Effects of maternal nutritional status on nutrient transporter expression in bovine utero-placental tissue on days 16 to 50 of gestation

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The objectives of this study were to determine the effect of a 40 percent global nutrient restriction on the messenger ribonucleic acid (mRNA) expression of nutrient transporters known for their roles in transporting arginine (CAT-1, CAT-2 and CAT-3) and glucose (GLUT1) across the uterine endometrium and fetal membranes to the fetus from days 16 to 50 of gestation. The results indicate that a 40 percent global nutrient restriction only affects mRNA expression of arginine transporter CAT-2 and not any other transporter investigated.

Summary

We hypothesized that maternal nutrition and day of gestation would impact mRNA expression of nutrient transporters *GLUT1, CAT-1, CAT-2* and *CAT-3* in beef heifers. Crossbred Angus heifers (n = 49) were synchronized, bred via artificial insemination (AI), assigned to nutritional treatment (CON = 100 percent of requirements for 1 pound/day of gain and RES = 60 percent of CON) and ovariohysterectomized on days 16, 34 or 50 of gestation (n = six to nine/day). Nonpregnant (NP) controls were not bred and ovariohysterectomized on day 16 of the synchronized estrous cycle (n = 6). The resulting arrangement of treatments was a 2 × 3 factorial + 1. Caruncle (CAR), intercaruncular endometrium (ICAR) and fetal membranes (FM) were obtained from the pregnant uterine horn immediately following ovariohysterectomy. For NP controls, only CAR and ICAR were obtained. The relative expression of the glucose transporter *GLUT1* and cationic amino acid transporters *CAT-1, CAT-2* and *CAT-3* was determined for each tissue utilizing NP-CAR and NP-ICAR tissue as the baseline. For FM, NP endometrium served as the baseline. We found no interaction of day and treatment in FM for any genes ($P \geq 0.05$). Expression of *GLUT1* and *CAT-1* showed a day effect, being greater ($P < 0.05$) in FM on days 34 and 50, compared with day 16. In CAR, we found no day × treatment interaction, and *CAT-3* expression tended ($P = 0.06$) to be greater in CON vs. RES heifers. Additionally, expression of *GLUT1, CAT-1* and *CAT-2* in CAR were greater ($P < 0.01$) on day 16, compared with days 34 and 50, day 34 compared with day 50, and days 16 and 34 compared with day 50, respectively. In ICAR, *CAT-2* showed a day ×

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treatment interaction, being greater ($P = 0.01$) on day 50 CON, compared with all other groups. Transporter *CAT-3* tended ($P = 0.09$) to be greater in day × treatment in ICAR on day 16 CON, compared with all other days and treatments. The expression of *GLUT1* was greater ($P < 0.01$) in ICAR on day 16 than all other days. Arginine transporter *CAT-1* was greater ($P < 0.01$) in ICAR on days 34 and 50, compared with day 16. These results partially support our hypothesis and indicate that day was a more influential factor for mRNA expression of utero-placental glucose and cationic amino acid transporters than maternal nutritional status in heifers during early pregnancy.

Introduction

Fetal growth is vulnerable to maternal dietary nutrient deficiencies during the first trimester of gestation (Wu et al., 2004). During the first 50 days of gestation, organogenesis is taking place. This time period of gestation is a critical developmental window with significant cellular and tissue differentiation. Nutritional influences may alter the mammalian phenotype through affecting gene regulatory mechanisms involved in DNA synthesis and replication, thus "imprinting" potential susceptibilities to chronic disease and metabolic issues into the genome (Waterland and Jirtle, 2004).

Currently, fetal undernutrition occurs in grazing livestock worldwide (Wu et al., 2004). Maternal undernutrition has been implicated in fetal growth restriction and altered placental growth, reduced amino acid and glucose transport, and increased apoptosis and autophagy, which overall can yield decreased fetal growth during gestation (Zhang et al., 2015).

Before the establishment of hemotrophic nutrition, the placenta is developing and the fetus begins to utilize increasing quantities of glucose and amino acids (Groebner et al., 2011). Thus, the expression of glucose and amino acid transporters in the utero-placenta becomes essential to the viability of the conceptus.

Therefore, we studied the utero-placental glucose transporter *GLUT1 (SLC2A1)*, which is present in most tissues throughout the body and is ubiquitous across species. The amino acid transporters investigated are *CAT-1, CAT-2* and *CAT-3* (*SLC7A1, SLC7A2* and *SLC7A3*), whose substrates are cationic amino acids such as arginine and lysine, which are directly linked to angiogenesis and cell proliferation.

