหรือการรักษาสภาวะความเข้มข้นของแอล-คาร์นิทีนและน้ำหนักตัวใน ช่วงระยะลูกสุกรกินนม ให้มุมมองเพื่อความเข้าใจประการหนึ่งถึงผลของแอล-คาร์นิทีนในลูกสุกรระยะดังกล่าว นอกจากนี้ผลการแสดงออกของจีนตัวขนส่งแอล-คาร์นิทีนในเนื้อเยื่อเต้านมจะนำไปสู่การศึกษากลไกการควบคุมทั้งในระดับเซลล์และอณูชีวโมเลกุลการขนส่งแอล-คาร์นิทีนจากแม่ไปสู่ลูกสุกรผ่านทางเต้านม

คำสำคัญ : แอล-คาร์นิทีน เนื้อเยื่อเต้านม การแสดงออกของจีน โอซีทีเอ็น-สอง ลูกสุกร

[1]ภาควิชาสัตวบาล   [2]ภาควิชาสรีรวิทยา คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ปทุมวัน กรุงเทพฯ 10330
*ผู้รับผิดชอบบทความ

Introduction

    The physiological significance of L-carnitine as a nutritional supplement has been reviewed and investigated because L-carnitine has led to increased piglet vitality at birth and improved not only growth performance during the suckling period (Ramanau et al., 2004; 2005) but also reproductive performance of sows (Eder et al., 2001). Carnitine (β-hydroxy-γ-4-n-trimethylaminobutyrate) is an amino acid derivative which is synthesized from lysine and methionine as precursors *in vivo*. It is necessary for the transfer of fatty acyl groups across the mitochondrial membrane into the mitochondrial matrix for beta-oxidation metabolism and subsequent production of adenosine triphosphate (ATP). The natural carnitine is the L-form (Robouche and Seim, 1998). Immediately after birth, L-carnitine plays a crucial role in energy production. The oxidation of fatty acids becomes an important source of energy for the neonatal energy homeostasis to maintain body temperature and for other metabolic processes. Thus the provision of acetyl units is essential in the neonate and is required for the synthesis of optimal hepatic ketone synthesis as an alternative energy source in developing the brain (Shennan et al., 1998). The early postnatal growth of piglets depends on their intake

of nutrients with colostrums and milk. Generally, sow's colostrums and milk are good sources of carnitine (Kerner et al., 1984) for suckling pigs because the capacity of the neonate to synthesize L-carnitine is very low during the early stages of lactation (Hahn, 1982). Adequate L-carnitine levels are critically important for the survival of newborn piglets and neonates and particularly, piglets born in non-optimal environment or with other injuries. In neonatal piglets, L-carnitine deficiency may result in more risk of immaturity of L-carnitine biosynthesis pathways or a lack of sufficient mammary transport.

During lactation, the mammary gland cell is differentiated into a highly active secretory tissue. Three carnitine transporters belong to the organic cation transport (OCT) family and have been identified as organic cationic/carnitine transporters (OCTN); OCTN1, OCTN2, and OCTN3. The OCTN2 is a unique transporter with dual modes of transport; one as a sodium-independent OCT and another in a sodium-dependent manner with high affinity carnitine (Wu et al., 1999). It is the agreement with carnitine transport in lactating rat mammary tissues that demonstrates sodium-dependent component (Shennan et al., 1998). In the MCF 12A human mammary gland epithelial cell line, Kwok et al (2006) reported that sodium-dependent uptake of carnitine is mediated at least in part by hOCTN2. Moreover, a congenital defect of the hOCTN2 gene causes primary systemic carnitine deficiency (SCD) known as autosomal recessive disease (Nezu et al., 1999). This lethal metabolic condition is associated with clinical responses such as cardiomyopathy, muscular weakness, hypoglycaemia and sudden death (Lahjouji et al., 2001). Therefore, this suggests an important role of OCTN2 transporter in the mammary gland.

