JOURNAL OF ANIMAL SCIENCE
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J ANIM SCI 2012, 90:3266-3273.
doi: 10.2527/jas.2011-4959 originally published online May 14, 2012

Plasma progesterone concentration in beef heifers receiving exogenous glucose, insulin, or bovine somatotropin[1]

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ABSTRACT: Three experiments were conducted to evaluate plasma concentrations of glucose, insulin, IGF-I, and progesterone (P4) in pubertal beef heifers receiving exogenous glucose, insulin, or sometribove zinc. All heifers used had no luteal P4 synthesis but received a controlled internal drug-releasing device containing 1.38 g of P4 to estimate treatment effects on hepatic P4 degradation. In Exp. 1, 8 pubertal, nulliparous Angus × Hereford heifers (initial BW = 442 ± 14 kg; initial age = 656 ± 7 d) were randomly assigned to receive, in a crossover design containing 2 periods of 10 h, intravenous (i.v.) infusions (10 mL) of insulin (1 μg/kg of BW; INS) or saline (0.9%; SAL). Treatments were administered via jugular venipuncture in 7 applications (0.15 μg insulin/kg BW per application) 45 min apart (from 0 to 270 min). Blood samples were collected immediately before each infusion as well as at −120, −60, 330, 390, and 450 min relative to the first infusion. Heifers receiving INS had greater ($P < 0.01$) plasma insulin, reduced ($P \leq 0.04$) plasma glucose and IGF-I, and similar ($P = 0.62$) plasma P4 concentrations compared with SAL heifers. In Exp. 2, the same heifers were assigned to receive, in a similar experimental design as Exp. 1, i.v. infusions (10 mL) of 1) insulin (1 μg/kg BW) and glucose (0.5 g/kg BW; INS+G) or 2) SAL. Heifers receiving INS+G had greater ($P \leq 0.02$) plasma insulin, glucose, and P4 but reduced ($P = 0.01$) plasma IGF-I concentrations compared with SAL heifers. In Exp. 3, the same heifers were assigned to receive, in a crossover design containing 2 periods of 14 d, subcutaneous (s.c.) injections of 1) 250 mg of sometribove zinc (BST) or 2) SAL. Blood samples were collected 3 h apart (0900, 1200, 1500, and 1800 h) from heifers on d 6, 8, and 10 relative to treatment administration (d 1). Heifers receiving BST had greater ($P < 0.01$) plasma glucose and IGF-I and similar ($P \geq 0.67$) plasma insulin and P4 concentrations compared with SAL heifers. Results from this series of experiments suggested that concurrent increases in glucose and insulin are required to reduce hepatic catabolism and increase plasma concentrations of P4 in bovine females.

Key words: beef heifers, glucose, insulin, insulin-like growth factor-I, progesterone

 J. Anim. Sci. 2012.90:3266–3273
doi:10.2527/jas2011-4959

INTRODUCTION

Nutrition, more specifically energy intake, is the environmental factor that most influences reproductive function in beef females (Mass, 1987). Several research studies demonstrated that energy intake can be positively associated with hastened attainment of puberty, decreased postpartum interval, and greater pregnancy rates (Wiltbank et al., 1962; Schillo et al., 1992; Looper et al., 2003; Pescara et al., 2010). Moreover, beneficial effects of energy intake on cattle reproduction are regulated, at least partially, by circulating hormones and metabolites such as glucose, insulin, and IGF-I (Wettemann et al., 2003).

As an example, insulin modulates circulating concentrations of progesterone (**P4**; Lopes et al., 2009), a steroid required for resumption of estrous cycles and establishment and maintenance of pregnancy (Spencer and Bazer, 2002; Looper et al., 2003). More specifi-

[1]The Eastern Oregon Agricultural Research Center, including the Burns and Union stations, is jointly funded by the Oregon Agricultural Experiment Station and ARS-USDA. Appreciation is expressed to Flavia Cooke and Arthur Nyman (Oregon State University) for their assistance during this study.