In this experiment, we tested the hypothesis that mRNA for glucose and cationic amino acid transporters in utero-placental tissues would be expressed differentially due to day of gestation and maternal nutritional status.

Experimental Procedures

Protocols described herein were approved by the North Dakota State University Institutional Animal Care and Use Committee. Crossbred Angus heifers (n = 49, about 15 months of age; average initial body weight [BW] = 722 pounds) were exposed to the 5-d CO-Synch + CIDR estrus synchronization protocol. Six heifers were not inseminated to serve as nonpregnant (NP) controls but received ovariohysterectomy for tissue collections on day 16 of the synchronized estrous cycle. The remaining heifers (n = six to nine/day of gestation/treatment) were bred by AI to a common sire at 12 hours after observed estrus and ovariohysterectomized at days 16, 34 or 50 of gestation.

Heifers were housed at the NDSU Animal Nutrition and Physiology Center. Heifers were acclimated to individual bunk feeding for two weeks before the beginning of the trial.

Immediately following AI, heifers were assigned randomly to one of two treatment groups. Control heifers (CON) received 100 percent of National Research Council (NRC, 2000) requirements for 0.45 kilogram per day (kg/d) gain to reach 80 percent of mature BW at first calving. Restricted heifers (RES) were placed on a 40 percent global nutrient restriction, which was accomplished by reducing total diet delivery 60 percent of the control delivery.

The diet was delivered via total mixed ration (TMR) and consisted of grass hay (73.4 percent neutral detergent fiber [NDF], 8 percent crude [CP]), corn silage (55.6 percent NDF, 6.3 percent CP), alfalfa haylage, (48.9 percent NDF, 16.4 percent CP), grain supplement, (32.6 percent NDF, 24.1 percent CP) and dried distillers grains (53.4 percent NDF, 31.3 percent CP), on a dry-matter (DM) basis.

Immediately following ovariohysterectomy (McLean et al., 2016), utero-placental tissues (caruncle, CAR; intercaruncular endometrium, ICAR; fetal membrane [chorioallantois], FM; cotyledon, COT; intercotyledonary placenta ICOT; and amnion, AMN) were obtained from the uterine horn containing the conceptus, as previously described (Grazul-Bilska et al., 2010, 2011). Fetal membranes also were collected only from heifers that were bred due to a lack of FM in NP controls.

On day 50 of gestation, FM was split into COT and ICOT. Amnion was collected only on day 50. Once collected, all tissues were frozen in liquid nitrogen-cooled isopentane and stored at minus 112 F.

The RNA was extracted from each tissue and purified. The level of mRNA expression of each transporter within the tissue was established using polymerase chain reaction (PCR) to determine differences in mRNA expression of the transporters across days of early gestation.

Results and Discussion

The mRNA expression of glucose transporter *GLUT1* was greater ($P < 0.01$) in AMN, compared with COT and ICOT (0.67 vs. 0.24 and 0.29, respectively; Table 1). Arginine transporter *CAT-1* mRNA expression was greater ($P = 0.02$) in AMN when compared with COT and ICOT (0.30 vs. 0.22 and 0.17, respectively; Table 1).

Cationic amino acid transporter *CAT-2* mRNA expression was greater ($P = 0.05$) in AMN, compared with ICOT (3.27 vs. 0.82, respectively). The level of expression of *CAT-3* was greater ($P < 0.01$) in AMN, compared with COT and ICOT (7.64 vs. 0.73 and 2.75, respectively)

We found no day × treatment interaction or main effect of treatment for any gene in FM ($P > 0.05$). Expression of *GLUT1* was greater ($P = 0.04$) on day 50 of gestation, compared with day 16 (0.38 vs. 0.15, respectively; Table 2). Cationic amino acid transporter *CAT-1* expression was greater ($P < 0.01$) on days 34 and 50, compared with day 16 (0.23 and 0.22 vs. 0.05, respectively; Table 2). The mRNA expression of *CAT-2* tended to be greater ($P = 0.09$) on day 50 of gestation, compared with day 16.

We also found no day × treatment interaction ($P \geq 0.05$) on the mRNA expression of *GLUT1, CAT-1, CAT-2* or *CAT-3* in CAR. Expression of *CAT-3* showed a tendency ($P = 0.06$) to be greater across day of gestation in CON vs. RES (2.60 vs. 1.16; Table 3). Expression of *GLUT1* was greater ($P < 0.01$) on day 16 of gestation, compared with days 34 and 50 (2.89 vs. 0.85 and 1.14 respectively).