Before the supplementation of L-carnitine in a neonatal diet, the carnitine status of neonatal piglets as to whether it could be conceivably sufficient to utilize the long-chain fatty acids as metabolic fuel to enhance growth performance because dietary carnitine is necessary to maintain normal carnitine concentration in the newborn (Borum, 1983). There are a few studies of the plasma L-carnitine concentration in neonatal piglets in conjunction with the observation of body weight changes during the 4 weeks of life. In order to assess carnitine concentration of piglets during the suckling period that receive carnitine from colostrum and milk as well as endogenous carnitine synthesis, we planned to raise the piglets with their sows but without feeding their a neonatal diet or creep feed. In addition, especially in lactating sows, no publication has described any carnitine transporters in these mammary cells, with particular attention to the OCTN2 gene. The expression of this gene may reflect an endogenous function in the formation and modulation of mammary tissue and milk composition. Therefore, the two purposes of the present study were to determine. Firstly, the weekly change in plasma L-carnitine concentration of the piglets correlated with body weight change over a 28 day suckling period and secondly, to investigate not only the mRNA expression of L-carnitine transporter OCTN2 in lactating porcine mammary tissue but also whether the expression levels of this gene in the mammary tissue of lactating sows are affected by the stages of lactation in view of the large changes in milk production that occur during early and peak lactation.

Materials and Method

Animals, diet and feeding: Six multiparous crossbred lactating sows (Landrace X Large White) were used in this study. Sows were moved to the farrowing crates 2 weeks prior to farrow and kept individually in farrowing crates. After farrowing (farrowing day = day 0), the piglets were cross-fostered as soon as possible after birth to achieve an equal number of piglets per sow. All sows were fed with commercially available sow diet for 5-6 kg/day during the lactation period. The proximate analysis of nutrient composition in the experimental diet was 15.27% crude protein, 4.03% fat, 3.46% fibre, 5.17% ash, 1.17% calcium, 0.63% phosphorous and 10.39% moisture. These sows

and piglets had free access to drinking water at all time from nipple drinkers. Suckling piglets were raised with their dams and received sowís milk throughout lactation but did not receive any creep feed until weaning on day 28 of lactation. The experimental protocol use in this study was approved by the Animal Care Committee guidelines of Chulalongkorn University.

Data recording, Body weight: Measurements of individual piglet body weight in each litter (5 lactating sows) were recorded on the first day of age and subsequently on the 3rd day and every week after birth.

Sample collection and analyses
Feed samples: Feed samples randomly collected and concentrations of nutrients in the diet were analyzed by the proximate analysis method (AOAC, 1990). Chemical analysis was conducted on duplicate samples.

Blood samples: Blood samples from 4 randomly selected piglets per litter per sow (5 lactating sows) were taken weekly on the first day until day 28, 2-3 h after feeding on the last morning. One ml of blood per piglet was drawn by puncture of the venous plexus in the jugular fossa and put into potassium ethylendiamine tetraacetate (K-EDTA) tubes. Blood samples were centrifuged at 3000 g for 10 min at 4°C. The plasma samples were separated, pooled together in one tube and stored at -20°C pending L-carnitine analysis. The plasma L-carnitine concentrations were assayed with a L-carnitine enzymatic UV test kit (Roche Cat. No.11242008001) by spectrophotometry (Shimadzu, UV-VIS-160A) at a 340 nm wavelength. All processes for L-carnitine analyses followed manufacturer instructions. In brief, 1 ml perchloric acid solution (0.6 M) was added to 1 ml cooled plasma, mixed properly and put on ice for 10 min for deproteinization. The deproteinized plasmas were centrifuged at 3000 g for 10 min at 4°C. Subsequently, 1 ml supernatant was taken into a fresh centrifuge tube and 0.2 ml potassium carbonate solution (1.2 M) was added, mixed properly, kept on ice for 20 mins and centrifuged at 3000 g for 5 min at 4°C. Finally, 1 ml of the separated supernatant was used to determine the L-carnitine profile with a commercial enzymatic kit.

Mammary tissues: Mammary tissue was obtained by incisional biopsy using an aseptic technique from the anterior glands of the udder that were usually suckled throughout lactation. Six lactating sows on day 9 and 19 of lactation (3 sow each) were tranquilized by intramuscular injection of Azaperone (4 mg/kg body weight; Stresnil®; JANSSEN Pharmaceutical N.V., Belgium). Before the incision biopsy, the udders were locally infused with 2% Xylocaine (OLIC, Thailand) at the incision site. The incision site was made approximately midway (4 to 5 cm) between the teat and the upper line of the udder. Care was taken not to perforate blood vessels visible on the gland. Approximately 15-mm elliptical incision at a depth of 5-10 mm, mammary tissues (1-5 g) were collected and immediately placed in a RNAlater tissue protection kit® (Qiagen, Hilden, Germany) to prevent RNA degradation. The subcutaneous tissue and skin were sutured with coated Vicryl® (NSW, Australia).

