

Effects of L-carnitine on fetal growth and the IGF system in pigs^{1,2}

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ABSTRACT: The effects of L-carnitine on porcine fetal growth traits and the IGF system were determined. Fourth-parity sows were fed a gestation diet with either a 50-g top dress containing 0 (control, n = 6) or 100 mg of L-carnitine (n = 6). At midgestation, fetuses were removed for growth measurements, and porcine embryonic myoblasts (PEM) were isolated from semitendinosus. Real-time quantitative PCR was used to measure growth factor messenger RNA (mRNA) levels in the uterus, placenta, muscle, hepatic tissue, and cultured PEM. A treatment × day interaction ($P = 0.02$) was observed for maternal circulating total carnitine. Sows fed L-carnitine had a greater ($P = 0.01$) concentration of total carnitine at d 57 than control sows. Circulating IGF-I was not affected ($P = 0.55$) by treatment. Supplementing sows with L-carnitine resulted in larger ($P = 0.02$) litters (15.5 vs. 10.8 fetuses) without affecting litter weight ($P = 0.07$; 1,449.6 vs. 989.4 g) or individual fetal weight ($P = 0.88$) compared with controls. No treatment effect was found for muscle IGF-I ($P = 0.36$), IGF-II ($P = 0.51$), IGFBP-3 ($P = 0.70$), or IGFBP-5 ($P = 0.51$) mRNA abundance. The abundance of IGF-I ($P = 0.72$), IGF-II ($P = 0.34$), and IGFBP-3 ($P = 0.99$) in hepatic

tissue was not influenced by treatment. Uterine IGF-I ($P = 0.46$), IGF-II ($P = 0.40$), IGFBP-3 ($P = 0.29$), and IGFBP-5 ($P = 0.35$) mRNA abundance did not differ between treatments. Placental IGF-I ($P = 0.30$), IGF-II ($P = 0.18$), IGFBP-3 ($P = 0.94$), and IGFBP-5 ($P = 0.42$) mRNA abundance did not differ between treatments. There was an effect of side of the uterus for IGF-I ($P = 0.04$) and IGF-II ($P = 0.007$) mRNA abundance; IGF-I mRNA abundance was greater in the left uterine horn than in the right uterine horn (0.14 and 0.07 relative units, respectively). Placental IGF-II mRNA abundance was greater ($P = 0.007$) in the left than in the right uterine horn (483.5 and 219.59, respectively). The abundance of IGFBP-3 was not affected by uterine horns in either uterine ($P = 0.66$) or placental ($P = 0.13$) tissue. There was no treatment difference for IGF-I ($P = 0.31$) or IGFBP-5 ($P = 0.13$) in PEM. The PEM isolated from sows fed L-carnitine had decreased IGF-II ($P = 0.02$), IGFBP-3 ($P = 0.03$), and myogenin ($P = 0.04$; 61, 59, and 67%, respectively) mRNA abundance compared with controls. These data suggest that L-carnitine supplemented to gestating sows altered the IGF system and may affect fetal growth and development.

Key Words: L-Carnitine, Insulin-Like Growth Factor, Insulin-Like Growth Factor Binding Proteins, Messenger RNA, Myoblasts, Pigs

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Introduction

The supplementation of gestating sows with L-carnitine results in an increased number of pigs born alive (Musser et al., 1999) and heavier BW at birth (Eder et al., 2001; Ramanau et al., 2002). Additionally, offspring

from sows fed L-carnitine had a larger cross-sectional area and more total muscle fibers in the semitendinosus muscle than piglets from controls (Musser et al., 2001). Thus, muscle growth seems to be enhanced by L-carnitine supplementation in gestating sow diets.

The IGF are polypeptides that stimulate proliferation and differentiation of skeletal muscle cells and are regulators of muscle growth and development. The IGF-II is considered to be an embryonic growth factor (Moses et al., 1980), whereas IGF-I regulates growth postnatally

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(Lee et al., 1991). The IGF are bound to IGFBP, which regulate their biological activity. The IGFBP-3 controls the bioavailability of IGF-I and protects the host from the acute insulin-like effects of free IGF (Zapf et al., 1986). In fetal pigs, IGFBP-3 messenger RNA (**mRNA**) was found in the liver, kidneys, and muscle tissues (Peng et al., 1996). The IGFBP-5 is produced during embryonic muscle development (Green et al., 1994), especially in the induction of myoblast differentiation (James et al., 1993; Rotwein et al., 1995).

Supplementing dams with L-carnitine increased circulating IGF-I concentrations at midgestation in swine (Musser et al., 1999), and total serum IGF-I and IGFBP-2, -3, and -4, and liver IGF-I concentrations in rats also were increased (Heo et al., 2001). Thus, results indicate that L-carnitine affects the IGF system.