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Received November 26, 2011.
Accepted March 20, 2012.

cally, insulin stimulates luteal P4 synthesis (Spicer and Echternkamp, 1995) and alleviates hepatic steroid catabolism (Murray, 1991; Lemley et al., 2008). Our research group recently reported that cows in adequate nutritional status receiving intravenous (**i.v.**) glucose infusion to increase circulating insulin concentrations had greater plasma P4 concentrations compared with cohorts receiving saline, which was attributed to reduced hepatic P4 degradation given that cows were ovariectomized and supplemented with exogenous P4 (Vieira et al., 2010). However, glucose supplementation also increases circulating concentrations of other hormones associated with reproductive and hepatic function, including glucose itself and IGF-I (Jones and Clemmons, 1995; Schroeder et al., 2006). Therefore, we hypothesized that the insulin-stimulated decrease in hepatic P4 catabolism may also be dependent on circulating glucose and IGF-I. On the basis of this rationale, 3 experiments were conducted to evaluate plasma concentrations of glucose, insulin, IGF-I, and P4 in beef females receiving exogenous insulin, insulin plus glucose, or ST.

MATERIALS AND METHODS

All experiments were conducted at the Oregon State University Eastern Oregon Agricultural Research Center (Burns, OR) from January to March 2011. Animals used were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Oregon State University, Institutional Animal Care and Use Committee.

Experiment 1

Animals. Eight pubertal, nulliparous Angus × Hereford heifers (initial BW = 452 ± 12 kg; initial age = 656 ± 7 d) were assigned to an estrus synchronization protocol (d −16 to 0 of the study) with the purpose of attaining heifers with no luteal P4 synthesis but similar and substantial plasma P4 concentrations originating from an exogenous source. Heifers received a 100-μg treatment of GnRH (Cystorelin; Merial Ltd., Duluth, GA) and a controlled internal drug-releasing device containing 1.38 g of P4 (CIDR; Pfizer Animal Health, New York, NY) on d −16, PG $F_{2\alpha}$ treatment (25 mg Lutalyse; Pfizer Animal Health) and CIDR removal on d −9, and a second GnRH treatment (100 μg) on d −7. On d 0, heifers received another PG $F_{2\alpha}$ treatment (25 mg) and a CIDR that remained in heifers throughout Exp. 1 (d 0 to 14). Transrectal ultrasonography examinations (5.0-MHz transducer, 500V; Aloka, Wallingford, CT) were performed immediately and 48 h after the second GnRH (d −7) and PG $F_{2\alpha}$ (d 0) treatments to verify ovulation and corpus luteum regression, respectively. All heifers used in this experiment responded to the hormonal treatment.

Insulin Infusion and Sampling. Heifer BW was recorded at the beginning and end of the experiment (d 0 and 14). On d 5, heifers were randomly assigned to receive, in a crossover design containing 2 periods of 10 h each (within d 6 and d 8), 1) i.v. insulin infusion (1 μg/kg BW; **INS**) or 2) i.v. saline infusion (0.9%; **SAL**). Bovine insulin solution (catalog number I0516; Sigma-Aldrich Co. LLC, St. Louis, MO) was dissolved into 10 mL of physiological saline immediately before infusions and was administered via jugular venipuncture in 7 applications (0.15 μg/kg BW per application) 45 min apart (0, 45, 90, 135, 180, 225, and 270 min), whereas SAL heifers concurrently received 10 mL of physiological saline (0.9%). The INS treatment used herein was based on the hourly insulin dose reported by Butler et al. (2003) that promptly and substantially increased plasma insulin concentrations. Blood samples were collected immediately before each infusion as well as at −120, −60, 330, 390, and 450 min relative to the first infusion. All heifers were fasted for 12 h before the beginning of each period and remained fasted during sampling to prevent any confounding effects between feed intake and infusion treatments on circulating concentrations of P4 (Vasconcelos et al., 2003). Immediately after the last blood collection of each period, heifers were offered their daily diet.

Experiment 2

Animals. Immediately after the end of Exp. 1 (d 14), the same heifers (mean BW = 456 ± 14 kg) received a new CIDR and were evaluated via transrectal ultrasonography (5.0-MHz transducer, 500V; Aloka) to confirm the absence of a corpus luteum. None of the heifers ovulated and developed a corpus luteum during Exp. 1 and were therefore immediately assigned to Exp. 2 (d 14 to 28).