Total RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR) analysis for the determination of carnitine transporter (OCTN2) expressions

Isolations of total RNA were extracted from the mammary tissue using an Aurum™ Total RNA Fatty and Fibrous Tissue Kit (BioRad, Hercules, USA) in accordance with the manufacturer's instruction. Total RNA concentration samples were verified for integrity and reverse transcribed into cDNA (complementary DNA). This reverse transcription step was performed using iScript™ Select cDNA Synthesis kit (BioRad, Hercules, USA). The synthesized cDNA products were used for RT-PCR, using IQ™ Supermix kit (BioRad, Hercules, USA) following the manufacturer's instructions. Gene sequences for the primer design were obtained from the

Table 1 Primer sequences used in region PCR amplification for detecting transcripts of porcine OCTN2, 18S rRNA, and CAT2A mRNA expression in lactating mammary tissues or liver tissues

Genes	Primer pairs 5' ⟶ 3'		GenBank Acc. no.	Amplicon size (bp)
OCTN2	CAT CTT TGT GAA CTG CTT CCT TTC	(F)	AB015050	206
	TGA CTC CAA ACT TGC CCA CCA TCA	(R)		
18S rRNA	CCG CGG TTC TAT TTT GTT GGT TTT	(F)	AF102857	399
	CGG GCC GGG TGA GGT TTC	(R)		
CAT2A	CAA GAC GGG GTC TGC ATA TT	(F)		
	TGC CAC ATT TCC TTT CAC AA	(R)	U76368	368

National Center for Biotechnology Information's GenBank. The primer pairs used in the subsequent PCR and their corresponding amplicon size are shown in Table 1. Gene sequences of OCTN2 and CAT2A were from human sequences. The PCR conditions were: initial denature at 95°C for 3 min, 3 step cycling amplifications for 40 cycles; at 94°C for 30 sec, at 58°C for 30 sec, and at 72°C for 1 min followed by stop reaction at 72°C for 10 min. The PCR products were subjected to electrophoreses to size-fractionate on a 1.8% agarose gel using a 1xTris Acetic acid EDTA (TAE) buffer. DNA bands were visualized with ethidium bromide staining. The CAT2A and 18S rRNA were used as negative controls and internal controls, respectively.

Semiquantitative reverse transcription-polymerase chain reaction for quantitative determination of carnitine transporter (OCTN2) expression

The relative abundance of a carnitine transporter mRNA was determined by semiquantitative RT-PCR using a IQ™ Supermix kit (BioRad, Hercules, USA). In multiplexed PCR reaction, 18S rRNA was used as an internal control that was amplified with OCTN2 gene. The final cDNA concentration of each sample was adjusted and the cDNA sample equalized to 1 μg/μl. Preliminary experiments were conducted to determine both the optimal amount of internal control primer and interested gene primer ratio and the number of PCR cycles that would

enable detected PCR amplification within the linear range of the PCR reaction for both groups of sows at early and peak lactation. Finally, thermal cycling was carried out as previously mentioned for 30 cycles. The intensities of both the OCTN2 and 18S rRNA band for each sample were measured by densitometry using the Scion Image software program. The relative quantitative RT-PCR expression levels of the OCTN2 gene which were normalized to the 18S rRNA were calculated and compared between two groups of sow mammary tissues at early and peak lactation. All assays were conducted in duplicate or triplicate.

Statistical analyses: Significant statistical analyses to determine the differences between age and L-carnitine levels as well as body weight changes were performed using ANOVA with a post hoc Student-Newman Keul multiple range test as a p value<0.05. The linear relationship between suckling ages and L-carnitine concentration was determined by the spearman method. The relative mRNA expressions of OCTN2/18S rRNA ration obtained from semiquantitative RT-PCR determination were analyzed by unpair t-test using the commercial computer program GraphPad Prism (Prism3).

Results

Plasma L-carnitine profile and body weight change

The results showed that the L-carnitine concentration of piglets after birth was 3.94±0.13 mg/L. Also, L-carnitine levels on the 7th day were significantly decreased ($p<0.05$) compared to those on the 1st day. On the 14th day, L-carnitine concentration increased significantly ($p<0.05$) again and maintained this until the weaning day (Fig.1). Body weight changes between birth and day 7 in piglets were not significant but the body weights on day 14 were significantly greater for the entire suckling period (day 28) than for the 1st week ($p<0.05$). Also, the increase in the plasma L-carnitine profile after 1 week of suckling was related to the body weight changes and increased at the same time (Fig. 2). To emphasize this, as is shown in Figure 3, the linear regression of suckling ages and L-carnitine levels had a significant and positive correlation ($R^2_{Adj}=0.1328$, $p<0.05$) but the coefficient of determination was rather small.

