

## IMPACTS OF SUPPLEMENTAL GLUCOGENIC PRECURSORS AND COW AGE ON POSTPARTUM RANGE COW PERFORMANCE

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**ABSTRACT:** Altering nutrient partitioning after calving from milk production to positive energy balance may improve reproductive performance. A 2004 study conducted at the Corona Range and Livestock Research Center evaluated responses of 2- (n = 17), 3- (n = 23), and 4-yr-old (n = 31) postpartum cows grazing native range (11.3% CP and 80% NDF, OM basis) to 3 protein supplements with increasing glucogenic potential (GP). Supplements were fed at 1,135 g·cow<sup>-1</sup>·d<sup>-1</sup> twice weekly for 65 d postpartum and provided: 1) 341 g CP, 142 g ruminally undegradable protein (RUP), 57 g GP (RUP0), 2) 341 g CP, 151 g RUP + 80 g propionate salt (NutroCAL™, Kemin Industries, Inc.), 121 g GP (RUP80), or 3) 341 g CP, 159 g RUP + 160 g propionate salt, 185 g GP (RUP160). A supplement × age interaction occurred for days to first estrus ( $P = 0.10$ ). Days to first estrus was longest for 2-yr-old cows fed RUP0 and then decreased with cow age ( $P \leq 0.04$ ), while for RUP80 and RUP160, return to estrus was similar for 2- and 3-yr-old cows ( $P \geq 0.16$ ) and shorter for 4-yr-old cows ( $P \leq 0.10$ ). Milk production exhibited a quadratic ( $P = 0.03$ ) response to increasing supplemental GP, with cows fed RUP80 producing the least amount of milk at 55 d postpartum (9,982, 8,439, and 9,620 ± 473 g/d for RUP0, RUP80, and RUP160, respectively). Milk production differences did not impact 205-d calf weight ( $P = 0.96$ ; 251 ± 5 kg). Days from BW nadir to estrus decreased linearly with cow age ( $P < 0.01$ ; 33, 22, and 1 ± 4 d for 2-, 3-, and 4-yr-old cows, respectively). Milk production increased linearly with cow age ( $P < 0.01$ ; 7,856, 9,407, and 10,777 ± 509 g/d for 2-, 3-, and 4-yr-old cows respectively). Calf 205-d weight reflected cow age differences in milk production (linear  $P < 0.01$ ; 229, 249, and 274 ± 6 kg for 2-, 3-, and 4-yr-old cows, respectively). Moderate amounts of supplemental GP shifted nutrients away from milk production. Older cows returned to estrus at the same time they reached BW nadir, while younger cows needed to regain weight to return to estrus. Glucogenic precursor addition to protein supplements decreased days to first estrus in postpartum 2-year-old range cows.

Key Words: Beef Cattle, Glucogenic, Reproduction

### Introduction

Protein supplementation is often necessary to meet maintenance nutrient requirements of cows grazing dormant range forage and greater nutrient demands during gestation and lactation amplify the need for supplementation. Young, supplemented range cows often experience a period of negative energy balance and weight loss before and after

parturition (Waterman et al., 2006), and poor reproductive performance of first- and second-calf cows is a challenge faced by cow-calf producers in the southwestern US and other regions. Supplementing ruminally undegradable protein (RUP) once ruminally degradable protein (RDP) requirements are met (NRC, 2000) can encourage nutrient repartitioning from lactation to synthesis of maternal tissues for maintenance, growth and reproduction by way of improved nutrient use (Hunter and Magner, 1988; Triplett et al., 1995; Waterman et al., 2006). It has been suggested that increased supply of protein as RUP may result in alterations in glucose supply and metabolism (Bell and Bauman, 1997; Waterman et al., 2006). Waterman et al. (2006) found that 2-yr-old cows fed protein supplement containing glucogenic precursors provided as glucogenic amino acids from RUP plus 100 g/d propionate salt (NutroCal™, Kemin Industries, Inc.) while grazing dormant range were more sensitive to insulin and returned to estrus 9 d earlier than cows fed traditional cottonseed meal-based protein supplements with no additional glucogenic precursors. The objectives of the current research was to investigate if 2-, 3-, and 4-yr-old postpartum cows would benefit if the amount of glucogenic precursors in the supplements was increased and to determine if cow age influenced response to increased supplemental glucogenic precursors. To accomplish these objectives, we evaluated return to estrus, milk production, weight change responses, and insulin sensitivity of postpartum 2-, 3-, and 4-yr-old range beef cows to supplements with increasing glucogenic potential (GP) provided as 0, 80, or 160 g/d propionate salt.

