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# Effect of Kisspeptin on Regulation of Growth Hormone

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# Effect of Kisspeptin on Regulation of Growth Hormone and Luteinizing Hormone in Lactating Dairy Cows

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**ABSTRACT**: Kisspeptin (KP), a neuroendocrine regulator of reproduction, is hypothesized to be an integrator of metabolism and hormones critical to the regulation of reproduction. Lactation is associated with enhanced growth hormone (GH) responsiveness and reduced fertility. Our study was designed to determine the effects of lactation on KP-stimulated GH and luteinizing hormone (LH) secretion. Five non-lactating and five lactating dairy cows were used in the study. Experiments were conducted with lactating cows at weeks 1, 5 and 11 after parturition. The experimental treatments (saline and KP [100 and 400 pmol/kg body weight]) were given intravenously and blood was collected and plasma was stored until later assay to determine concentrations of GH, LH, progesterone and non-esterified fatty acids. We found that neither dose of KP stimulated an increase in GH secretion. The low dose of KP increased ($P<0.05$) LH concentrations only in lactating cows. The higher dose of KP elicited an increase in circulating LH concentrations in both lactating and non-lactating cows. The lower dose of KP increased ($P<0.05$) the area under the curve for LH only in cows during week 5 of lactation, and the area under the curve of LH following the highest dose of KP was greater ($P<0.05$) in cows during week 5 of lactation than that for the other groups of cows. In summary, lactation status and stage of lactation did not change the sensitivity of the GH system to KP. However, an effect of stage of lactation on KP-stimulated LH secretion was detected in the dairy cows. Study of the KP system during lactation in dairy cows may provide critical insights into the mechanisms for lactation-associated changes in the reproductive axis.

**Key words**: growth hormone, kisspeptin, lactation, luteinizing hormone

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## INTRODUCTION

Kisspeptin (KP) is a regulator of reproductive function in a number of species (Tena-Sempere, 2006). However, recent studies have suggested a possible additional role for KP in the regulation of growth hormone (GH) secretion (Gutierrez-Pascual et al., 2007; Kadokawa et al., 2008b; Whitlock et al., 2008) and a potential link between metabolism, growth, lactation, and reproduction (Crown et al., 2007).

Initial studies revealed a stimulatory effect of KP on GH release from cultured rat and bovine pituitary cells (Gutierrez-Pascual et al., 2007; Kadokawa et al., 2008b). KP stimulates GH release at doses ranging from $10^{-5}$ to $10^{-10}$ mol/L (Gutierrez-Pascual et al., 2007; Kadokawa et al., 2008b). Moreover, there is

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an increase in calcium influx in somatotropes stimulated by KP (Gutierrez-Pascual et al., 2007). These results suggest that KP may induce regulation of GH release *in vitro*. Kadokawa (2008a) first reported an effect of KP in regulating GH release *in vivo*, where intravenous (IV) injections of a high dose of KP (1 mg) into prepubertal female cattle significantly increased circulating concentrations of GH (Kadokawa et al., 2008a). Subsequently, in a study of the effect of KP on secretion of sex steroids, IV injection of KP at doses similar to those used to elicit LH release in sheep (Caraty et al., 2007) and $1/20^{th}$ the dose used in prepubertal cattle (Kadokawa et al., 2008a) and ovariectomized cows (lacking reproductive steroids) failed to increase circulating concentrations of GH (Whitlock et al., 2008). However, when cows were treated with progesterone, estradiol, or a combination of these two sex steroids, KP induced an increase in plasma GH concentrations (Whitlock et al., 2008).

In addition to the effects on GH, KP neurons may have a role in signaling body energy reserves to multiple neuroendocrine axes (Smith et al., 2006). Fasting alters hypothalamic expression of *Kiss*1 and *Kiss*1*r* and changes the sensitivity of the hypothalamic-pituitary-gonadal axes of fasted animals to KP (Castellano et al., 2005). Sensitivity of the hypothalamic-pituitary axis to KP may also be different during lactation (Roa et al., 2006). Lactating rats have lower expression of *Kiss*1 mRNA and KP protein in the hypothalamus compared with that in non-lactating control rats (Yamada et al., 2007).