Detection of OCTN2 mRNA expression

Lactating mammary tissues of sows were analyzed by RT-PCR. According to GenBank information, the DNA product sizes of the OCTN2-specific gene and the 18S rRNA (positive control) were detected as expected with a size in the specific band of 206 and 399 base pairs, respectively. Even though CAT2A which was used as a negative control was not detected in porcine mammary tissue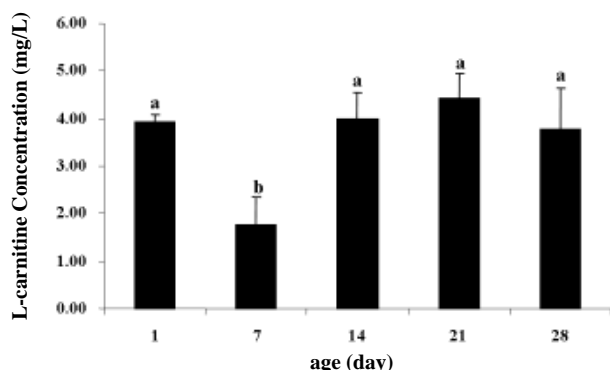s in agreement with the results reported by Laspiur et al (2004), it was expressed in the pig liver tissues as a specific band of 368 base pairs (Fig. 4).

Relative mRNA abundance of OCTN2/18S rRNA ratio in porcine mammary tissues at early and peak lactation
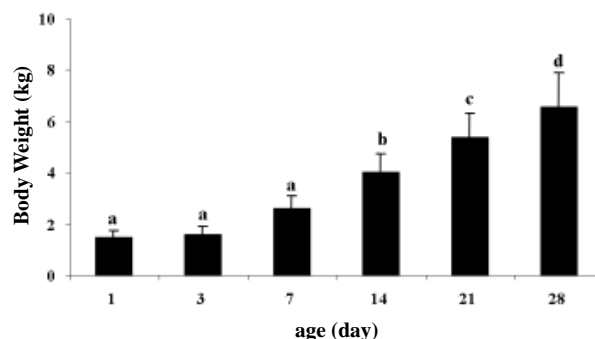
Semiquantitative RT-PCR analyses to determine the expression level of OCTN2 mRNA showed the relative mRNA abundance of this carnitine transporter (Fig 5). It tended towards the lower expression observed in lactating mammary tissues between sows at the peak rather than those at the early lactation (t(4)=1.80, $p=0.065$).