The effects of supplementing L-carnitine to gestating sows on IGF-I, IGF-II, IGFBP-3, and IGFBP-5 mRNA abundance at midgestation have not been elucidated. Consequently, we examined these IGF-system growth factors in fetal muscle and hepatic tissue, embryonic myoblasts, and in maternal circulation and reproductive tissues from gestating sows that were supplemented with L-carnitine.

Materials and Methods

Animals

All animal procedures were reviewed and approved by the Kansas State University Animal Care and Use Committee. Twelve fourth-parity sows (PIC, Franklin, KY; C 22 sows; BW = 250.7 kg) were artificially inseminated (PIC; 327 MQ) 12, 24, and 36 h after the onset of estrus. Sows were housed in individual crates (1.83 × 0.55 m) in an environmentally controlled gestation barn at the Kansas State University Swine Teaching and Research Center from breeding to midgestation. Sows were blocked by BW at breeding and allotted randomly to one of two dietary treatments. All sows were fed once daily 2.0 kg (as-fed basis) of a corn-soybean meal based gestation diet (Table 1) and received a 50-g top dress containing either 0 (control, n = 6) or 100 mg of L-carnitine (L-carnitine, n = 6; Carniking, Lonza Group, Inc., Fair Lawn, NJ) from 1 to 54.5 to 59 d of gestation. Day 1 was considered 12 h after the first insemination. Surgeries on one randomly selected L-carnitine sow and one randomly selected control sow were on 54.5 to 59 d of gestation because facilities allowed for preparation of porcine embryonic myoblasts (**PEM**) from two sows per day. Sows were allowed ad libitum access to water.

Blood Samples

At d 0, 28, and approximately 57 (d 54.5 to 59) of gestation, blood was collected by jugular venipuncture for determination of total and free carnitine and IGF-I. Blood samples were collected in both heparinized and untreated tubes and were placed on ice until centrifuged

Table 1. Composition of gestation diet, as-fed basis

Ingredient	%
Corn	85.06
Soybean meal (47% CP)	10.89
Mono-calcium phosphate	1.85
Limestone	1.05
Salt	0.50
Minerals and vitamins ^a	0.65
Calculated analyses	
Lysine	0.55
CP	12.30
Ca	0.80
P	0.70
Fat	3.64
Fiber	1.67

^aProvided per kilogram of complete feed: 11,023 IU of vitamin A; 1,653.45 IU of vitamin D₃; 44.09 IU of vitamin E; 0.0044 g of menadi-one; 0.000004 g of vitamin B₁₂; 0.0099 g of riboflavin; 0.033 g of pantothenic acid; 0.0551 g of niacin; 0.5510 g of choline; 0.00022 g of biotin; 0.0017 g of folic acid; 0.0152 g of pyridoxine; 0.1653 g of Zn; 0.0397 g of Mn; 0.1653 g of Fe; 0.0165 g of Cu; 0.0030 g of I; and 0.0030 g of Se.

(2,500 × g for 20 min at 4°C) or refrigerated (4°C) 48 h before centrifugation, respectively. Plasma or sera were then separated and frozen (−20°C) until analysis. The concentrations of free and total plasma carnitine (Parvin and Pande, 1977) and serum IGF-I (Active IGF-I with extraction, DSL-5600, Diagnostic Systems Laboratories, Inc., Webster, TX) were determined.

Surgical Protocol and Collection of Samples

A hysterectomy was completed on each sow between d 54.5 to 59 of gestation. Eighteen hours before hysterectomy, sows were transported 5.5 km from Kansas State University Swine Teaching and Research Center to the surgery suite on campus, where sample collections were performed 24 h after the last feeding and 12 h after drinking. Sows were anesthetized i.v. with sodium thio-pental (8 mg/kg; Abbott Laboratories, North Chicago, IL) before surgery and a surgical plane of anesthesia was maintained by inhalation of 2% halothane (Halocarbon Laboratories, River Edge, NJ). Additionally, atropine sulfate (0.04 to 0.08 mg/kg; Phoenix Pharmaceutical St. Joseph, MO) was administered i.m. to decrease salivation. A ventro-lateral incision was made to gain access to the abdominal cavity. The ovarian pedicles and uterine stump, at the level of the cranial cervix, were ligated before removal of the uterus. Once the uterus was removed, the muscle layers and skin were closed with absorbable sutures. The number of fetuses in both horns of the uterus was determined. Fetal pigs were immediately removed under aseptic conditions and rapidly transported to a laminar flow hood, where myogenic cells were isolated according to procedures described previously (Pampusch et al., 1990; Hembree et al., 1991, 1996). Briefly, semitendinosus muscle from the right hind limb of each fetus was aseptically excised and washed with warm (37°C) Earle's balanced salt solution