Negative energy balance alters the KP axis (Castellano et al., 2005, Smith et al., 2006). During early lactation, high producing dairy cows are in a state of negative energy balance, which is also characterized by an increased sensitivity of the GH axis (Sartin et al., 1985; Sartin et al., 1986). Therefore, this study was designed to test the hypothesis that KP increases plasma GH concentrations in high producing dairy cows during early lactation independent of exogenous sex steroids. Moreover, the effects of lactation on KP-mediated LH release in large domestic species, particularly cattle, are not known. Therefore, an additional objective of the present study was to determine whether lactation status and stage of lactation affect plasma LH concentrations following treatment of dairy cows with KP.

## MATERIALS AND METHODS

### *Animals*

All procedures were approved by the Auburn University Institutional Animal Care and Use Committee. Five non-lactating (5.10 ± 0.76 [SEM] years; 576.5 ± 19.1 kg body weight [BW]) and five lactating (4.14 ± 0.56 years; 608.3 ± 10.9 kg BW] multiparous Holstein cows were used in the study. Cows were housed at the Auburn University Veterinary Teaching Dairy and exposed to ambient temperatures and photoperiod throughout the experimental period of 6 months (November to April). Before calving, lactating cows had a typical dry period (60 d). Three weeks before parturition, lactating cows were fed an anionic diet with pH of the urine determined periodically to allow for adjustments in the ration. After calving, the lactating cows were milked twice daily and individually fed grain and alfalfa hay. The cows also had access to pasture and *ad libitum* Coastal Bermuda Grass hay following each milking. The diet of lactating cows consisted of approximately 24 kg dry matter (30:70, forage:grain), 1.80 Mcal NEL/kg, 18% crude protein and 29% NDF. Non-lactating cows were fed the same grain as lactating cows along with *ad libitum* access to Coastal Bermuda Grass hay. Both groups were fed diets to meet their daily nutrient requirements (NRC, 2001).

### *Experimental design*

The effects of lactation and stage of lactation on GH and LH concentrations after treatment with KP (KP-10, human Metastin 45-54, 4389-v; Peptide Institute, Inc., Osaka, Japan) were determined. Human KP-10 was used because these experiments were initiated before rat and mouse Kp-10 was available. Bovine, rat, and mouse Kp-10 are identical and differ from human KP-10 by one amino acid. However, previous studies with ruminants indicated that there are no appreciable differences in responses to human and murine KP (Caraty et al., 2007).

Experiments were conducted with lactating cows at weeks 1, 5 and 11 of lactation after parturition and with non-lactating cows over the 6-month experimen-

tal period. Except for experiments with lactating cows in the 1st week of lactation (before resumption of estrous cycles and ovarian activity), all other experiments were conducted when cows were in the luteal phase of the estrous cycle to reduce variability in responses to KP-10 because of differences in circulating concentrations of sex steroids and the stage of estrous cycle. Estrous cycles were synchronized using dinoprost tromethamine (25 mg, IM) administered every 2 weeks beginning on day 28 of lactation from the date of calving through week 11 of lactation. Ovaries were trans-rectally palpated and concentrations of progesterone in blood were determined during each experimental period. This was performed to ensure the presence of a functional corpus luteum indicated by concentrations of progesterone ≥ 1 ng/mL. Cows were fitted with sterile indwelling jugular catheters (18 gauge; Ico-Rally, Palo Alto, CA, USA) the day before each experiment was initiated. Catheters were kept patent by infusion with heparinized physiological saline (10 IU/mL; 3 mL) every 12 h during an experimental week. Physiological saline (vehicle) and doses of KP-10 (100 and 400 pmol/kg BW) were derived from results from previous experiments with sheep and cattle (Caraty et al., 2007; Whitlock et al., 2008). A stock solution of 0.5 to 5 μg/μL KP-10 in physiological saline was diluted to the desired concentration in a final volume of 3 mL immediately before each treatment dose was delivered IV via a jugular catheter. Treatments were administered between 09:00 and 10:00 (following the morning milking for lactating cows) in random order successively to each cow in each group with a 48 h interval maintained between treatments. Lactating cows were given all treatments in each experimental week of lactation (1, 5 and 11 weeks) and non-lactating cows received all treatments during a single week. Blood was collected at −30, −15, 0, 5, 10, 15, 20, 30, 45, 60, 75 and 90 min relative to the IV injection of physiological saline or KP-10 in physiological saline. Plasma was stored at −20℃ until further assay to determine concentrations of GH, LH, and progesterone. Plasma from a blood sample collected before each experimental treatment was stored at −80℃ until later assay to determine concentrations of nonesterified fatty acids (NEFA) and glucose.