### Materials and Methods

The experiment was conducted during spring and summer 2004 at the Corona Range and Livestock Research Center, Corona, NM (average elevation = 1900 m; average annual precipitation = 400 mm) located 300 km northeast of Las Cruces, NM. Predominant forages in experimental pastures included blue grama (*Bouteloua gracilis*) and wolftail (*Lycurus phleoides*), as well as other less dominant grasses and forbs (Forbes and Allred, 2001). Three ruminally cannulated cows were used to collect diet samples for analysis of CP (AOAC, 2000) and NDF (Van Soest et al., 1991), which averaged 11.3% and 80%, respectively (OM basis).

All animal handling and experimental procedures were conducted in accordance with guidelines of the Institutional Animal Care and Use Committee of New Mexico State University. Cows (n = 71) were 2 (n = 17), 3

( $n = 23$ ), and 4 ( $n = 31$ ) years of age and predominantly Angus with some Hereford influence. First-calf heifers were wintered separately from older cows and all cows were wintered on pasture with protein supplementation. Within age, cows were assigned to treatment by calving date so that similar age distribution and days postpartum were reflected in each treatment group. Supplements were cubed and milled at Hi-Pro Feeds, Friona, TX and were individually fed at a rate of  $1135 \text{ g}\cdot\text{cow}^{-1}\cdot\text{d}^{-1}$  for 65 d postpartum. Supplementation ceased when breeding season began (15 May). Supplements provided 1) 341 g CP, 142 g RUP, 57 g GP (**RUP0**), 2) 341 g CP, 151 g RUP + 80 g propionate salt (NutroCAL™, Kemin Industries, Inc.), 121 g GP (**RUP80**), or 3) 341 g CP, 159 g RUP + 160 g propionate salt, 185 g GP (**RUP160**). Glucogenic potential was calculated by the equation of Preston and Leng (1987), where 40% of the RUP is considered to be glucogenic (Overton et al., 1999; Vanhatalo et al., 2003). NutroCal™ contains 80% propionate, which was assumed to be 95% glucogenic (Steinour and Bauman, 1988).

Blood samples were collected twice weekly on supplementation days (Monday and Friday) via coccygeal venipuncture beginning approximately 40 d postpartum for analysis of progesterone to determine days to first estrus (2 or more consecutive progesterone concentrations  $> 1 \text{ ng/mL}$ ). Samples were analyzed for progesterone by solid-phase radioimmunoassay (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA) as described by Schneider and Hallford (1996). Inter- and intra-assay coefficients of variation were less than 10%. Cows were diagnosed for pregnancy via rectal palpation at weaning (24 September).

A subsample of cows ( $n = 20$ ) were milked with a portable milking machine approximately 56 d postpartum on a day following supplementation using a modified weigh-suckle-weigh technique (Appeddu et al., 1997). Milk weight was recorded to estimate milk yield. Milk subsamples were collected in preservative-coated vials for analysis of protein, lactose, butterfat, and solids non-fat by an independent laboratory (Pioneer Dairy Labs, DHIA, Artesia, NM).

Cows were weighed weekly until termination of breeding season and at weaning. Days to BW nadir were determined from lowest BW obtained postpartum. Pre-planned intervals of weight change were calculated and included beginning of supplementation to BW nadir, BW nadir to end of supplementation, BW nadir to end of breeding, end of supplementation to end of breeding, and end of breeding to weaning. Body condition scores (**BCS**; 1 = emaciated, 9 = obese) were assigned to each cow by visual observation and palpation at beginning and end of supplementation, beginning and end of breeding season, and at weaning. Calf birth weights were obtained in the field within 3 d after birth with a portable platform scale, and body weight was measured at branding and weaning. Calf weights at branding were adjusted to average calf age at branding (45 d) and adjusted 205-d weaning weights were calculated with no adjustment for sex of calf or age of dam.