Table 2. Primers and probes used for real-time quantitative polymerase chain reaction

Gene	Genbank Accession No.		Sequence
IGF-I	M31175	Forward	TCTTCTACTTGGCCCTGTGCTT
		Reverse	GCCCCACAGAGGGTCTCA
		Probe	6FAM-CCTTCACCAGCTCTGCCACGGC-TAMRA
IGF-II	X56094	Forward	CCGGACAACCTCCCCAGATA
		Reverse	CGTTGGGCGGACTGCTT
		Probe	6FAM-CCCCTGGGCAAGTCTTCCGC-TAMRA
IGFBP-3	AF085482	Forward	AGCACGGACACCCAGAACTT
		Reverse	CGGCAAGGCCCGTATTC
		Probe	6FAM-TCCTCTGAGTCCAAGCGGAGA-TAMRA
IGFBP-5	U41340	Forward	GGCAGAGGCCGTGAAGAAG
		Reverse	CAGTCCCCCAGCAACT
		Probe	6FAM-CCGCAGAAAGAAGCTGACCCAGTCC-TAMRA
Myogenin	U14331	Forward	AGTGCCCTTTCGCTACA
		Reverse	ACTCTTCGTCTCTCAAAGTCTTTCTTT
		Probe	6FAM-CCCTGGCTCAGGGACAACTCAGGA-TAMRA

(**EBSS**), pH 7.4. Excised fetal muscle tissue was pooled for each sow and minced with scissors, after which it digested with 10 volumes (vol/wt of minced muscle) of 0.2% (wt/vol) trypsin in Ca-Mg-free EBSS. After a 1-h incubation period, the digested tissue was pelleted by centrifugation ($400 \times g$). The pellet was suspended in EBSS and again centrifuged ($400 \times g$). The resultant pellet was suspended in Dulbecco's modified Eagle medium (**DMEM**; Gibco, Grand Island, NY) containing 10% (vol/vol) fetal calf serum (**FCS**; Gibco) to give 0.4 g of original tissue weight/mL of medium and then sequentially filtered through 149- and 74- μ m mesh Nitex cloth. The filtrate was preplated on 75-cm² tissue culture flasks and incubated for 1 h at 37°C, 5% CO₂, 95% air in a water saturated environment. Unattached cells were removed and pelleted by centrifugation ($1,400 \times g$) and then suspended in DMEM containing 10% FCS and 10% (vol/vol) dimethylsulfoxide. Aliquots were placed in polypropylene cryogenic vials (8 to 10 vials of pooled fetal myogenic cells were obtained for each sow) and frozen at -80°C and stored in a liquid N₂ tank, as previously described by Johnson et al. (2003). Uterine and placental samples were excised at the median fetus from both the left and right uterine horns and placed into 5 mL of RNALater (Ambion, Austin, TX) and stored at 4°C in polypropylene tubes for 8 h until they were processed.

Fetal Weights, Lengths, and Tissues

Individual fetuses were weighed. The crown-to-rump length of each fetus was measured. To calculate total litter weight, the sum of individual fetus weights was determined per litter.

Semitendinosus muscle from the left hind limb and hepatic tissue from the left lobe was excised from each fetus, individual identity preserved, and placed into 5 mL of RNALater. The samples were stored at 4°C for 8 h until they were processed.

Sample Preparation and Tissue RNA Isolation

Tissue samples were homogenized in 10 mL of a 5 M guanidine thiocyanate, 50 mM Tris·HCl, 25 mM EDTA, 0.5% lauryl sarcosine, and 1% β -mercaptoethanol solution (**Solution D**), followed by rapid freezing in liquid N₂ and storage at -80°C for later RNA isolation. Total RNA was isolated according to Chomczynski and Sacchi (1987). Briefly, sodium acetate (2 M; pH 4.0), phenol, and chloroform/isoamyl alcohol (24:1) were added to a 2-mL aliquot of homogenized muscle sample. Samples were vortexed, chilled on ice for 15 min, and centrifuged at $10,000 \times g$ for 20 min at 4°C. The aqueous layer was transferred to a new tube and reextracted following the procedure described previously. After the second extraction, the aqueous layer was transferred to a new tube, mixed with cold isopropanol, chilled on ice for 15 min and centrifuged at $10,000 \times g$ for 20 min at 4°C. The resulting pellets were dissolved in Solution D and precipitated with 75% ethanol and dissolved in diethyl pyrocarbonate-treated water. The concentration of RNA was determined by absorbance at 260 nm. Total RNA (1 μ g) with ethidium bromide was loaded onto a 1% agarose-formaldehyde gel and subjected to electrophoresis to allow visualization of 28S and 18S ribosomal RNA to assess the integrity of RNA. After RNA integrity was assessed, samples were treated with DNase to remove any contaminating genomic DNA using a commercially available kit (DNA-free, Ambion, Austin, TX). TaqMan reverse transcription reagents, MultiScribe reverse transcriptase (Applied Biosystems, Foster City, CA) were used to produce complementary DNA (**cdNA**) from 1 μ g of total RNA. Random hexamers were used as primers in cdNA synthesis.