***Hormone assays***

Plasma GH and LH concentrations were determined using reagents in a double-antibody RIA provided by the National Hormone and Pituitary Program of NIDDK, as previously described (Sartin et al., 1985; Coleman et al., 1993). The limit of detection was 1.25 ng/mL and 0.125 ng/mL for GH and LH, respectively. The intra- and inter-assay coefficients of variation were 8.4% and 13.8% for GH, and 5.7% and 15% for LH, respectively. Glucose and NEFA concentrations were determined using commercially available enzymatic-colorimetric methods (Autokit Glucose [Code No. 439-90901] and NEFA-HR[2] [Code No. 999-34691; 991-34891; 993-35191]; Wako Diagnostics, Wako Chemicals USA, Richmond, VA, USA) (Sartin et al., 1988; Accorsi et al., 2005). The limits of detection and intra-assay coefficients of variation were 12.5 mg/dL and 5.0% for glucose and 0.016 mEq/L and 4.5% for NEFA, respectively. Progesterone concentrations were determined using the Coat-a-Count Progesterone RIA kit (Siemens, Los Angeles, CA, USA), which is a reliable validated method for quantification of progesterone in plasma from cows (Reimers et al., 1991); The limit of detection and intra- and inter-assay coefficients of variation for the progesterone assay were 0.1 ng/mL, 5.3%, and 6.4%, respectively.

***Statistics***

The effects of lactational status (lactating or not lactating) and week of lactation (non-lactating, weeks 1, 5, and 11) on mean daily milk yields, and NEFA, glucose, and progesterone plasma concentrations were determined using ANOVA GLM procedures of SAS (Statistical Analysis Systems Institute, Cary, NC, USA). The model included treatment, week of lactation, and first-order interactions. To determine the effect of KP-10 and lactation on plasma GH and LH concentrations, data were subjected to ANOVA with repeated measures using the MIXED procedures of SAS. The models included treatment, week of lactation, time, and all first- and second-order interactions, with a compound symmetric function used to model the covariance structure for repeated measures. If a treatment by week-time interaction was significant ($P<0.05$), effects of treatment by week within time were compared using the SLICE option of the LSMEANS statement of SAS. Mean concentration, total area under the curve (TAUC), and incremental area under the curve (iAUC) for plasma LH and GH at fixed periods were subjected to ANOVA

using GLM procedures of SAS. Periods 1 and 2 were defined as the 30 min before and 90 min after IV with physiological saline or KP-10 in physiological saline, respectively.

## RESULTS

Daily milk yields (kg/d) for lactating cows during weeks 5 and 11 were greater (Table 1; $P < 0.01$) than yields during week 1. Fat mobilization, assessed by NEFA plasma concentrations, was affected by lactational status and week of lactation ($P < 0.001$). NEFA plasma concentrations were greatest in cows during the first week of lactation (Table 1; $P < 0.05$), and cows in the 5th week of lactation had the second greatest concentrations ($P < 0.05$). In non-lactating cows and cows in week 11 of lactation, NEFA concentrations were not different (Table 1; $P = 0.19$). Circulating glucose concentrations were affected by lactational status and week of lactation ($P < 0.001$). Glucose concentrations were greatest (Table 1; $P < 0.001$) in the non-lactating group, but there was no significant difference among groups because of the effect of the week of lactation. Lactational status and week of lactation affected plasma progesterone concentrations ($P < 0.001$), and they were highest in week 11 of lactation (Table 1; $P < 0.01$). However, there was no difference in progesterone concentrations between non-lactating and lactating cows during week 5 of lactation ($P = 0.76$). As expected, cows in the 1st week of lactation had the lowest ($P < 0.05$) progesterone concentrations as they had not yet resumed ovarian cyclicity.

