Effects of realimentation after nutrient restriction during early to midgestation on maternal and fetal circulating serum insulin and pancreas histology

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The objective of this study was to examine the effects of nutrient restriction followed by realimentation during early to late gestation on fetal and maternal serum insulin concentrations and pancreatic histology. The results indicate that nutrient restriction negatively impacts the concentration of serum insulin in the jugular vein from cows at day 85 of gestation, while cows at later stages showed no significant differences. The fetal pancreas exhibited a shift in islet size due to maternal diet at day 140 but was not impacted during late gestation.

Summary

Multiparous cross-bred cows (n = 46; 1,281 ± 150 pounds) were assigned randomly to dietary treatments on day 30 of gestation. Treatments were: control (C; 100 percent National Research Council [NCR]; n = 18) and nutrient restriction (R; 60 percent NRC; n = 34). On day 85 of gestation, cows were slaughtered (C; n = 6 and R; n = 6) or remained on control (CC; n = 12), restricted (RR; n = 12) or realimented to control (RC; n =11). On day 140, cows were slaughtered (CC; n = 6; RR; n = 6; RC; n = 5), remained on control (CCC; n = 6; RCC; n = 5) or realimented to control (RRC; n = 6). On day 254, all remaining cows were slaughtered. At the time of slaughter, blood was collected from the maternal jugular vein and umbilical cord. Serum was separated from each sample and analyzed for insulin concentration. The pancreas also was removed from maternal and fetal animals and a subsample prepared for histological examination of number and size of pancreatic islets and insulin concentration.

Introduction

The dietary requirements of gestating animals vary based on several factors such as stage of pregnancy, number of offspring and overall animal health. Nutrient availability in feedstuffs also fluctuates throughout the year, making ration balancing challenging and can lead to nutrient restriction. Realimentation frequently is implemented to regain lost productivity, but the timing of realimentation is vital when considering any irreversible effects of nutrient restriction on the developing fetus (Freetly et al., 2000).

One area of concern is the impact nutrient restriction has on insulin production. Insulin is a peptide hormone central to regulating carbohydrate, protein and fat metabolism. During nutrient restriction, insulin secretion is suppressed. Insulin can be restored, however, if dietary levels are returned to an adequate state (Hearing, 2004).

Determining the stage of gestation when insulin production is most vulnerable to dietary restriction is important for establishing an effective realimentation strategy. Several studies have noted a substantial increase in insulin levels during midgestation of ewes, and the levels then decrease as pregnancy progresses to its final term (Vernon et al., 1981). Pododar et al. (2014) reported serum insulin levels increasing from day 90 to 150 of gestation and then decreasing as pregnancy continues. While the majority of fetal growth occurs in the last trimester, Aldoretta et al. (1998) suggested that the fetal pancreas develops during the first three to four months of pregnancy, allowing for the production of measurable fetal insulin concentrations by midgestation.

Recognizing critical developmental periods, along with understanding how insufficient maternal nutrition can affect the fetus, may allow optimal timing for realimentation so that fetal development and animal productivity are not impacted negatively.

Experimental Procedures

On day 30 of pregnancy, multiparous beef cows (n = 46; 1,281 ± 150 pounds) were assigned randomly to dietary treatments: control (C; 100 percent NRC; n = 18) and nutrient restriction (R; 60 percent NRC; n = 34). On day 85 of gestation, cows were slaughtered (C; n = 6 and R; n = 6), or remained on control (CC; n = 12), restricted (RR; n = 12) or realimented to control (RC; n =11). On day 140, cows were slaugh-

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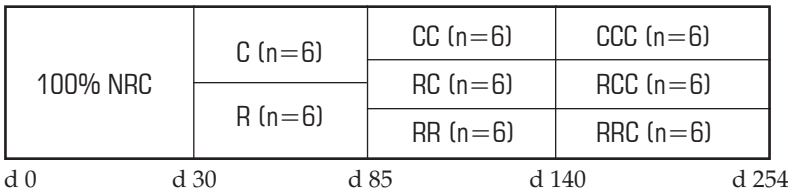
tered (CC; n = 6; RR; n = 6; RC; n = 5), remained on control (CCC; n = 6; RCC; n = 5) or realimented to control (RRC; n = 6). On day 254, all remaining cows were slaughtered (Figure 1).