Glucose + Insulin Infusion and Sampling. Heifer BW was recorded at the beginning and end of the experiment (d 14 and 28). On d 20, heifers were randomly assigned to receive, in a crossover design containing 2 periods of 10 h each (within d 20 and 22), 1) i.v. infusion containing insulin (1 μg/kg BW) and glucose (0.5 g/kg BW; **INS+G**) or 2) i.v. saline infusion (0.9%; **SAL**). Glucose (catalog number G8270; Sigma-Aldrich Co.) and bovine insulin solution (catalog number I0516; Sigma-Aldrich Co.) were dissolved into 10 mL of physiological saline immediately before infusions and were administered via jugular venipuncture in 7 applications (0.07 g/kg BW and 0.15 μg/kg BW per application for glucose and insulin, respectively) 45 min apart (0, 45, 90, 135, 180, 225, and 270 min), whereas SAL heifers

concurrently received 10 mL of physiological saline (0.9%). The INS+G treatment used herein was based on the hourly insulin dose reported by Butler et al. (2003) and the total glucose dose reported by Vieira et al. (2010). Blood samples were collected immediately before each infusion as well as at −120, −60, 330, 390, and 450 min relative to the first infusion. As in Exp. 1, heifers were fasted for 12 h before the beginning and during sampling and were offered their daily diet immediately after the last blood collection of each period.

Experiment 3

Animals. Immediately after the end of Exp. 2 (d 28), heifers (mean BW = 462 ± 14 kg) received a new CIDR and were evaluated via transrectal ultrasonography (5.0-MHz transducer, 500V; Aloka) to confirm the absence of a corpus luteum. Again, none of the heifers ovulated and developed a corpus luteum during Exp. 2 and were therefore immediately assigned to Exp. 3 (d 28 to d 55).

Somatotropin Application and Sampling. Heifer BW was recorded at the beginning and end of the experiment (d 28 and 55). On d 28, heifers were randomly assigned to receive, in a crossover design containing 2 periods of 14 d each (d 28 to 42 and d 42 to 56), 1) subcutaneous (**s.c.**) injection containing 250 mg sometribove zinc (**BST**; Posilac; Elanco, Greenfield, IN) or 2) s.c. saline injection (0.9%; **SAL**). Treatments were applied once, at 0800 h, during the first day of each period (d 28 and 42). Heifers also received a new CIDR at the beginning of the second period concurrently with treatment administration (d 42). The BST treatment was developed on the basis of previous research demonstrating that application of 250 mg of sometribove zinc every 14 d increases plasma IGF-I concentrations in beef heifers (Buskirk et al., 1996).

Four blood samples were collected 3 h apart (from 0900 to 1800 h) from heifers on d 33, 35, 37 (period 1) and 47, 49, and 51 (period 2) of the experiment. Similar to Exp. 1 and Exp. 2, all heifers were fasted for 12 h before the beginning and during each collection day to prevent any confounding effects between feed intake and treatments on circulating concentrations of P4 (Vasconcelos et al., 2003). Heifers were offered their daily diet immediately after the last blood collection of each day (1800 h).

Diets

During all experiments, heifers were individually offered (as-fed basis) 12 kg of mixed alfalfa-grass hay, 1.0 kg of ground corn, and 0.5 kg of camelina meal in the morning (0700 h). Heifers also received a complete commercial mineral and vitamin mix (14% Ca, 10% P, 16% NaCl, 1.5% Mg, 6000 mg/kg Zn, 3200 mg/kg Cu, 65 mg/kg I, 900 mg/kg Mn, 140 mg/kg Se, 136 IU/g of vitamin A, 13 IU/g of vitamin D_3, and 0.05 IU/g of vitamin E) and water for ad libitum consumption. Nutritional composition of the hay, corn, and camelina meal were analyzed by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY) using samples collected at the beginning of Exp. 1. All samples were analyzed by wet chemistry procedures for concentrations of CP [method 984.13; Association of Official Analytical Chemists (**AOAC**), 2006], ADF (method 973.18 modified for use in an Ankom 200 fiber analyzer; Ankom Technology Corp., Fairport, NY; AOAC, 2006), and NDF (Van Soest et al., 1991; method for use in an Ankom 200 fiber analyzer; Ankom Technology Corp.) with no inclusion of Na sulfite or α-amylase. Calculations of TDN used the equation proposed by Weiss et al. (1992), whereas NE_m and NE_g were calculated with the equations proposed by NRC (1996). The nutritional quality of hay, corn, and camelina meal was estimated at (DM basis), respectively, 54%, 89%, and 95% TDN; 60%, 15%, and 32% NDF; 40%, 4%, and 22% ADF; 1.1, 2.2, and 2.6 Mcal/kg of NE_m; 0.6, 2.1, and 2.4 Mcal/kg of NE_g; and 12.8%, 9.8%, and 34.7% CP.