The mRNA expression of CAT-1 was greater ($P < 0.01$) on day 34, compared with days 16 and 50 (5.22 vs. 1.47 and 0.51 respectively; Table 3). Additionally, mRNA expression of *CAT-1* tended to be greater ($P = 0.09$) in the post-implantation (days 34 and 50) vs. implantation (day 16). Expression of cationic amino acid transporter *CAT-2* was greater ($P = 0.02$) on day 34, compared with day 16 of gestation (14.67 vs. 4.36, respectively). In addition, *CAT-2* mRNA expression showed a tendency ($P = 0.06$) to be greater in pregnant vs. NP heifers.

The expression of *CAT-2* showed a day × treatment interaction ($P = 0.01$) being greater, with day 50 CON heifers having greater expression, compared with days 16 and 50 RES and day 34 CON heifers (Table 4). The cationic amino acid transporter *CAT-3* tended ($P = 0.09$) to be greater in day 16 CON, compared with all other days and treatments.

The mRNA expression of *GLUT1* was greater ($P < 0.01$) on day 16 of gestation compared with day 34 (2.11 vs. 0.75). Arginine and Lysine transporter *CAT-1* was greater ($P < 0.01$) on days 34 and 50, compared with day 16 (14.62 and 11.13 vs. 0.58, respectively). Additionally, *CAT-1* mRNA expression was greater in ICAR ($P < 0.01$) in pregnant, compared with NP heifers (8.78 vs. 1, respectively).

Fertilization rates for first-service AI are approximately 90 percent in beef heifers (Bridges et al., 2013); however, by day 30 of gestation, only 50 to 60 percent of heifers maintain a viable pregnancy. Moreover, Thatcher et al., (1994) indicated that up to 40 percent of all embryonic loss occurs before day 40 of gestation in sheep and cattle.

Glucose and amino acids, specifically arginine, are crucial for proper energy metabolism and growth, and are key regulators of mTOR, which is linked to angiogenesis and cell proliferation, causing increased fetal growth and mitigating apoptosis (Tan and Miyamoto, 2016

The expression of all transporters investigated was greatest on day 50 in AMN, compared with COT and ICOT. Amniotic fluid contains the nutrient reserve from which the conceptus draws to meet its energetic and growth requirements prior to transplacental exchange. The reported data further demonstrate the increased emphasis on transport of nutrients across the AMN to provide nutrients to the conceptus.

Table 1. Relative expression of nutrient transporters *GLUT1, CAT-1, CAT-2* and *CAT-3* in AMN, COT and ICOT on day 50 of gestation using NP endometrium as a baseline value set to 1.

Gene[1]	AMN[2]	COT[3]	ICOT[4]	SEM[5]	P-value[6]
GLUT1	0.67[a]	0.24[b]	0.29[b]	0.07	< 0.01
CAT-1	0.30[a]	0.22[b]	0.17[b]	0.03	0.02
CAT-2	3.27[a]	1.42[ab]	0.82[b]	0.66	0.05
CAT-3	7.64[a]	0.73[b]	2.75[b]	1.38	< 0.01

[1]Gene = *GLUT1* – Glucose transporter solute carrier family 2 member 1. *CAT-1, CAT-2* and *CAT-3* – Cationic amino acid transporters of arginine and lysine, solute carrier family 7 member 1, 2 and 3.
[2]Amnion taken on day 50 of gestation.
[3]Cotyledons taken from fetal membranes on day 50 of gestation.
[4]Intercotyledonary tissue (fetal membrane tissue not including cotyledons; taken from fetal membranes on day 50 of gestation).
[5]Average SEM was used within gene; AMN n = 11, COT n = 14, ICOT n =14
[6]Probability values for the effect of tissue on level of expression of individual genes.
[a-b]Means within gene without a common superscript differ by tissue ($P \leq 0.05$).

Before the establishment of transplacental exchange, nutrient transporters are the only method of supplying nutrients to the conceptus. Therefore, evaluating the concentration of nutrients in the maternal and fetal fluids (serum, histotroph, and allantoic and amniotic fluids) is of interest to determine whether nutrient restriction during early gestation affects nutrient concentrations in maternal and fetal fluids or nutrient transport capacity, thereby altering the abundance of nutrients available for transport to the conceptus.