Discussion

In this study, the piglets at birth had significantly higher concentrations of total carnitine in their plasma when compared to those on day 7. This might have been due to higher concentration of total L-carnitine in sows' colostrum than in milk. This finding concurs with results of a recent study that have demonstrated that sows' colostrum concentrations of total L-carnitine and free L-carnitine were about 151 and 10.1 µmol/l, respectively (Birkenfeld et al., 2006). Consequently, the concentrations of total L-carnitine and free L-carnitine in sow's milk on day 7 were about 98 and 44.4 µmol/l, respectively (Birkenfeld et al., 2006). Another possible reason found that the high plasma total L-carnitine concentration of the piglets at birth might be due to an transplacental supply of L-carnitine to the foetuses as L-carnitine can cross the placenta (Lahjouji et al., 2004;
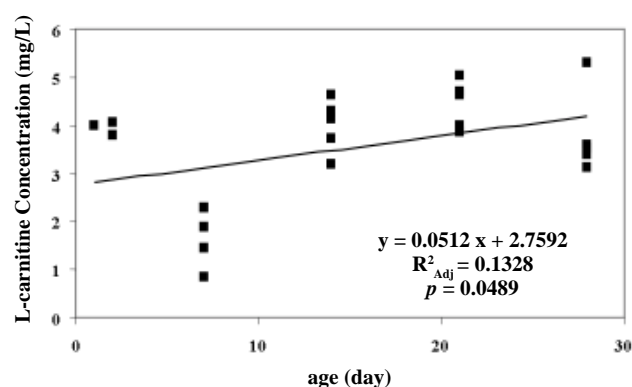
Figure 1 L-carnitine concentrations in plasma of piglets born to and suckled by normal sows on days 1, 7, 14, 21, and 28. Values are expressed as means±SD (n=5).[a,b] Means with different superscripts differ significantly ($p<0.05$).
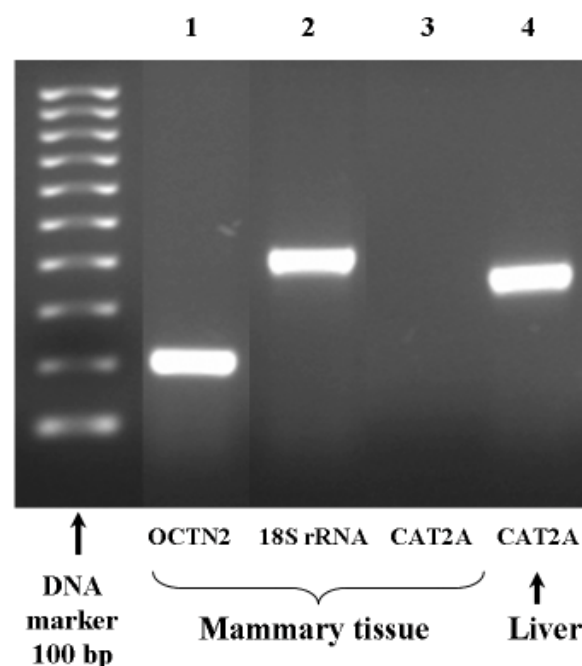
Figure 2 Body weight changes of piglets during the suckling period. Values are expressed as means±SD (n = 5).[a, b, c, d] Means with different superscripts differ significantly ($p<0.05$).
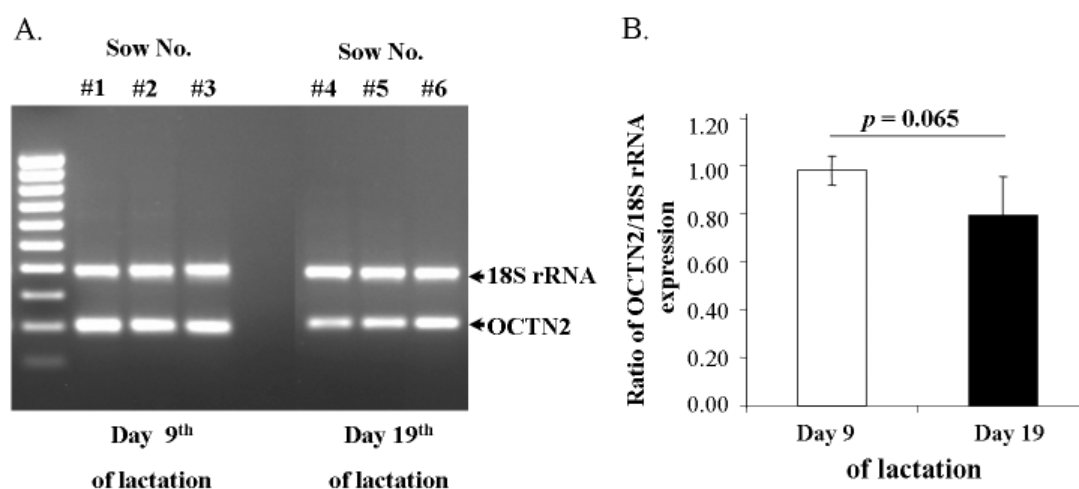
Grube et al., 2005). In addition, L-carnitine concentration, on the 14th day, increased significantly ($p < 0.05$) again at the same level found on day 1 and maintained or slightly decrease its level until the weaning day (day 28). This is in agreement with the research study of Birkenfeld et al. (2006) who demonstrated that the plasma L-carnitine concentrations of piglets on day 14 were no longer different when compared to those on day 1 as value 12.3 and 12.1 µmol/l, respectively. The provision of L-carnitine to the neonate, via milk, is very important because it is known that the endogenous synthesis of carnitine is extremely low during the first week of life, increasing thereafter (Coffey et al., 1991). This is in agreement with Seccombe et al. (1978) who reported that in newborn rats, plasma levels of carnitine increase rapidly after birth and decrease only when the pups are weaned

Figure 4 PCR amplification of sequences comprising cDNA from porcine mammary and liver tissues by RT-PCR. Amplification products of 40 cycles were subjected to electrophoresis on an agarose gel at 18 ml/well and visualized with ethidium bromide staining. The mRNA of the target gene; OCTN2 (lane 1), and the internal control gene; 18S rRNA (lane 2), a were expressed in sow mammary tissues. CAT2A was used as the negative control gene and was not expressed in sow mammary tissue (lane 3) but expressed in the liver (lane 4). The figure is representative of 3 independent experiments.