Blood Analysis. Blood samples were collected via jugular venipuncture into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing 158 United States Pharmacopeia units of freeze-dried sodium heparin, immediately placed on ice, and centrifuged at 2,500 × *g* for 30 min at 4°C for plasma collection. Plasma was frozen at −80°C on the same day as collection.

Glucose concentrations were determined using a quantitative colorimetric kit (catalog number G7521; Pointe Scientific, Inc., Canton, MI). Insulin concentrations were determined using a bovine-specific commercial ELISA kit (B1009; Endocrine Technologies Inc., Newark, CA). Concentrations of IGF-I and P4 were determined using human-specific commercial ELISA kits with 100% cross-reactivity with the respective bovine hormone. Nevertheless, the IGF-I procedure (SG100; R&D Systems, Inc., Minneapolis, MN) was previously validated for bovine samples by our research group (Moriel et al., 2012). The P4 procedure (11-PROHU-E01; Alpco Diagnostics, Salem, NH) was also validated for bovine samples using plasma pools collected from yearling beef steers that were charcoal-stripped (Sharpe and Cooper, 1984), enriched with known concentrations of P4 (0.0, 0.5, 1.0, 2.5, 5.0, and 10.0 ng/mL), and included into each assay as quality controls. Across all assays, the mean r^2 value between the expected and observed results among pools with known P4 concentrations was 0.993 ± 0.002, and mean P4 concentrations for each pool were 0.02 ± 0.01, 0.50 ± 0.04, 1.03 ± 0.03, 2.7 ± 0.11, 5.59

± 0.11, and 12.29 ± 0.30 ng/mL for, respectively, pools enriched with 0.0, 0.5, 1.0, 2.5, 5.0, and 10.0 ng/mL of P4. Across all experiments, the intra- and interassay CV were, respectively, 3.2% and 2.2% for glucose, 3.3% and 10.6% for insulin, 2.0% and 4.2% for IGF-I, and 3.9% and 4.3% for P4.

Statistical Analysis. All data were analyzed using the MIXED procedure (SAS Inst., Inc., Cary, NC) and Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects. Heifer was considered the experimental unit for all analysis. The model statement used for Exp. 1 and 2 contained the effects of treatment, time, and the resultant interaction in addition to period as an independent variable. Data obtained before treatment application (−120, −60, and 0 min before infusion) were averaged and used as covariate. Heifer was used as a random variable. The specified term for the repeated statement was time, and heifer (treatment × period) was included as the subject. The covariance structure used was autoregressive, which provided the smallest Akaike information criterion and hence the best fit for all variables analyzed. Results are reported as covariately adjusted least-squares means if the covariate was significant ($P \leq 0.05$) and were separated by LSD. The model statement used for Exp. 3 contained the effects of treatment, day, time, and all interactions, in addition to period as an independent variable. Heifer was used as a random variable. The specified term for the repeated statement was time, and heifer (treatment × day × period) was included as the subject. The covariance structure used was also autoregressive by providing the smallest Akaike information criterion for all variables analyzed. The model statement used for change in heifer BW during the entire study contained the effects of day, whereas heifer was used as a random variable. Results are reported as least-squares means and separated using LSD. For all analysis, significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and ≤ 0.10. Results are reported according to treatment effects if no interactions were significant or according to the greatest-order interaction detected.