Cell Culture and RNA Isolation

To establish cultures from frozen stocks (stocks contained pooled fetal myogenic cells for each sow), rapidly

thawed cell suspensions were diluted with 25 mL of DMEM containing 10% (vol/vol) FCS and antibiotic/antimycotic. A cell solution (10 mL, 3,290 cells/cm²) was plated on 100-mm dishes coated with Basement Membrane Matrigel (diluted 1:27 [vol/vol] in DMEM; Becton Dickinson Labware, Bedford, MA). All cultures were maintained at 37°C, 5% CO₂, 95% air in a water saturated environment. After a 24-h attachment period, the plates were rinsed twice with 5 mL of DMEM. Cultures were refed with DMEM containing 10% FCS (7 mL/100 mm plate).

At 96-h after plating, total RNA was isolated from cells on the 100-mm plates (Absolutely RNA Microprep kit; Stratagene, La Jolla, CA). The concentration of RNA was determined by absorbance at 260 nm. TaqMan reverse transcription reagents and MultiScribe reverse transcriptase (Applied Biosystems) were used to produce cDNA from 1 µg of total RNA. Random hexamers were used as primers in cDNA synthesis.

Real-Time Quantitative PCR

Real-time quantitative PCR was used to estimate the quantity of IGF-I, IGF-II, IGFBP-3, IGFBP-5, and myogenin mRNA relative to the quantity of 18S ribosomal RNA in total RNA isolated from tissue samples and cell cultures. Measurement of the relative quantity of cDNA was carried out using TaqMan Universal PCR Master Mix (Applied Biosystems), 900 nM of the appropriate forward and reverse primers, 200 nM of appropriate TaqMan detection probe, and 1 µL of the cDNA mixture. The porcine specific IGF-I, IGF-II, IGFBP-3, IGFBP-5, and myogenin forward and reverse primers and TaqMan detection probes were synthesized using published GenBank sequences (Table 2). Commercially available eukaryotic 18S ribosomal RNA primers and probe were used as an endogenous control (Applied Biosystems; Genbank Accession No. X03205). Assays were performed in an ABI Prism 7000 sequence detection system (Applied Biosystems), using thermal cycling criteria recommended by the manufacturer (50 cycles of 15 s at 95°C and 1 min at 60°C). Relative abundance of the IGF-system and myogenin genes was normalized with the 18S endogenous control using the Δ -CT method and is expressed in relative units. Titration of 18S, IGF-I, IGF-II, IGFBP-3, IGFBP-5, and myogenin primers against increasing amounts of cDNA gave linear responses with slopes of -3.3 to -3.9.

Statistical Analyses

Statistical analyses for blood concentrations were performed with the Mixed procedure of SAS (SAS Inst., Inc., Cary, NC). A split-plot analysis was conducted to account for repeated measurements that included the fixed effects of treatment and day of bleeding as the repeated measure. The Satterthwaite adjustment was used for the degrees of freedom. Gestational growth data also were analyzed using the Mixed procedure of SAS. The