A glucose tolerance test (**GTT**) was conducted approximately 65 d postpartum on a subsample of cows ( $n$

$= 20$ ; same cows used for milk production) on a day after supplementation. A 50% dextrose solution was infused at  $0.5 \text{ mL/kg BW}$  via indwelling jugular catheter inserted the morning of the GTT. Blood samples were collected at  $-1, 0, 3, 6, 9, 12, 15, 20, 40, 60, 80, 100, 120, 140, 160,$  and  $180 \text{ min}$  relative to infusion. Glucose was analyzed with a commercial kit (enzymatic endpoint method, Thermo DMA, Louisville, CO). Insulin was analyzed by solid-phase radioimmunoassay (DCP kit, Diagnostic Products Corp., Los Angeles, CA) as validated by Reimers et al. (1982). Intra- and inter-assay coefficients of variation were less than 10%. Serum glucose and insulin areas under the curve (**AUC**) were calculated using trapezoidal summation. Glucose half-life was estimated by determining time required for 50% decrease in peak serum glucose concentration.

Data were analyzed as a completely randomized design by analysis of variance using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) with cow as the experimental unit, using the Kenward-Roger degrees of freedom method. The model included fixed effects of supplement, cow age, and their interaction. Covariates were used when appropriate and included calving date, days on supplement, and calf gender. When appropriate, orthogonal polynomial contrasts were used to test for linear and quadratic effects of increasing supplemental glucogenic precursors and cow age. Pregnancy data were analyzed using the GENMOD procedure of SAS. Significance was determined at  $P \leq 0.10$ .

## Results and Discussion

A supplement  $\times$  age interaction occurred for days to first estrus ( $P = 0.10$ ; Table 1). Two-yr-old cows fed RUP0 took longer to return to estrus than any other group ( $P \leq 0.04$ ), and RUP0-fed cows exhibited decreasing days to first estrus with increasing cow age. Cows fed RUP80 and RUP160 had similar patterns of return to estrous cyclicity where days to first estrus were similar for 2- and 3-yr-old cows ( $P \geq 0.16$ ) and shorter for 4-yr-old cows ( $P \leq 0.10$ ). Increasing glucogenic precursor delivery to postpartum cows had the most beneficial effect on return to estrus in 2-yr-old cows. Pregnancy rates were above 95% for all supplement groups, and all cows fed RUP80 were pregnant in the fall. Calving intervals were slightly over a year in length and were similar ( $P = 0.99$ ) regardless of supplement group.

Milk production exhibited a quadratic ( $P = 0.03$ ; Table 2) response to increasing supplemental GP, with cows fed RUP80 producing the least amount of milk at 55 d postpartum. Milk lactose and solids non-fat production exhibited the same quadratic trend ( $P \leq 0.03$ ) as 24-h milk production, while milk butterfat and protein production were similar regardless of supplement group ( $P \geq 0.23$ ). Decreased milk production when 80 g of propionate salt was added to the supplement suggests that this combination of glucogenic precursors (metabolizable protein from RUP plus propionate salt) shifted nutrient partitioning away from milk production. Similarly, Waterman et al. (2006) found a 9% decrease in milk production when cows were fed

bypass protein + 100 g/d propionate salt compared with bypass protein without additional propionate. In contrast however, Waterman et al. (2006) found a 25% reduction in butterfat secretion for cows supplemented with additional propionate while butterfat was similar for all treatment groups in the current experiment. Similar results to Waterman et al. (2006) were observed for dairy cows fed calcium propionate in a total mixed ration; decreased milk fat was attributed to decreased adipose tissue mobilization (Mandebvu et al., 2003). Milk production differences did not impact calf weights at branding ( $P = 0.69$ ) or weaning (205-d weight;  $P = 0.96$ ).

Cow BW were similar for all supplement groups at all measurement times during the experiment ( $P \geq 0.58$ ; Table 2). Days to BW nadir increased linearly ( $P < 0.01$ ) with increasing supplemental glucogenic precursors, but magnitude of BW nadir was similar across supplement groups ( $P = 0.87$ ), and all other BW change intervals were similar as well ( $P \geq 0.52$ ). Days from BW nadir to first estrus were similar for all supplement groups ( $P = 0.21$ ). Cow BCS were similar for all treatment groups at beginning and end of supplementation ( $P \geq 0.68$ ), and tended ( $P = 0.15$ ) to exhibit a quadratic relationship ( $P = 0.06$ ) at termination of breeding, when RUP80-fed cows had the highest BCS. A supplement  $\times$  cow age interaction occurred at weaning ( $P = 0.10$ ; Table 3). Two-yr-old cows fed RUP80 had higher BCS at weaning than their counterparts. For 3-yr-old cows, the RUP 160 group had higher BCS than RUP0-fed cows, with RUP80-fed cows intermediate. Four-yr-old cows had similar BCS at weaning regardless of supplement.