RESULTS AND DISCUSSION

Recent research studies demonstrated that insulin may increase circulating concentrations of P4 in bovine females by alleviating hepatic P4 catabolism. Postpartum dairy cows receiving continuous i.v. infusion of insulin and glucose for 4 d had reduced expression of hepatic enzymes associated with catabolism of P4, such as CYP2C and CYP3A (Murray, 1991), compared with cohorts infused with saline (Butler et al., 2003; Lemley et al., 2008). However, these authors did not evaluate plasma P4 concentrations to directly assess the relationship among increased plasma insulin, reduced CYP2C and CYP3A expression, and circulating P4. Our research group recently reported that nonlactating cows in adequate nutritional status and receiving i.v. glucose infusion for 3 h had greater plasma concentrations of insulin and P4 compared with cohorts receiving saline (Vieira et al., 2010). This outcome was attributed to reduced hepatic P4 degradation given that cows were ovariectomized and supplemented with exogenous P4. However, glucose supplementation may also increase circulating concentrations of other hormones associated with reproductive and hepatic function in cattle, including glucose itself and IGF-I (Rutter et al., 1989; Jones and Clemmons, 1995; Schroeder et al., 2006). Therefore, this series of experiments was conducted using beef heifers with no luteal P4 synthesis and receiving exogenous P4 to estimate if insulin increases plasma P4 concentrations by reducing hepatic P4 catabolism independently of plasma concentrations of glucose and IGF-I. One could speculate that adrenal or follicular P4 synthesis (Spicer et al., 1993; O'Connor et al. 2000), as well as mobilization of P4 stored in adipose tissues (Hamudikuwanda et al., 1996), may have contributed to plasma P4 concentrations of heifers evaluated herein. However, recent research studies demonstrated that follicular contribution to circulating P4 is negligible (Cerri et al., 2011), whereas short-term fasting is not expected to stimulate stress-induced synthesis of adrenal P4 or release of P4 from adipose tissues (Rodrigues et al., 2010)

Experiment 1

Heifer BW did not change ($P = 0.51$; data now shown) during the experimental period (442 and 456 kg of BW on d 0 and 14; SEM = 14), indicating that heifers were in adequate nutritional status. A treatment effect was detected ($P < 0.01$) for plasma insulin (Table 1). Insulin concentrations before treatment infusion were significant covariates ($P < 0.01$) but did not differ ($P = 0.61$; data not shown) between INS and SAL heifers (1.06 vs. 1.09 ng/mL, respectively; SEM = 0.85). As expected from the experimental design, mean plasma insulin concentration during the experimental period was greater ($P < 0.01$) for INS compared with SAL heifers (Table 1).

A treatment × time interaction was detected ($P = 0.01$) for plasma glucose (Figure 1). Glucose concentrations before treatment infusion were significant covariates ($P = 0.01$) but did not differ ($P = 0.57$; data not shown) between INS and SAL heifers (77.1 vs. 78.2 mg/dL, respectively; SEM = 2.1). After the initial infusion, plasma glucose decreased for INS heifers (time effect;

Table 1. Plasma concentrations of glucose, insulin, IGF-I, and progesterone (P4) in beef heifers receiving intravenous infusion of insulin (1 μg/kg BW; INS, n = 8) or saline (0.9%; SAL, n = 8) in Exp. 1[1–3]

Item[4]	INS	SAL	SEM	*P*-value
Glucose, mg/dL	68.2	79.0	1.3	<0.01
Insulin, ng/mL	1.40	0.99	0.10	<0.01
IGF-I, ng/mL	145	154	3	0.04
P4, ng/mL	3.74	3.84	0.27	0.65

[1]Bovine insulin was dissolved into 10 mL of physiological saline (0.9%) immediately before infusions and was administered via jugular venipuncture in 7 applications (0.15 μg/kg BW per application) 45 min apart.

[2]Plasma samples were collected immediately before each infusion as well as at −120, −60, 330, 390, and 450 min relative to the first infusion.

[3]Beef heifers with no luteal P4 synthesis and receiving exogenous P4 from a controlled internal drug-releasing device containing 1.38 g of P4 (CIDR, Pfizer Animal Health, New York, NY).

[4]Least-squares means covariately adjusted to values obtained before the first infusion (−120, −60, and 0 min).