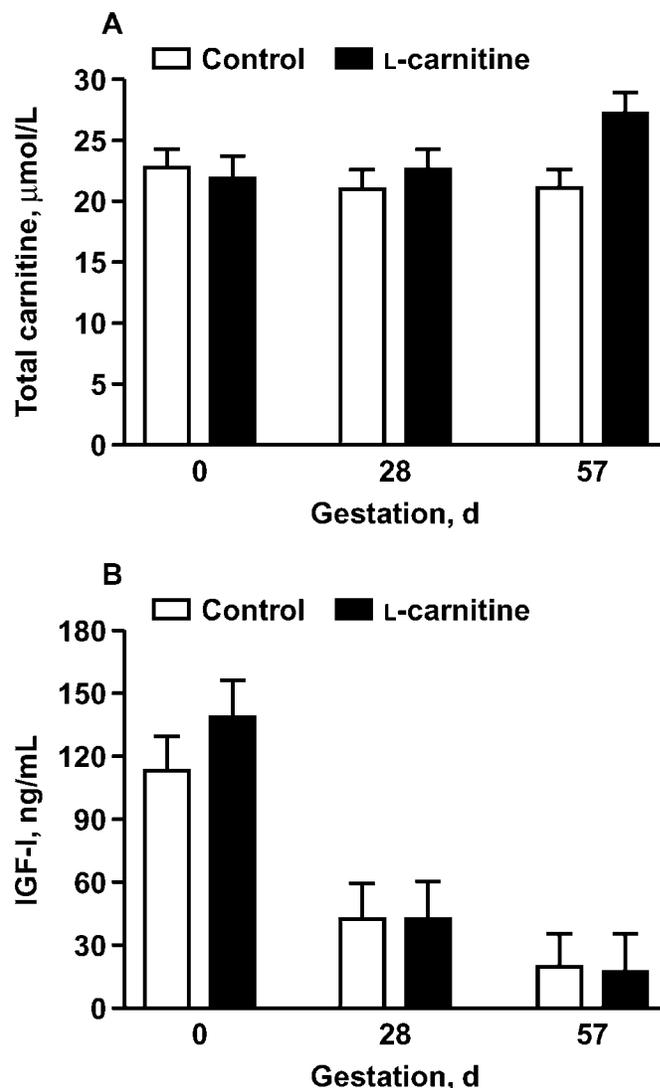


Figure 1. Plasma total carnitine (A) and serum IGF-I concentrations (B) of unsupplemented (control) and supplemented (100 mg/kg of L-carnitine) sows at various times of gestation. A feeding treatment \times day of gestation interaction was detected ($P = 0.02$). Circulating total carnitine concentrations in sows were greater ($P = 0.01$) than in control sows on d 57 of gestation. Circulating IGF-I concentrations in sows were not affected ($P = 0.55$) by feeding treatment on any of the days sampled. There was a gestation day affect ($P < 0.001$). On d 0 and 28 of gestation, circulating IGF-I concentrations were greater than on d 57.

model included treatment for litter size and the addition of a covariate (gestation day) for the remaining growth traits. For all of the genes evaluated in tissues, mRNA concentrations for each fetus were averaged for each sow by uterine horn by the Means procedure of SAS. These data and the mRNA abundance of maternal reproduction tissues were then analyzed with the Mixed procedure. Fixed effects were treatment and uterine horn, whereas sow was the random effect. The statistical

Table 3. Influence of L-carnitine supplementation to gestating sows (54.5 to 59 d) on fetal growth traits^a

Variable	Control	L-Carnitine, 100 mg/d	P-value
No. of fetuses/litter	10.8 ± 1.2	15.5 ± 1.4	0.02
Total litter weight, g ^b	989.4 ± 170.5	1,449.6 ± 195.7	0.07
Individual fetus weight, g	91.4 ± 4.7	92.4 ± 5.4	0.88
Crown-to-rump length, cm	13.6 ± 0.2	13.2 ± 0.2	0.09

^aValues are least squares means ± SEM.

^bTo calculate total litter weight, the sum of individual fetus weights was determined per litter.

model for the cell culture gene abundance data included the fixed effect of treatment and the random effect of animal. Unless otherwise stated, all treatment means were separated ($P < 0.05$) using the LSD procedure when the respective F -tests were significant ($P < 0.05$). The Pearson correlation coefficients were calculated for the growth measurements and reproductive tissue mRNA abundance.

Results

A treatment × gestation day interaction ($P = 0.02$) was observed for maternal circulating total carnitine (Figure 1A). Sows fed L-carnitine had greater ($P = 0.01$) concentration of total carnitine at d 57 than did control sows. No treatment × gestation day interaction ($P = 0.55$; Figure 1B) was observed for circulating IGF-I; however, a day effect ($P < 0.001$) was detected. The circulating IGF-I concentration was greater on d 0 (126.1 ng/mL) of gestation than on d 28 ($P < 0.001$) or 57 ($P < 0.001$; 42.9 and 18.5 ng/mL, respectively).

Supplementing sows with L-carnitine resulted in larger ($P = 0.02$) litters compared with litters from control sows (Table 3). Total litter weights ($P = 0.07$), individual fetus weight ($P = 0.88$), and crown-to-rump length ($P = 0.09$) did not differ between the two feeding treatments.