Cows had similar response to GTT regardless of supplement ( $P \geq 0.32$ ; Table 2). Glucose half-lives for all cows were similar to those in Waterman et al. (2006) and these authors determined that cows fed supplements with increased GP in the form of additional metabolizable protein (i.e., RUP) and/or propionate salt had shorter glucose half-lives and were more sensitive to insulin than cows fed a traditional cottonseed meal-based supplement with no additional glucogenic precursors (avg 63 vs 100 min, respectively). All cows in the present study were fed supplements with additional GP from metabolizable protein and/or propionate salt and exhibited similar glucose half-lives to those of the previous study. Cows in both studies were considered insulin-resistant, as glucose half-lives were approximately 2-fold higher than the normal value of 35 min described by Kaneko (1997).

All 2-yr-old cows were pregnant in the fall, with all pregnancy rates above 90% (Table 4). The lowest pregnancy rates were for 3-yr-old cows. Calving intervals were slightly over a year in length for all cow ages ( $P = 0.49$ ), which might be unexpected based on shorter days to first estrus observed for 4-yr-old cows.

Milk production and milk constituents increased linearly with cow age ( $P < 0.01$ ). Three-yr-old cows produced ~20% more milk than 2-yr-old cows, and 4-yr-old cows produced ~15% more milk than 3-yr-olds. These milk production differences resulted in a linear increase in calf branding and 205-d weights with cow age ( $P < 0.01$ ). For each year increase in cow age, a corresponding 20-25 kg increase in calf 205-d weight was observed.

Cow BW at supplementation start increased linearly ( $P < 0.01$ ) with cow age as expected. At subsequent measurement times, BW increased in a quadratic fashion with increasing cow age. Throughout the experiment, 3-yr-old cows were approximately 73 kg heavier than 2-yr-old cows, compared to a 24 kg BW difference between 3- and 4-yr-old cows. Cows lost increasing amounts of weight from start of supplementation to BW nadir with increasing cow age ( $P < 0.01$ ). Similar gains were observed from BW nadir to end of supplementation regardless of age ( $P = 0.70$ ), but a quadratic pattern of BW gain was observed for the period from BW nadir to end of breeding ( $P \leq 0.01$ ). During this time period, 3-yr-old cows gained more weight than 2- or 4-yr-old cows. A similar trend ( $P = 0.15$ ) was noted from the end of supplementation to end of breeding, but all cows gained similar amounts of weight from the end of breeding to weaning ( $P = 0.69$ ). Days to BW nadir were similar ( $P = 0.46$ ) regardless of cow age. Days from BW nadir to estrus decreased linearly with cow age ( $P < 0.01$ ). Two-yr-old cows returned to estrus approximately 1 mo after reaching BW nadir, compared to 3-yr-old cows who returned to estrus approximately 3 wk after BW nadir. Interestingly, 4-yr-old cows returned to estrus at essentially the same time as they reached BW nadir, implying that BW loss has less of an impact on reproductive performance in mature cows. Cow BCS at start of supplementation exhibited a quadratic ( $P = 0.10$ ) pattern to increasing cow age, where 4-yr-olds were in higher body condition than 2- and 3-yr-old cows. At the end of supplementation and end of breeding, cow BCS increased linearly with cow age ( $P \leq 0.02$ ).

Glucose AUC, insulin AUC, and insulin:glucose ratio were similar ( $P \geq 0.53$ ) for all cows, regardless of age. A weak linear trend (overall  $P = 0.24$ ; linear  $P = 0.10$ ) was detected for decreasing glucose half-life with increasing cow age, implying increased metabolic efficiency.