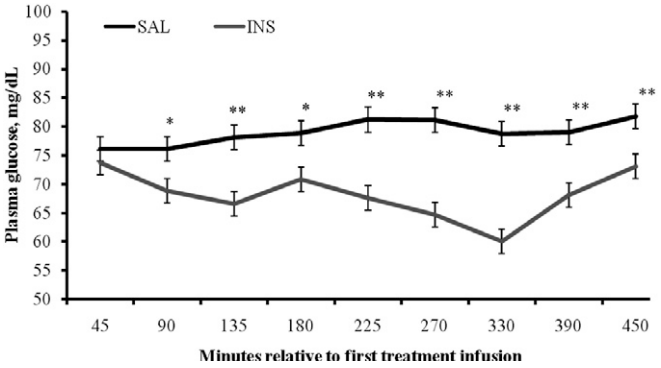

Figure 1. Plasma glucose concentrations (±SEM) of heifers receiving intravenous infusions containing 10 mL of physiological saline (0.9%; n = 8) or 0.15 μg/kg BW of insulin (INS; n = 8). Bovine insulin solution was dissolved into 10 mL of physiological saline (0.9%) immediately before infusions and was administered via jugular venipuncture in 7 applications (0.15 μg/kg BW per application) 45 min apart (0, 45, 90, 135, 180, 225, and 270 min). Values obtained from samples collected at −120 and −60 min and immediately before infusion at 0 min served as covariate ($P < 0.01$) but did not differ ($P = 0.57$) between INS and saline (SAL) heifers (77.1 vs. 78.2 mg/dL, respectively; SEM = 2.1). Therefore, results reported are covariately adjusted least-squares means. A treatment × time interaction was detected ($P < 0.01$). Treatment comparison within time: ** $P < 0.01$, * $P = 0.01$.

$P < 0.01$) and did not change for SAL heifers (time effect; $P = 0.53$). Hence, plasma glucose concentrations were greater ($P \leq 0.02$) for SAL vs. INS heifers beginning at 90 min relative to the initial infusion (Figure 1). Moreover, mean plasma glucose concentration during the experimental period was reduced ($P < 0.01$; Table 1) for INS compared with SAL heifers (68.2 vs. 79.0 mg/dL, respectively; SEM = 1.3). Concurring with our findings, Kegley et al. (2000) and Prior et al. (1984) also reported that i.v. insulin infusion reduced circulating glucose concentrations in beef cattle, given that insulin directly estimates the uptake of glucose by body tissues (Nussey and Whitehead, 2001; Nelson and Cox, 2005).

A treatment effect was detected ($P = 0.04$) for plasma IGF-I (Table 1). Plasma IGF-I concentrations before treatment infusion were significant covariates ($P < 0.01$) but did not differ ($P = 0.50$; data not shown) between INS and SAL heifers (158 vs. 161 ng/mL, respectively; SEM = 9). However, INS heifers had reduced ($P = 0.04$) mean plasma IGF-I concentrations compared with SAL heifers during the experimental period (Table 1). Insulin stimulates hepatic IGF-I synthesis by enhancing binding of GH to hepatic GH receptors (Houston and O'Neill, 1991; Molento et al., 2002) and modulates circulating IGF-I concentrations by regulating activity of IGFBP (McGuire et al., 1995), whereas concentrations of insulin typically correlate positively with IGF-I concentrations in cattle (Keisler and Lucy, 1996; Webb et al., 2004; Cooke et al., 2007).Conversely, Douglas et al. (1991) reported that plasma IGF-I concentrations were not altered in fasted lambs receiving i.v. insulin infusion. Nevertheless, the present study was not designed to evaluate the effects of insulin administration on the bovine somatotropic axis, particularly because short-term experiments may not adequately assess this subject (McGuire et al., 1995). Therefore, additional research is warranted to understand why administration of exogenous insulin reduced plasma IGF-I concentration in the heifers evaluated herein.

The goal of Exp. 1 was to evaluate if insulin administration would increase plasma P4 concentrations in beef heifers in adequate nutrient balance by reducing hepatic P4 catabolism independently of circulating concentrations of glucose and IGF-I. However, no treatment effects were detected ($P = 0.62$) for plasma P4 concentrations (Table 1). Therefore, insulin itself may not be capable of alleviating hepatic P4 catabolism and, consequently, increasing circulating concentrations of this hormone. Accordingly, research studies documenting the role of insulin on hepatic expression of P4 catabolic enzymes (Lemley et al., 2008) and resultant plasma P4 concentrations (Vieira et al., 2010) included glucose infusion into the experimental design. Therefore, results from Exp. 1 suggested that circulating glucose and perhaps IGF-I potentially modulate the effects of insulin on hepatic steroid catabolism and subsequent circulating P4 concentrations in bovine females in adequate nutritional status.