**Table 1.** Least square means for days in milk, milk production, and concentrations of NEFA, glucose and progesterone for non-lactating and lactating cows at weeks 1, 5, and 11 of lactation (n = 5 cows group)

| Items | Groups | | | | |
|---|---|---|---|---|---|
| | NL | Week 1 | Week 5 | Week 11 | SEM |
| DIM, d | NA | 8.000[a] | 35.000[b] | 74.400[c] | 0.800 |
| Milk, kg/d | NA | 28.710[a] | 35.790[b] | 37.660[b] | 1.670 |
| NEFA, mEq/L | 0.093[a] | 0.491[b] | 0.348[c] | 0.183[a] | 0.047 |
| Glucose, mg/dL | 73.800[a] | 53.000 | 55.200 | 57.900 | 1.900 |
| Progesterone, ng/mL | 3.180[a] | 0.120[b] | 2.780[a,b] | 7.240[c] | 0.940 |

Abbreviations: DIM, days in milk; NA, not applicable; NL, non-lactating and non-pregnant cows.

[a,b,c]Least squared means in rows with different superscripts are different ($P < 0.05$).

Lactational status and week of lactation affected basal GH concentrations ($P = 0.0004$). As indicated by TAUC, pre-treatment (−30 to 0 min) GH concentrations were greater in cows during the 1st ($P = 0.01$) and 5th ($P < 0.0001$) weeks of lactation compared with those in non-lactating cows (Fig. 1). Basal GH concentrations were greater ($P \leq 0.009$) in cows during the 5th week of lactation compared with those in non-lactating cows and cows in the 11th week of lactation (Fig. 1). Although basal GH concentrations during the 1st and 11th weeks of lactation were not different ($P = 0.43$), there tended to be a difference ($P = 0.06$) between the 1st and 5th weeks of lactation (Fig. 1). There was no effect of treatment ($P = 0.90$) or treatment by time ($P = 0.82$) on GH concentrations in any group of cows (lactating and non-lactating) (Fig. 2). For GH concentrations between 0 and 90 min following saline and KP-10 treatments, there was an effect of lactational status and week of lactation ($P = 0.03$), but not treatment ($P = 0.70$) (saline vs. KP-10) or their interactions ($P = 0.76$, Fig. 3).

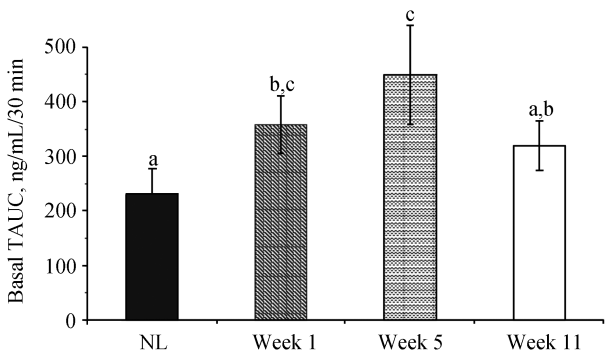


**Fig. 1.** Total areas under the curve (TAUCs) for basal plasma GH concentrations in non-lactating (NL) and lactating (weeks 1, 5, and 11 of lactation) Holstein cows (n = 5/group) from −30 to 0 min prior to treatment (mean ± pooled SEM). TAUCs with different letters are significantly different ($P < 0.05$).