$$y = 0.0512\,x + 2.7592$$
$$R^2_{Adj} = 0.1328$$
$$p = 0.0489$$

Figure 3 Linear relationships between suckling ages and L-carnitine concentration (n=3-5)

Figure 5 The relative mRNA abundance of OCTN2 in each sow by semiquantitative RT-PCR using 18S rRNA as internal control. The gel figure (A) and bar chart (B) are representative of 3 independent experiments. Values are expressed as means±SD (n=3 in each group)

and fed on a dry diet. Consistent with L-carnitine in rat milk, it is initially high and then falls precipitously (Robles-Valdes et al., 1976). Also, neonates are dependent on external sources of carnitine. Interestingly, plasma L-carnitine concentrations after day 14 to day 28 were no longer different in piglets suckled by sows. This might be a physiological regulation of an adequate L-carnitine level for the normal growth rate. Another growth parameter of suckling pigs related to the increase in the plasma L-carnitine profile after 1 week was body weight change. The body weight changes also increased significantly ($p<0.05$) from the 14th to 28th day of the suckling period. Therefore, L-carnitine status was responsible for piglet growth during the suckling period, at least after the second week because they had higher plasma L-carnitine concentrations again on day 14. Moreover, the linear correlation of suckling ages and L-carnitine levels was significant and a positive correlation ($R^2_{Adj}=0.1328$, $p<0.05$). The decrease in L-carnitine concentration on day 7 might only pretend that a positive correlation between age and plasma concentration exists. Also, an important role of carnitine in energy production has led to considerable interest in its action on neonatal piglets. Any reduction in the ability of them to oxidize fatty acids may lead to a reduction in some metabolic processes with the increased risk of hypoglycaemia, hypothermia and distress found generally within the 1 week of piglet life. In addition, greater fatty acid oxidation leads to increased energy and heat production on the part of the piglets that might in turn have increased their suckling persistence during the first few days after birth (Birkenfeld et al., 2006).

More than 20 years ago, Kerner et al. (1984) reported that sows' colostrum and milk contain unusually high amounts of carnitine. For this reason, the supplementation of L-carnitine to suckling pigs is done to increase L-carnitine in plasma and tissue for improving growth performance. However, according to the limited research evaluating carnitine status in suckling pigs, the biosynthesis and blood levels of L-carnitine seem to be lower in neonates than in adults (Borum, 1981). This reflects a poor ability to synthesize L-carnitine at the very time when adequate levels are critically required. Carnitine is synthesized in the body but the enzymes needed to do this may be at low levels in piglets. O'Donnell

et al. (2002) found that carnitine synthesis in the neonate is limited by low levels of gamma-butyrobetaine hydroxylase.

In mammary cells, Shennan et al. (1998) reported that there are at least two components of L-carnitine uptake; a saturable Na+-dependent component and a non-saturable Na+-independent component, of approximately 20%. This study is the first report to investigate and show the mRNA expression of L-carnitine transporter (OCTN2) in lactating sow mammary tissue. Detection of carnitine transporter gene expression in the porcine lactating mammary cells is a necessary first step in the identification of this transporter protein and its function and localization. Functional studies in rat, ovine and human mammaries have postulated the presence of the carnitine transporters in the mammary cells (Shennan et al., 1998; Zammit et al., 1998; Kwok et al., 2006). The transport and provision of L-carnitine into milk is important for normal growth and the development of the suckling piglets. However, L-carnitine transport may involve transporters other than OCTN2. In human, lactating mammary epithelial cell expression of OCTN1 and OCTN2 corroborates the functional studies (Shennan et al., 1998; Zammit et al., 1998). Another transporter that might play a role in the sodium-dependent carnitine transport is the amino acid transporter $B^{0,+}$ ($ATB^{0,+}$). It functions as a low-affinity/high-capacity transporter for carnitine (Nakanishi et al., 2001). In the present study, the physiological regulation of OCTN2 gene expression from early and peak lactation showed a trend towards decreasing after early lactation. Alcorn et al. (2002) reported that OCTN2 gene expression in human mammary epithelial cells was found the down regulation of OCTN2 expression from early to late lactation cycle milk. This regulation of OCTN2 mRNA expression may be related to lactation stages. Possibly, mammary epithelial cells predominated in accounting for the apparent down-regulation of OCTN2 expression in late lactation stage. This is in agreement with result of Shennan et al. (1998) demonstrated that the activity of sodium-dependent L-carnitine transport decreases with progressing lactation and maturation of an infant's capacity to synthesize L-carnitine. For functional study, L-carnitine uptake was significantly lower in mammary tissues taken from rats during peak lactation compared

with those from early lactation (Shennan et al., 1998). In the early stages of lactation both in rats and ovines, the lactating mammary gland is able to generate a substantial transepithelial or to maintain its L-carnitine concentration gradient in milk (Snoswell and Linzell., 1975).