Total RNA was isolated from individual fetal muscle and hepatic tissues, as well as from the uterus and pla-

centa. In fetal muscle, IGF-I ($P = 0.36$), IGF-II ($P = 0.51$), IGFBP-3 ($P = 0.70$), and IGFBP-5 ($P = 0.51$) mRNA abundance were not affected by maternal feed treatment or by the uterus horn (IGF-I ($P = 0.61$), IGF-II ($P = 0.28$), IGFBP-3 ($P = 0.68$), and IGFBP-5 ($P = 0.82$; Table 4). Fetal hepatic IGF-I ($P = 0.72$), IGF-II ($P = 0.34$), and IGFBP-3 ($P = 0.99$) mRNA abundance were not influenced by either treatment or uterine horn (IGF-I [$P = 0.17$], IGF-II [$P = 0.51$], and IGFBP-3 [$P = 0.58$]; Table 4).

No differences in uterine IGF-I ($P = 0.46$), IGF-II ($P = 0.40$), IGFBP-3 ($P = 0.29$), and IGFBP-5 ($P = 0.35$) mRNA abundance were found for treatment or uterine horn (IGF-I [$P = 0.26$], IGF-II [$P = 0.58$], IGFBP-3 [$P = 0.66$], and IGFBP-5 [$P = 0.32$] mRNA abundance) of the uterus (Table 5). In addition, no treatment effect was detected for placental IGF-I ($P = 0.30$), IGF-II ($P = 0.18$), IGFBP-3 ($P = 0.94$), and IGFBP-5 ($P = 0.42$) mRNA abundance. There was an effect of uterine horn for IGF-I ($P = 0.04$) and IGF-II ($P < 0.001$) mRNA abundance in placental tissue. The abundance of IGF-I mRNA was greater ($P = 0.04$) in the left uterine horn than in the right uterine horn (0.14 and 0.07 relative units, respectively), and the abundance of placental IGF-II mRNA also was greater ($P = 0.007$) in the left than in the right uterine horn (483.50 and 219.59 relative units, respectively). The abundance of IGFBP-3 was not affected ($P = 0.13$) by the uterine horn, but there was a tendency ($P = 0.07$) for the left uterine horn to have greater abundance of

Table 4. Influence of L-carnitine supplementation to gestating sows (54.5 to 59 d) and uterine horn on least squares means for fetal muscle and hepatic tissue messenger RNA abundance of insulin-like growth factor system genes^a

Variable	L-Carnitine, mg/d:		0		100		SEM	P-value		
	Uterine horn:	0	0	100	100	Trt ^b		Horn	Trt × Horn	
Muscle ^c										
IGF-I		1.28	1.35	1.98	1.70	0.40	0.36	0.61	0.41	
IGF-II		679.30	701.57	898.55	676.22	118.23	0.51	0.28	0.19	
IGFBP-3		3.83	4.18	4.95	4.20	1.08	0.70	0.68	0.26	
IGFBP-5		12.78	13.69	16.44	14.94	2.69	0.51	0.82	0.37	
Hepatic tissue										
IGF-I		0.02	0.02	0.02	0.02	0.004	0.72	0.17	0.51	
IGF-II		375.00	279.01	416.36	460.50	83.70	0.34	0.51	0.10	
IGFBP-3		2.08	1.91	2.03	1.97	0.33	0.99	0.58	0.80	

^amRNA abundance values are expressed in relative units.

^bTreatment.

^cTotal RNA was isolated from semitendinosus muscle from the left hind limb.

Table 5. Influence of L-carnitine supplementation to gestating sows and uterine horn on least squares means for uterine and placental messenger RNA abundance of insulin-like growth factor system genes^{a,b}

Variable	L-Carnitine, mg/d: Uterine horn:					SEM	P-value		
		0 Left	0 Right	100 Left	100 Right		Trt ^c	Horn	Trt × Horn
Uterus									
IGF-I		1.92	0.97	2.24	1.64	0.80	0.46	0.26	0.80
IGF-II		25.79	28.52	11.83	11.23	16.57	0.40	0.58	0.39
IGFBP-3		15.00	22.09	11.1	8.41	8.87	0.29	0.66	0.28
IGFBP-5		6.26	2.35	2.50	2.21	2.51	0.35	0.32	0.39
Placenta									
IGF-I		0.12	0.05	0.16	0.09	0.04	0.30	0.045	0.92
IGF-II		552.89	286.34	414.18	152.85	110.5	0.18	0.007	0.97
IGFBP-3		33.88	14.46	31.71	18.48	14.67	0.94	0.13	0.76
IGFBP-5		1.45	0.76	2.70	0.82	0.91	0.42	0.07	0.37

^aUterine and placental samples were excised at the median fetus from both the left and right uterine horns; the left uterine horn had 6.75 fetuses, and the right uterine horn had 6.42 fetuses.

^bmRNA abundance are expressed in relative units.