At the time of slaughter, blood from the maternal jugular vein and umbilical cord was collected into Vacutainer tubes. The blood was allowed to clot and then centrifuged at 2,000 × g for 20 minutes to separate the serum from whole blood. Serum insulin was assayed in duplicate using an Immulite 1000 (Diagnostic Products Corp., Los Angeles, Calif.). Additionally, at the time of slaughter, maternal and fetal pancreatic tissue was removed. A representative area from the body of the pancreas was collected and prepared for histological examination of insulin concentration and islet size. Pancreatic sections were analyzed with a Zeiss Imager.M2 epifluorescence microscope using a 10x objective and AxioCam HR camera with a Zeiss piezo automated stage.

Figure 1. Experimental design.



Results and Discussion

At day 85, insulin concentrations were greater ($P = 0.02$) in jugular serum in the restricted cows than in cows that had received 100 percent of nutrient requirements. This may be due to the cow producing more insulin to compensate for the lack of nutrients and to maintain an appropriate level of insulin for the developing fetus. Unfortunately, we were unable to measure umbilical cord insulin at day 85 because the fetuses were too small and, therefore, collecting umbilical cord blood was not possible.

Maternal jugular insulin was not affected by dietary treatments at day 140 and 254 of gestation. Umbilical cord insulin at these time points also was not impacted, possibly demonstrating that the developing fetus at these stages was able to sustain a stable serum insulin level.

In terms of the endocrine pancreas, dietary treatment did not influence the number or size of islets in cows at any stage of gestation. In fetuses at day 140, however, nutrient restriction decreased ($P = 0.04$) the number of islets between the measurements of 4,225 and 12,674 micrometers[2]. This difference was not apparent at day 254.

No differences between insulin concentrations and islet size for fetuses at day 254 suggests that the realimentation of cows allowed the fetuses to be rescued from the effects of nutrient restriction. This, too, was observed when examining the fetal musculature of the animals in this study (Gonzalez et. al., 2013).

Another thought is that the insulin concentration of serum and pancreatic tissue is unaffected by nutrient restriction at day 254; however, past research results would suggest this theory is unlikely because insulin is a major anabolic hormone of infancy, and studies in nonruminants and ruminants have shown developmental differences between control and nutrient-restricted animals (Garofano et al., 1999 and Long et al., 2010).

Finally, one may suspect that the level of restriction was not severe enough to cause significant differences among the treatments at day 254. Further research is necessary on the effects of nutrient restriction and realimentation on pancreatic development in the calf and later in life.

Table 1. Influence of nutrient restriction on maternal serum insulin and islet histology on day 85.

	Dietary Treatments		*P*-Value
	C[a]	R[b]	Trt
Jugular vein serum insulin, µIu/ml	4.77 ± 2.114	10.4 ± 2.062	0.02
	Pancreatic Insulin		
Percent insulin	0.645 ± 0.3438	0.983 ± 0.3826	0.47
Number of islets between the following measurements:			
6.34 to 1,055 µm^2	494 ± 172.7	223 ± 189.2	0.32
1,056 to 2,112 µm^2	30.0 ± 8.20	31.7 ± 8.79	0.88
2,113 to 4,224 µm^2	16.8 ± 6.66	33.6 ± 6.66	0.12
4,225 to 12,674 µm^2	19.0 11.11	21.0 ± 12.00	0.89
Total islets	551 ± 186.2	312 ± 203.9	0.39

[a]Fed at 100% of NRC recommendations from day 30 to 85.
[b]Fed at 60% of NRC recommendations from day 30 to 85.

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Table 2. Influence of nutrient restriction and refeeding on maternal serum insulin and islet histology on day 140.

	Dietary Treatments			*P*-Value
	CC[a]	RC[b]	RR[c]	Trt
Jugular vein serum insulin, µIu/ml	9.62 ± 2.666	4.55 ± 2.908	10.5 ± 2.667	0.22
	Pancreatic Insulin			
Percent insulin	0.713 ± 0.2411	0.948 ± 0.2695	0.942 ± 0.2411	0.50
Number of islets between the following measurements:				
6.34 to 1,055 µm^2	358 ± 82.9	323 ± 99.0	316 ± 82.9	0.91
1,056 to 2,112 µm^2	37.5 ± 10.7	24.2 ± 12.5	45.7± 10.7	0.33
2,113 to 4,224 µm^2	28.3 ± 9.65	24.6 ± 10.78	36.7 ± 9.66	0.47
4,225 to 12,674 µm^2	18.2 ± 6.98	15.0 ± 7.87	28.9 ± 6.98	0.18
Total islets	441 ± 74.7	368 ± 91.5	439 ± 74.7	0.80

[a]Fed at 100% of NRC recommendations from day 30 to 140.
[b]Fed at 60% of NRC recommendations from day 30 to 85 and 100% of NRC recommendations from day 85 to 140.
[c]Fed at 60% of NRC recommendations from day 30 to 140.