Experiment 2

Similar to Exp. 1, BW did not change ($P = 0.55$; data now shown) during the experimental period (456 and 468 kg of BW on d 14 and 28; SEM = 14). Treatment effects were detected for plasma glucose ($P = 0.01$) and insulin ($P < 0.01$; Table 2). Glucose and insulin concentrations before treatment infusion were not significant covariates ($P > 0.34$) and hence did not differ ($P > 0.23$; data not shown) between INS+G and SAL heifers (69.2

vs. 70.5 mg/dL for glucose, respectively; SEM = 2.2 and 2.1 vs. 2.4 ng/mL for insulin, respectively; SEM = 1.6). As expected from the experimental design, mean plasma glucose and insulin concentrations during the experimental period were greater ($P \leq 0.01$) for INS+G compared with SAL heifers (Table 2). In addition, administration of glucose to INS+G heifers increased, at least numerically, plasma insulin concentrations to values greater than detected for INS heifers in Exp. 1 (Tables 1 and 2), given that glucose infusion stimulates synthesis and release of endogenous insulin (Nelson and Cox, 2005; Vieira et al., 2010).

A treatment effect was detected ($P = 0.01$) for plasma IGF-I (Table 2). Plasma IGF-I concentrations before treatment infusion were significant covariates ($P < 0.01$) but did not differ ($P = 0.57$; data not shown) between INS+G and SAL heifers (149 vs. 147 ng/mL, respectively; SEM = 7). Similar to Exp.1, INS+G heifers had reduced ($P = 0.01$) mean plasma IGF-I concentrations compared with SAL heifers during the experimental period (Table 2). Other researchers reported that cattle receiving i.v. infusions of insulin and glucose had similar (Molento et al. 2002) or greater circulating IGF-I concentrations compared with cohorts receiving saline (McGuire et al., 1995; Butler et al., 2003). Therefore, treatment effects detected in Exp. 2 for plasma IGF-I concentrations were unexpected and, as reported in Exp. 1, deserve proper and further investigation.

A treatment effect was detected for plasma P4 ($P = 0.02$). Plasma P4 concentrations before treatment infusion were significant covariates ($P < 0.01$) but did not differ ($P = 0.49$; data not shown) between INS+G and SAL heifers (2.65 vs. 2.75 ng/mL, respectively; SEM = 0.28). During the experimental period, INS+G heifers had greater ($P = 0.02$) mean P4 concentration compared with SAL heifers (Table 2). The goal of Exp. 2 was to evaluate if supplemental glucose modulates the effects of insulin infusion on plasma P4 concentrations by reducing hepatic P4 catabolism. In fact, we also expected that INS+G heifers would also have greater plasma IGF-I concentrations compared with SAL heifers, whereas IGF-I also influences hepatic function and could potentially modulate hepatic steroid catabolism (Rutter et al., 1989; Jones and Clemmons, 1995). Nevertheless, results from Exp. 2 suggest that i.v. insulin infusion increased plasma P4 concentrations by reducing hepatic P4 catabolism only when supplemental glucose is provided. Therefore, results from Exp. 2 combined with those reported by Lemley et al. (2008) and Vieira et al. (2010) suggest that circulating glucose modulates the effects of insulin on hepatic steroid catabolism and subsequent circulating P4 concentrations in bovine females in adequate nutritional status.

Table 2. Plasma concentrations of glucose, insulin, IGF-I, and progesterone (P4) in beef heifers receiving intravenous infusion containing insulin (1 μg/kg of BW) and glucose (0.5 g/kg of BW; INS+G, n = 8) or saline (0.9%; SAL, n = 8) in Exp. 2[1–3]

Item[4]	INS+G	SAL	SEM	*P*-value
Glucose, mg/dL	133.9	76.8	16.4	0.01
Insulin, ng/mL	3.65	2.12	0.32	<0.01
IGF-I, ng/mL	134	142	2	0.01
P4, ng/mL	2.88	2.52	0.11	0.02

[1]Glucose and bovine insulin were dissolved into 10 mL of physiological saline (0.9%) immediately before infusions and were administered via jugular venipuncture in 7 applications (0.07 g/kg BW and 0.15 μg/kg BW per application for glucose and insulin, respectively) 45 min apart.