^cTreatment.

IGFBP-5 mRNA than the right uterine horn (2.07 and 0.79 relative units, respectively) in placental tissue.

The relative abundance of uterine IGF-II and IGFBP-3 mRNA was negatively correlated to fetal weight ($P = 0.01$) and crown-to-rump length ($P = 0.01$), as well as IGFBP-3 to number of fetuses per horn ($P < 0.01$; Table 6). In contrast, in placental tissue, IGF-I was positively correlated ($P < 0.01$) to number of fetuses per horn.

Total RNA was isolated from the PEM cultures at 96 h after plating. In other studies in our laboratory, these primary cultures of PEM were shown to be approximately 80% positive at 72 h after plating for a muscle-specific cell surface antigen, neural cell adhesion molecule, utilizing flow cytometry with the 5.1H11 monoclonal antibody (unpublished data). Following RNA isolation at 96 h in culture, levels of IGF-I, IGF-II, IGFBP-3, IGFBP-5, and myogenin mRNA were determined (Table 7). There was no difference between treatments for the abundance of IGF-I ($P = 0.31$) or IGFBP-5 ($P = 0.13$); however, PEM isolated from sows fed L-carnitine had decreased mRNA abundance of IGF-II ($P = 0.02$), IGFBP-3 ($P = 0.03$), and myogenin ($P = 0.04$; 61, 59, and 67%,

respectively) compared with PEM isolated from control sows.

Discussion

Supplementing gestating sows with L-carnitine resulted in increased maternal circulating total carnitine at midgestation. These results are supported by those of Musser et al. (1999), who reported that sows supplemented 100 mg/d L-carnitine had increased plasma carnitine concentrations on d 60 and 90 of gestation. Heo et al. (2001) injected streptozotocin-induced diabetic rats with 50, 100, or 200 mg/kg BW of L-carnitine every other day for 4 wk. Similarly, Heo et al. (2001) reported that serum carnitine concentrations increased linearly with increasing L-carnitine concentrations. Thus, supplementation of exogenous L-carnitine seems to increase concentrations of circulating L-carnitine, resulting in an increased availability of carnitine for biological activities. L-Carnitine, along with other metabolites, is involved in protein synthesis (Owen et al., 2001), glucose homeosta-

Table 6. Pearson correlation between fetal growth traits and uterine and placental insulin-like growth factor system mRNA abundance

Item	Weight	P-value	Length ^a	P-value	No. ^b	P-value
Uterus						
IGF-I	0.09	0.69	0.09	0.69	0.12	0.61
IGF-II	-0.52	0.01	-0.61	<0.01	-0.07	0.74
IGFBP-3	-0.5	0.01	-0.58	0.01	-0.56	<0.01
IGFBP-5	-0.04	0.85	-0.13	0.58	0.12	0.60
Placenta						
IGF-I	0.21	0.37	-0.05	0.81	0.65	<0.01
IGF-II	0.04	0.87	-0.05	0.82	0.11	0.63
IGFBP-3	0.008	0.97	0.17	0.47	0.14	0.53
IGFBP-5	0.12	0.61	0.33	0.14	0.24	0.30

^aFetal crown-to-rump length.

^bNumber of fetuses per horn; the left horn had 6.75 fetuses, and the right horn had 6.42 fetuses.

Table 7. Growth factor messenger RNA abundance in porcine embryonic muscle cells at 96 h of culture^{a,b}

Gene	Control	L-Carnitine, 100 mg/d	P-value
IGF-I	3.47 ± 0.42	6.31 ± 2.64	0.31
IGF-II	21.68 ± 4.82	8.44 ± 1.39	0.02
IGFBP-3	5.62 ± 1.19	2.30 ± 0.45	0.03
IGFBP-5	22.49 ± 6.17	11.24 ± 3.00	0.13
Myogenin	4.02 ± 1.03	1.33 ± 0.42	0.04

^aGene expression levels are expressed in relative units; n = 6 per treatment.

^bValues are least squares means ± SEM.

sis (De Gaetano et al., 1999), and β -oxidation (McGarry and Brown, 1997).