In conclusion, these results are able to explain or provide insight into the fact that the supplemented L-carnitine of the early-weaned piglet (2-3 weeks of age) had no effect on growth performance because they should have adequate L-carnitine concentration probably from milk and endogenous carnitine synthesis to support normal growth. The essential functions of carnitine in metabolism were realized by the endogenous carnitine synthesis (Eder, 2000). In addition, supplemental L-carnitine in early life can affect fat metabolism since L-carnitine influences the flux rate of beta-oxidation in newborn pigs (Kempen and Odle, 1995). Therefore, a factor in the variation of response to added dietary L-carnitine in neonatal piglets may be the amount of L-carnitine in milk, milk products and age of piglets. L-carnitine may be of significant nutritional benefit for very low birth weight infants and piglets (O'Donnell et al., 2002; Birkenfeld et al., 2005) although it is available in breast milk. However, some further investigation is needed to confirm L-carnitine efficacy in neonatal piglets *in vivo*. In addition, a major carnitine transporter gene, named OCTN2, has also been shown to express and has tended to be decreased by physiological change at the early to peak milking cycle in lactating porcine mammary tissues. The mRNA expression of OCTN2 may suggest a significant endogenous role for it during lactation. However, the function of this protein transporter in the sow mammary gland remains to be elucidated.

Acknowledgements

References

Alcorn, J., Lu, X., Moscow, J.A. and McNamara, P.J. 2002. Transporter gene expression in lactating and nonlactating human mammary epithelial cells using real-time reverse transcription-polymerase chain reaction. J. Pharmacol. Exp. Ther. 303: 487-496.

AOAC., 1990. Official Methods of analysis. Association of Official Analytical Chemists. 15th ed. Association of Official Analytical Chemists Inc., Washington, DC, USA.

Birkenfeld, C., Kluge, H. and Eder, K. 2006. L-carnitine supplementation of sows during pregnancy improves the suckling behavior of their offspring. Br. J. Nutr. 96: 334-342.

Birkenfeld, C., Ramanau, A., Kluge, H., Spilke, J. and Eder, K. 2005. Effect of dietary L-carnitine supplementation on growth performance of piglets from control sows or sows treated with L-carnitine during pregnancy and lactation. J. Anim. Physiol. Anim. Nutr. 89: 277-283.

Borum, P.R. 1981. Possible carnitine requirement of the newborn and the effect of genetic disease on the carnitine requirement. Nutr. Rev. 39: 385-390.

Borum, P.R. 1983. Carnitine. Ann. Rev. Nutr. 3: 233-259.

Coffey, T.M., Shireman, R.B., Herman, D.L. and Jons, E.E. 1991. Carnitine status and lipid utilization in neonatal piglets fed diets low in carnitine. J. Nutr. 121: 1047-1053.

Eder, K. 2000. L-carnitine supplementation and lipid metabolism of rats fed a hyperlipidaemic diet. J. Anim. Physiol. Anim. Nutr. 83: 132-140.

Eder, K., Ramanau, A. and Kluge, H., 2001. Effect of L-carnitine supplementation on performance parameters in gilts and sows. J. Anim. Physiol. Anim. Nutr. 85: 73-80.

Grube, M., Schwabedissen, H.M., Draber, K., Prager, D., Moritz, K.U., Linnemann, K., Fusch, C., Jedlitschky, G. and Kroemer, H.K. 2005. Expression, localization and function of the carnitine transpoter OCTN2 (slc22a2) in human placenta. Drug Metabol. Dispos. 33: 31-37.

Hahn, P. 1982. Development of lipid metabolism. Ann. Rev. Nutr. 2: 91-111.

Kempen, T.A.T.G. and Odle, J. 1995. Carnitine affects octanoate oxidation to carbon dioxide and dicarboxylic acids in colostrum-deprived piglets: in vivo analysis of mechanisms involved based on CoA- and carnitine-ester profiles. J. Nutr. 125: 238-250.

Kerner, J., Froseth, J.A., Miller, E.R. and Bieber, L.L. 1984. A study of the acylcarnitine content of sows' colostrum, milk, and newborn piglet tissues: Demonstration of high amounts of isovalerylcarnitine in colostrum and milk. J. Nutr. 114: 854-861.