## Implications

Glucogenic precursor addition to protein supplements decreased days to first estrus in postpartum 2-year-old range cows, but did not have the same effect in 3- and 4-yr-old cows. Moderate amounts of supplemental GP shifted nutrients away from milk production regardless of cow age. Older cows returned to estrus at the same time they reached BW nadir, while younger cows needed to regain weight to return to estrus. Strategic supplementation with a combination of glucogenic precursors may be best suited to shift nutrient partitioning in young postpartum range cows grazing dormant forage, and may serve as a tool to enhance cow longevity and sustainability of extensively managed ranches in the western United States.

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Table 1. Supplement × cow age interaction ( $P = 0.10$ ) for days to first estrus for 2-, 3-, and 4-yr-old postpartum cows grazing native range and fed supplements containing increasing amounts of glucogenic potential (0, 80, 160 g/d propionate salt).

Cow Age	Supplement					
	RUP0	SEM	RUP80	SEM	RUP160	SEM
2	90 <sup>ax</sup>	6	68 <sup>bx</sup>	7	70 <sup>bxy</sup>	8
3	70 <sup>ay</sup>	6	63 <sup>ax</sup>	6	74 <sup>ax</sup>	5
4	46 <sup>az</sup>	5	50 <sup>ay</sup>	5	55 <sup>ay</sup>	5

<sup>a,b</sup> Within row, values with different superscripts differ ( $P \leq 0.10$ ).

<sup>x,y</sup> Within column, values with different superscripts differ ( $P \leq 0.10$ ).

Table 2. Effect of supplements containing increasing amounts of glucogenic potential (0, 80, or 160 g/d propionate salt) on reproduction, milk production, calf weight, cow weight and body condition score, and glucose tolerance test responses for 2-, 3-, and 4-yr-old postpartum cows grazing native range.

Response	Supplement						<i>P</i> -value	Contrast	
	RUP0	SEM	RUP80	SEM	RUP160	SEM		Linear	Quadratic
Pregnancy Rate, %	96	--	100	--	96	--	0.24	--	--
Ratio	22/23	--	24/24	--	24/25	--	--	--	--
Calving interval, d	370	3	371	3	371	3	0.98	0.86	0.99
Milk, g/d									
24-h production	9,982	443	8,439	473	9,620	437	0.08	0.56	0.03
Butterfat	342	27	297	28	361	25	0.26	0.62	0.13
Protein	271	16	232	16	262	15	0.23	0.67	0.10
Lactose	513	28	412	29	485	26	0.06	0.47	0.02
Solids non-fat	879	49	718	50	835	44	0.09	0.51	0.03
Calf BW, kg									
Branding	69	2	69	2	71	2	0.69	0.51	0.57
Weaning (205-d)	252	5	250	5	250	5	0.96	0.86	0.84
Cow BW, kg									
Begin supplementation	415	9	412	9	402	9	0.60	0.32	0.80
BW Nadir	361	7	355	8	350	8	0.58	0.30	0.97
End supplementation	395	9	393	9	389	9	0.89	0.64	0.90
End breeding	446	9	450	9	441	9	0.80	0.69	0.59
Weaning	500	10	504	11	500	11	0.96	0.96	0.77
Cow BW change, kg									
Begin supp – Nadir	-54	4	-56	4	-53	4	0.87	0.87	0.61
Nadir – End supp	34	5	37	5	39	5	0.70	0.40	0.95
Nadir – End breed	85	5	93	5	88	5	0.52	0.70	0.28
End supp – End breed	52	6	56	6	49	6	0.76	0.75	0.50
End breed – Wean	54	4	54	4	58	4	0.75	0.53	0.67
Days to BW nadir	45	1	47	1	50	1	0.01	< 0.01	0.42
Days from nadir to estrus	24	4	14	4	18	4	0.21	0.26	0.18
Cow BCS									
Begin supplementation	4.2	0.10	4.2	0.10	4.1	0.10	0.82	0.58	0.75
End supplementation	4.4	0.11	4.5	0.11	4.4	0.11	0.68	0.68	0.43
End breeding	4.6	0.12	4.9	0.12	4.5	0.12	0.15	0.54	0.06
GTT Response									
Glucose half-life, min	62	13	60	14	66	13	0.95	0.82	0.82
Glucose AUC	6,434	1,002	6,635	1,047	7,317	1,002	0.81	0.54	0.85
Insulin AUC	171	16	174	17	157	16	0.75	0.55	0.65
Insulin:glucose ratio	0.034	0.004	0.028	0.005	0.024	0.005	0.32	0.15	0.82

Table 3. Supplement  $\times$  cow age interaction ( $P = 0.10$ ) for body condition score at weaning for 2-, 3-, and 4-yr-old postpartum cows grazing native range and fed supplements containing increasing amounts of glucogenic potential (0, 80, 160 g/d propionate salt).