Serum IGF-I concentrations did not differ in sows with or without supplementation of L-carnitine. Previous research (Musser et al., 1999) has indicated that sows supplemented L-carnitine during gestation had increased circulating IGF-I concentrations on both d 60 and 90 of gestation compared with control sows. However, in the previous study, similar to the current results, circulating IGF-I decreased dramatically as gestation progressed. Therefore, the treatment-induced increased circulating IGF-I on d 60 and 90 of gestation was more a maintenance in circulating IGF-I relative to the d 0 concentrations (Musser et al., 1999). Heo et al. (2001) also investigated the effects of L-carnitine on IGF-I and -II and the IGFBP in rats. Total serum IGF-I concentrations increased in the rats given L-carnitine; however, L-carnitine had no effect on IGF-II concentrations. Feeding L-carnitine to gestating sows was beneficial for maintenance of fetal growth and development under situations of increased litter size at midgestation. Greater litter weights from sows supplemented L-carnitine throughout gestation also were reported by Musser et al. (1999), Eder et al. (2001), and Ramanau et al. (2002). Individual fetal weight was unchanged between the two treatments in the present study, but the number of fetuses per litter increased in sows supplemented with L-carnitine. The L-carnitine was fed after ovulation and, hence, may increase the availability of nutrients to sustain more embryos early in gestation and result in the observed increase in the number of fetuses at midgestation. Eder et al. (2001) also suggested that L-carnitine supplementation did not affect the number of fertilized oocytes. In addition to fetal weight, fetus crown-to-rump length was not affected by feeding L-carnitine, even though there was an increase in total litter weight. Therefore, the increased fetus number was not at the expense of fetal growth performance at midgestation, a relationship that is normally inversely related (Vonnahme et al., 2002).

In pregnant porcine uterus, IGF-I production decreases as gestation progresses (Song et al., 1996). There is greater abundance of IGF-II within the placenta compared with the uterus (Sterle et al., 1998). Sterle et al. (1998) suggested that the increased abundance of IGF-II mRNA may inhibit synthesis of IGF-I by the uterus

through a local IGF negative feedback loop. In the current study, there was no treatment effect on either uterine or placental IGF-I and-II abundance. These IGF-II results indicate that uterus IGF-I production was not affected, which also was supported by the similar IGF-I uterus values for both treatments.

The placental growth factor mRNA abundance was greater in tissue from the left uterine horn than the right uterine horn. To our knowledge, this is the first report that IGF system mRNA abundance varies by uterine horn. These findings are very interesting and suggest that the differences observed for the uterine horn effect in placental mRNA abundance and not in the uterine growth factors may reflect the local influence of the conceptus.

The IGF system is instrumental throughout fetal growth and development (DeChiara et al., 1991) and specifically, IGF-I and IGF-II modulate porcine fetal muscle development (Gerrard et al., 1998). Therefore, comprehension of developmental changes in IGF-I and II gene abundance during fetal development is valuable for determining how various nutritional and environmental strategies may be applied to influence developing skeletal muscle tissue. In the current study, supplementation of L-carnitine during gestation had no effect on the gene abundance of IGF-I, IGF-II, IGFBP-3, or IGFBP-5 in either skeletal muscle or hepatic tissue at midgestation. In addition to determining the effect of maternal L-carnitine on tissue gene transcript abundance of the IGF components, we isolated mononucleated myoblasts from hindlimb muscles opposite to that used for tissue gene transcript abundance. These isolated mononucleated myoblasts will ultimately differentiate into existing primary fibers, aid in the formation of secondary muscle fibers, or may become a population of satellite cells important in supporting postnatal muscle hypertrophy (Hembree et al., 1991; Rehfeldt et al., 2001). It is well established that IGF-I, IGF-II, and specific IGFBP, such as IGFBP-3 and IGFBP-5, have potent proliferative and differentiation-promoting effects on muscle cells (Florini et al., 1991; Hembree et al., 1996; Chakravarthy et al., 2000; Johnson et al., 2003). The expression of the IGF-II gene by myoblasts also induces the expression of the myogenin gene, which leads to the differentiation of muscle cells to form postmitotic myotubes (Florini et al., 1991). Porcine embryonic myoblasts isolated from sows fed L-carnitine had significant changes in the gene expression profiles of the IGF components and myogenin in total RNA isolated from these primary cultures at 96 h. Most striking were the significant decreases in mRNA abundance for IGF-II and myogenin in PEM cultures established from sows supplemented with L-carnitine during gestation. These data support that maternal supplementation of L-carnitine affected the gene expression of key growth factor and transcription factor genes, which ultimately will regulate the proliferation and differentiation status of these important myogenic precursor cells. These changes in mRNA levels are occurring during a time in which these mononucleated cells will

differentiate into the secondary fibers. Previous research has suggested that maternal supplementation of L-carnitine during gestation affected the number of secondary fibers in the neonate (Musser et al., 2001). We believe these dramatic changes in mRNA levels for IGF-I, IGF-II, IGFBP-3, and myogenin in cultured PEM will result in delayed differentiation of these cells to existing fibers and prolonged proliferation. This process could give rise to increased fiber numbers at birth due to increased number of embryonic myoblasts.

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