[2]Plasma samples were collected immediately before each infusion as well as at −120, −60, 330, 390, and 450 min relative to the first infusion.

[3]Beef heifers with no luteal P4 synthesis and receiving exogenous P4 from a controlled internal drug-releasing device containing 1.38 g of P4 (CIDR; Pfizer Animal Health, New York, NY).

[4]Least-squares means covariately adjusted to values obtained before the first infusion (−120, −60, and 0 min).

Experiment 3

Similar to Exp. 1 and 2, BW did not change ($P = 0.72$; data now shown) during the experimental period (468 and 476 kg of BW on d 28 and 55; SEM = 14). As expected, BST heifers had greater ($P < 0.01$) mean plasma IGF-I concentrations compared with SAL heifers (Table 3), given that sometribove zinc has been shown to increase IGF-I synthesis and circulating concentrations in cattle (Buskirk et al., 1996; Bilby et al., 1999). Heifers receiving BST had greater ($P < 0.01$) plasma glucose but similar ($P = 0.76$) plasma insulin concentrations compared with SAL heifers (Table 3). The effects of sometribove zinc administration on circulating concentrations of glucose and insulin in cattle have been variable, either by increasing (de la Sota et al., 1993; Chase et al., 2011), decreasing (Azza et al., 2010), or not altering (Neathery et al., 1991; Schwarz et al., 1993) one or both variables. In the present study, the increase in plasma glucose concentrations in BST heifers despite similar insulin concentrations can be attributed to decreased insulin sensitivity caused by sometribove zinc administration (Dunshea et al., 1995).

The main goal of Exp. 3 was to determine if circulating IGF-I also modulates hepatic P4 catabolism and consequent plasma P4 concentrations given that this hormone directly regulates hepatocyte activity (Jones and Clemmons, 1995). However, mean plasma P4 concentrations were similar ($P = 0.67$) between BST and SAL heifers (Table 3), suggesting that hepatic P4 catabolism in bovine females in adequate nutritional status is not directly regulated by circulating IGF-I.

Table 3. Plasma concentrations of glucose, insulin, IGF-I, and progesterone in beef heifers receiving subcutaneous injection containing 250 mg sometribove zinc (BST; n = 8) or saline (0.9%; SAL, n = 8) in Exp. 3[1,2]

Item	BST	SAL	SEM	*P*-value
Glucose, mg/dL	73.0	69.6	1.6	<0.01
Insulin, ng/mL	1.44	1.65	0.51	0.76
IGF-I, ng/mL	248	143	6	<0.01
Progesterone, ng/mL	3.07	3.13	0.15	0.67

[1]Blood samples were collected 3 h apart (0900, 1200, 1500, and 1800 h) from heifers on d 6, 8, and 10 relative to administration (d 1) of saline or sometribove zinc (Posilac; Elanco, Greenfield, IN).

[2]Beef heifers with no luteal P4 synthesis and receiving exogenous P4 from a controlled internal drug-releasing device containing 1.38 g of P4 (CIDR; Pfizer Animal Health, New York, NY).

Conclusion

Results collectively suggest that the effects of insulin on hepatic P4 degradation and circulating P4 concentrations in bovine females in adequate nutritional status are dependent on circulating glucose but not IGF-I. Alternatively, Lemley et al. (2008) also reported that hepatic expression of CYP2C and CYP3A decreased linearly according to the dose of insulin infusion (0.0, 0.3, or 1.0 g/kg BW of insulin). Therefore, it can be speculated that differences detected in plasma insulin concentrations between SAL and INS heifers in Exp. 1 were not sufficient to impact plasma P4 concentrations. Nevertheless, results reported herein indicate that nutritional and management alternatives to increase circulating concentrations of glucose and insulin may benefit reproductive function of females in adequate nutritional status by increasing circulating concentrations of P4.

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