Kwok, B., Yamauchi, A., Rajesan, R., Chan, L., Dhillon, U., Gao, W., Xu, H., Wang, B., Takahashi, S., Semple, J., Tamai, I., Nezu, J., Tsuji, A., Harper, P. and Ito, S. 2006. Carnitine/xenobiotics transporters in the human mammary gland epithelia, MCF12A. Am. J. Physiol. Integr. Comp. Physiol. 290: R793-R802.

Lahjouji, K., Elimrani, I., Lafond, J., Leduc, L., Qureshi, I.A. and Mitchell, G.A. 2004. L-carnitine transport in human placental brush-border membranes is mediated by the sodium-dependent organic cation transporter OCTN2. Am. J. Physiol. Cell. Physiol. 287: C263-C269.

Lahjouji, K., Mitchell, G.A. and Qureshi, I.A. 2001. Carnitine transport by organic cation transporters and systemic carnitine deficiency. Mol. Genet. Metab. 73: 287-297.

Laspiur, J.P., Burton, J.L., Weber, P.S.D., Kirkwood, R.N. and Trottier, N.L. 2004. Amino acid transporters in porcine mammary gland during lactation. J. Dairy. Sci. 87: 3235-3237.

Nakanishi, T., Hatanaka, T., Huang, W., Prasad, P.D., Leibach, F.H., Ganapathy, M.E. and Ganapathy, V. 2001. Na$^+$ - and Cl-couple active transport of carnitine by the amino acid transporter ATB$^{(0,+)}$ from mouse colon expressed in HRPE cell and Xenopus oocytes. J. Physiol. 532: 297-304.

Nezu, J., Tamai, I., Oku, A., Ohashi, R., Yabuuchi, H, Hashimoto, N., Nikaido, H., Sai, Y., Koizumi, A., Shoji, Y., Takada, G., Matsuishi, T., Yoshino, M., Kato, H., Ohura, T., Tsujimoto, G., Hayakawa, J., Shimane, M and Tsuji, A. 1999. Primary systemic carnitine deficiency is caused by mutations in a gene encoding sodium ion-dependent carnitine transporter. Nat. Genet. 21: 91-94.

O'Donnell, J., Finer, N.N., Rich, W., Barshop, B.A. and Barrington, K.J. 2002. Role of L-carnitine in apnea of prematurity; a randomized control trial. Pediatrics. 109: 622-626.

Ramanau, A., Kluge, H. and Eder, K. 2005. Effects of L-carnitine supplementation on milk production, litter gains and backfat thickness in sows with a low energy and protein intake during lactation. Br. J. Nutr. 93: 717-721.

Ramanau, A., Kluge, H., Spilke, J. and Eder, K. 2004. Supplementation of sows with L-carnitine during pregnancy and lactation improves growth of the piglets during the suckling period through increased milk production. J. Nutr. 134: 86-92.

Robles-Valdes, C., McGarry, J.D. and Foster, D.W. 1976. Maternal-fetal carnitine relationship and neonatal ketosis in the rat. J. Biol. Chem. 251: 6007-6012.

Robouche, C.J., and Seim, H. 1998. Carnitine metabolism and its regulation in microorganisms and mammals. Ann. Rev. Nutr. 18: 39-61.

Seccombe, D.W., Hahn, P. and Novak, M. 1978. The effect of diet and development on blood levels of free and esterified carnitine in the rat. Biochem. Biophys. Acta. 528: 483- 489.

Shennan, D.B., Grant, A., Ramsay, R.R., Burns, C. and Zammit, V.A. 1998. Characteristics of L-carnitine transport by lactating rat mammary tissue. Biochem. Biophys. Acta. 1393: 49-56.

Snoswell, A.M and Linzell, J.L. 1975. Carnitine secretion into milk of ruminants. J. Dairy. Res. 42: 371-380.

Wu, X., Huang, W., Prasad, P.D., Seth, P., Rajan, D.P., Leibach, F.H., Chen, J., Conway, S.J. and Ganapathy, V. 1999. Functional characteristics and tissue distribution pattern of organic cation transporter 2(OCTN2), and cation/carnitine transporter. J. Pharmacol. Exp. Ther. 290: 1482-1492.

Zammit, V.A., Grant, A., Ramsay, R.R., Burns, C. and Shennan, D.B. 1998. Expression of a sodium-dependant L-carniting transporter in lactating rat mammary tissue. Biochem. Soc. Trans. 26: S96.