We interpret these data to imply that a 40 percent global maternal nutritional restriction may affect the mRNA expression of some *(CAT-2)* but not all utero-placental nutrient transporters investigated in this study. The effects of day of gestation on the mRNA expression of glucose and cationic amino acid transporters reflect the changing nutrient supply and demand curve necessary for proper conceptus growth. Moreover, additional knowledge in this area will facilitate increased efficiencies of beef cattle production and contribute to meeting the projected world food demands.

Acknowledgments

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Table 2. Level of expression of nutrient transporters *GLUT1*, *CAT-1*, *CAT-2* and *CAT-3* in fetal membranes (FM) due to CON and RES dietary treatments from days 16 to 50 of gestation in beef heifers using NP endometrium as a baseline value set to 1.

			Day of Gestation[2]					*P* – values[3]		
Gene[1]	Trt[4]		16	34	50	Trt[5]	SEM[6]	Day	Trt	Day × Trt
GLUT1	CON		0.11	0.25	0.38	0.25	0.08	0.04	0.70	0.90
	RES		0.19	0.27	0.38	0.28				
		Day[7]	0.15[h]	0.26[gh]	0.38[g]					
CAT-1	CON		0.04	0.22	0.22	0.16	0.17	< 0.01	0.70	0.99
	RES		0.05	0.24	0.23	0.17				
		Day	0.05[h]	0.23[g]	0.22[g]					
CAT-2	CON		0.42	0.84	1.97	1.08	0.66	0.09	0.87	0.82
	RES		0.24	1.16	1.57	0.99				
		Day	0.33	1.00	1.77					
CAT-3	CON		0.08	3.94	5.20	3.07	2.21	0.39	0.61	0.57
	RES		2.38	0.93	3.02	2.11				
		Day	1.23	2.43	4.11					

[1]Gene = *GLUT1* – Glucose transporter solute carrier family 2 member 1. *CAT-1, CAT-2* and *CAT-3* – Cationic amino acid transporters of arginine and lysine, solute carrier family 7 member 1, 2 and 3.

[2]Day of Gestation = number of days after AI. Each gene of interest expression value is reported as a fold change in relation to NP endometrium level of gene expression.

[3]Probability values for the effect of day, treatment and day × treatment on the level of expression of individual genes.

[4]CON = Heifers fed a diet that meets 100% of NRC requirements to gain 1 pound daily. RES = Heifers restricted to 60% of CON diet.

[5]Mean gene expression of treatment group across day of gestation within tissue and gene of interest.

[6]Average SEM used within gene. d 16 CON n = 7, d 16 RES n = 7, d 34 CON n = 6, d 34 RES n = 9, d 50 CON n = 7, d 50 RES n = 7

[7]Mean gene expression across treatment within day and gene of interest.

[a-c]Means within gene and tissue without a common superscript differ in day × treatment ($P \leq 0.05$).

[g-h]Means within row lacking common superscript differ in main effect of day ($P \leq 0.05$).

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Table 3. Level of expression of nutrient transporters *GLUT1*, *CAT-1*, *CAT-2* and *CAT-3* in caruncular CAR tissue due to CON and RES dietary treatments from d 16 to 50 of gestation and in non-pregnant (NP) controls set to 1.

								P - values[3]					
		Day of Gestation[2]					NP vs.	16 vs.	34 vs.			Day x
Gene[1]	Trt[4]	16	34	50	Trt[5]	SEM[6]	Preg	34 and 50	50	Day	Trt	Trt
GLUT1	CON	2.40	0.93	1.38	1.57	0.44	0.21	< 0.01	0.47	< 0.01	0.77	0.23
	RES	3.38	0.76	0.89	1.67							
	Day[7]	2.89[g]	0.85[h]	1.14[h]								
CAT-1	CON	1.08	5.24	0.53	2.28	1.03	0.21	0.09	< 0.01	< 0.01	0.78	0.90
	RES	1.85	5.20	0.49	2.51							
	Day	1.47h	5.22[g]	0.51[h]								
CAT-2	CON	5.76	14.37	7.98	9.37	3.63	0.06	0.02	0.04	0.02	0.77	0.89
	RES	2.95	14.97	7.58	8.50							
	Day	4.36[h]	14.67[g]	7.78[gh]								
CAT-3	CON	1.29	2.23	4.28	2.60	1.09	0.44	0.24	0.11	0.20	0.06	0.90
	RES	0.45	0.99	2.05	1.16							
	Day	0.87	1.61	3.16								

[1]Gene = *GLUT1* – Glucose transporter solute carrier family 2 member 1. *CAT-1, CAT-2, and CAT-3* – Cationic amino acid transporters of arginine and lysine, solute carrier family 7 member 1, 2, and 3.