Cow Age	Supplement					
	RUP0	SEM	RUP80	SEM	RUP160	SEM
2	4.4 <sup>ax</sup>	0.14	4.9 <sup>bxy</sup>	0.16	4.5 <sup>ax</sup>	0.16
3	4.3 <sup>ax</sup>	0.15	4.6 <sup>bx</sup>	0.13	4.7 <sup>bx</sup>	0.12
4	4.9 <sup>aby</sup>	0.11	5.0 <sup>ay</sup>	0.10	4.7 <sup>bx</sup>	0.11

<sup>a,b</sup> Within row, values with different superscripts differ ( $P \leq 0.10$ ).

<sup>x,y</sup> Within column, values with different superscripts differ ( $P \leq 0.10$ ).

Table 4. Effect of cow age on reproduction, milk production, calf weight, cow weight and body condition score, and glucose tolerance test responses for 2-, 3-, and 4-yr-old postpartum cows grazing native range and fed supplements containing increasing amounts of glucogenic potential (0, 80, or 160 g/d propionate salt).

Response	Cow Age						<i>P</i> -value	Contrast	
	2	SEM	3	SEM	4	SEM		Linear	Quadratic
Pregnancy Rate, %	100	--	91	--	97	--	0.30	--	--
Ratio	17/17	--	21/23	--	30/31	--	--	--	--
Calving interval, d	369	4	368	3	373	2	0.49	0.50	0.45
Milk, g/d									
24-h production	7,856	509	9,407	476	10,777	477	< 0.01	< 0.01	0.88
Butterfat	244	29	347	30	408	28	< 0.01	< 0.01	0.60
Protein	208	15	250	16	308	16	< 0.01	< 0.01	0.71
Lactose	383	30	475	31	553	29	< 0.01	< 0.01	0.86
Solids non-fat	653	51	815	53	964	50	< 0.01	< 0.01	0.92
Calf BW, kg									
Branding	61	2	68	2	79	2	< 0.01	< 0.01	0.38
Weaning (205-d)	229	6	249	5	274	4	< 0.01	< 0.01	0.62
Cow BW, kg									
Begin supplementation	340	12	418	9	472	8	< 0.01	< 0.01	0.27
BW Nadir	297	9	368	8	399	6	< 0.01	< 0.01	0.04
End supplementation	335	12	406	9	436	8	< 0.01	< 0.01	0.07
End breeding	390	10	466	9	482	7	< 0.01	< 0.01	0.01
Weaning	445	12	519	10	539	9	< 0.01	< 0.01	0.04
Cow BW change, kg									
Begin supp – Nadir	-42	5	-50	4	-72	3	< 0.01	< 0.01	0.12
Nadir – End supp	33	5	37	5	39	4	0.70	0.38	0.86
Nadir – End breed	79	5	100	5	87	4	0.01	0.26	< 0.01
End supp – End breed	46	7	62	6	48	5	0.14	0.76	0.05
End breed – Wean	53	6	54	4	58	4	0.69	0.51	0.72
Days to BW nadir	47	1.0	47	1.0	48	0.9	0.46	0.42	0.46
Days from nadir to estrus	33	4	22	4	1	3	< 0.01	< 0.01	0.24
Cow BCS									
Begin supplementation	4.0	0.13	4.0	0.09	4.5	0.08	< 0.01	< 0.01	0.10
End supplementation	4.1	0.14	4.4	0.11	4.8	0.10	< 0.01	< 0.01	0.70
End breeding	4.5	0.15	4.6	0.12	4.9	0.10	0.03	0.02	0.45
GTT Response									
Glucose half-life, min	81	13	59	13	49	14	0.24	0.10	0.72
Glucose AUC	7,655	1,002	6,555	1,002	6,175	1,047	0.57	0.32	0.77
Insulin AUC	174	16	164	16	164	17	0.87	0.67	0.79
Insulin:glucose ratio	0.026	0.005	0.033	0.005	0.027	0.005	0.53	0.88	0.27