Table 3. Influence of nutrient restriction and refeeding on maternal serum insulin and islet histology on day 254.

	Dietary Treatments			*P*-Value
	CCC[a]	RCC[b]	RRC[c]	Trt
Jugular vein serum insulin, µIu/ml	12.3 ± 3.069	12.7 ± 3.172	15.1 ± 3.011	0.69
	Pancreatic Insulin			
Percent insulin	0.680 ± 0.2120	1.02 ± 0.212	0.622 ± 0.1936	0.37
Number of islets between the following measurements:				
6.34 to 1,055 µm^2	400 ± 155.8	540 ± 144.8	280 ± 138.2	0.30
1,056 to 2,112 µm^2	53.5 ± 6.36	39.1 ± 5.86	37.9 ± 5.55	0.11
2,113 to 4,224 µm^2	26.7 ± 6.59	43.8 ± 6.08	28.2 ± 5.76	0.08
4,225 to 12,674 µm^2	14.5 ± 8.92	31.2 ± 8.08	16.1 ± 7.52	0.28
Total islets	496 ± 164.7	658 ± 154.0	357 ± 147.8	0.22

[a]Fed at 100% of NRC recommendations from day 30 to 254.
[b]Fed at 60% of NRC recommendations from day 30 to 85 and 100% of NRC recommendations from day 85 to 140.
[c]Fed at 60% of NRC recommendations from day 30 to 140 and 100% of NRC recommendations from day 140 to 254.

Table 4. Influence of nutrient restriction and refeeding on umbilical serum insulin and fetal islet histology on day 140.

	Dietary Treatments			*P*-Value
	CC[a]	RC[b]	RR[c]	Trt
Umbilical cord serum insulin, µIu/ml	76.8 ± 7.67	73.6 ± 7.67	75.7 ± 7.63	0.96
	Pancreatic Insulin			
Percent insulin	7.54 ± 2.039	9.10 ± 2.208	7.11 ± 1.896	0.60
Number of islets between the following measurements:				
6.34 to 1,055 µm^2	10,085 ± 1,436.3	6,303 ± 1,547	7,408 ± 1,535	0.21
1,056 to 2,112 µm^2	853 ± 141.6	643 ± 152.6	508 ± 151.3	0.25
2,113 to 4,224 µm^2	299 ± 53.4	174 ± 57.5	154 ± 57.0	0.15
4,225 to 12,674 µm^2	71.2 ± 9.39[a]	33.3 ± 10.11[b]	42.2 ± 10.03[b]	0.037
≥12,675 µm^2	2,985 ± 453.1	2,468 ± 708.9	2,752 ± 588.9	0.83

[a]Fed at 100% of NRC recommendations from day 30 to 140.
[b]Fed at 60% of NRC recommendations from day 30 to 85 and 100% of NRC recommendations from day 85 to 140.
[c]Fed at 60% of NRC recommendations from day 30 to 140.

Table 5. Influence of nutrient restriction and refeeding on umbilical serum insulin and fetal islet histology on day 254.

	Dietary Treatments			*P*-Value
	CCC[a]	RCC[b]	RRC[c]	Trt
Umbilical cord serum insulin, µIu/ml	18.5 ± 2.99	21.2 ± 3.42	18.4 ± 3.03	0.79
	Pancreatic Insulin			
Percent insulin	6.70 ± 6.414	12.2 ± 3.54	8.23 ± 2.990	0.67
Number of islets between the following measurements :				
6.34 to 1,055 µm^2	3,426 ± 837.1	1,960 ± 461.8	2,831 ± 390.3	0.35
1,056 to 2,112 µm^2	322 ± 66.8	345 ± 61.0	398± 52.9	0.46
2,113 to 4,224 µm^2	121 ± 47.7	127 ± 26.3	144 ± 22.2	0.85
4,225 to 12,674 µm^2	45.0 ± 29.6	41.4 ± 16.36	48.3 ± 13.8	0.95
≥12,675 µm^2	300 ± 19.1	331 ± 10.6	324 ± 8.9	0.47

[a]Fed at 100% of NRC recommendations from day 30 to 254.
[b]Fed at 60% of NRC recommendations from day 30 to 85 and 100% of NRC recommendations from day 85 to 140.
[c]Fed at 60% of NRC recommendations from day 30 to 140 and 100% of NRC recommendations from day 140 to 254.