[2]Day of Gestation = number of days after insemination. Each gene expression is given as a fold change in relation to NP level of expression set to 1.

[3]Probability values for effect of d, treatment, and day × treatment on level of expression of individual genes. Probability values for the contrast of mRNA level of expression of NP vs. Preg (all days of gestation), d 16 of gestation vs. d 34 and 50 of gestation, and d 34 vs. d 50 of gestation.

[4]CON = Heifers fed a diet that meets 100% of NRC requirements to gain 1 pound daily. RES = Heifers restricted to 60% of CON diet

[5]Mean level of expression of treatment group across day of gestation within tissue and gene of interest.

[6]Average SEM was used within gene. NP n = 6, d 16 CON n = 7, d 16 RES n = 7, d 34 CON n = 6, d 34 RES n = 9, d 50 CON n = 7, d 50 RES n = 7

[7]Mean level of expression across treatment within day and gene of interest.

[a-c]Means within gene and tissue without a common superscript differ in day × treatment ($P \leq 0.05$).

[g-h]Means within row without a common superscript differ in main effect of day ($P \leq 0.05$).

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Table 4. Level of expression of nutrient transporters *GLUT1*, *CAT-1*, *CAT-2* and *CAT-3* in intercaruncular ICAR tissue due to CON and RES dietary treatments from d 16 to 50 of gestation and in non-pregnant (NP) controls set to 1.

								P - values[3]					
			Day of Gestation[2]					NP vs.	16 vs.	34 vs.			Day x
Gene[1]	Trt[4]		16	34	50	Trt[5]	SEM[6]	Preg	34 and 50	50	Day	Trt	Trt
GLUT1	CON		2.44	0.63	1.77	1.61	0.39	0.43	< 0.01	0.10	< 0.01	0.26	0.42
	RES		1.77	0.87	1.08	1.24							
		Day[7]	2.11^{g}	0.75^{h}	1.43^{gh}								
CAT-1	CON		0.65	13.86	9.94	8.15	2.59	< 0.01	< 0.01	0.13	< 0.01	0.56	0.89
	RES		0.51	15.37	12.33	9.41							
		Day	0.58^{h}	14.62^{g}	11.13^{g}								
CAT-2	CON		6.83^{ab}	2.31^{c}	7.78a	5.64	1.43	0.04	0.88	0.56	0.22	0.68	0.01
	RES		3.10b^{c}	6.08^{abc}	3.17^{bc}	4.11							
		Day	4.97	4.19	5.48								
CAT-3	CON		9.69	1.55	5.53	5.59	1.89	0.09	0.12	0.27	0.13	0.45	0.09
	RES		3.87	4.25	5.05	4.39							
		Day	6.78	2.90	5.29								

[1]Gene = *GLUT1* – Glucose transporter solute carrier family 2 member 1. *CAT-1, CAT-2,* and *CAT-3* – Cationic amino acid transporters of arginine and lysine, solute carrier family 7 member 1, 2, and 3.

[2]Day of Gestation = number of days after insemination. Each gene expression is given as a fold change in relation to NP level of expression set to 1.

[3]Probability values for effect of d, treatment, and day × treatment on level of expression of individual genes. Probability values for the contrast of mRNA level of expression of NP vs. Preg (all days of gestation), d 16 of gestation vs. d 34 and 50 of gestation, and d 34 vs. d 50 of gestation.

[4]CON = Heifers fed a diet that meets 100% of NRC requirements to gain 1 pound daily. RES = Heifers restricted to 60% of CON diet.

[5]Mean level of expression of treatment group across day of gestation within tissue and gene of interest.

[6]Average SEM was used within gene. NP n = 6, d 16 CON n = 7, d 16 RES n = 7, d 34 CON n = 6, d 34 RES n = 9, d 50 CON n = 7, d 50 RES n = 7

[7]Mean level of expression across treatment within day and gene of interest.

[a-c]Means within gene and tissue without a common superscript differ in day × treatment ($P \leq 0.05$).

[g-h]Means within row without a common superscript differ in main effect of day ($P \leq 0.05$).