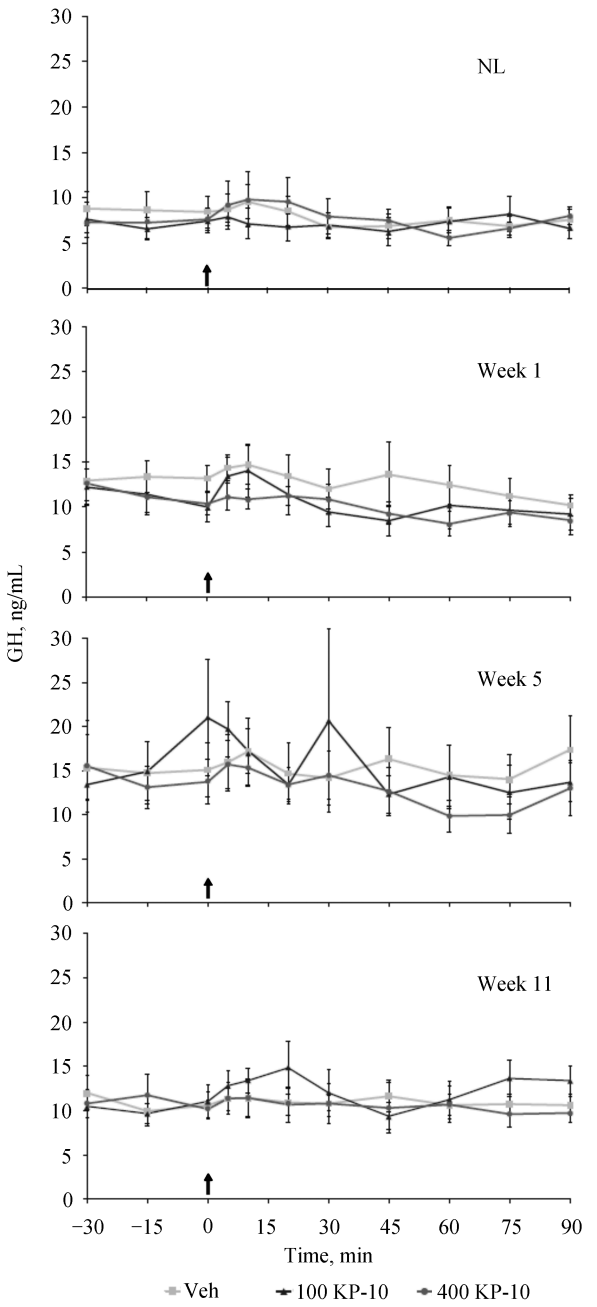


**Fig. 2.** Effect of lactational status and week of lactation on KP-10-induced changes in plasma GH concentrations in Holstein cows (n = 5/group; mean ± SEM). No effects of treatment or time by treatment effects were detected ($P > 0.05$). The arrows indicate the time of IV administration of saline or KP-10.

There was an effect of lactation and stage of lactation on KP-10-stimulated plasma LH concentrations ($P = 0.001$, Fig. 4). The greatest dose of KP-10 (400 pmol/kg BW) increased ($P < 0.05$) LH concentrations compared with saline treatment in all groups of lactating and non-lactating cows (Fig. 4). Following the highest KP-10 dose, LH concentrations were greater than those in saline controls at 5 min post-treatment in all lactating cows ($P < 0.05$), but values did not increase until the second sample at 10 min post-treatment for non-lactating cows ($P = 0.01$). In both lactating and non-lactating cows, LH concentrations were greater ($P \leq 0.03$) up to the 45 min post-treatment sample than those for cows given saline. Unlike the highest dose of KP-10, the low dose of KP-10 (100 pmol/kg BW) did not increase plasma LH concentrations in non-lactating cows, as the lowest P value ($P = 0.085$) occurred 20 min post-treatment compared with saline controls. However, the low KP-10 dose increased LH concentrations compared with those with saline treatment in all groups of lactating cows, and the duration of the LH response was greatest for cows during weeks 1 and 5 of lactation (Fig. 4).

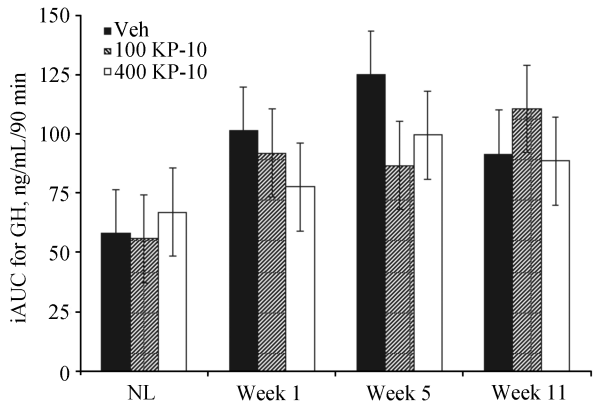


**Fig. 3.** Incremental areas under the curve (iAUCs) for plasma GH concentrations in non-lactating (NL) and lactating cows at weeks 1, 5 and 11 of lactation. The values are for samples of plasma from Holstein cows (n = 5/group; mean ± pooled SEM) taken between 0 and 90 min post-treatment with either physiological saline (vehicle), or 100 or 400 pmol/kg body weight KP-10. There were no significant effects ($P > 0.05$).

The iAUC for LH in the period from 0 to 90 min following KP-10 treatment was determined to assess the magnitude of the LH response for the different treatment groups (Fig. 5). There was no effect of lactational status or week of lactation on the iAUC for LH following treatment with physiological saline ($P \geq 0.61$). The greatest KP-10 dose (400 pmol/kg) increased the iAUC for plasma LH compared with saline controls for all time periods and both groups ($P \leq 0.048$). However, the lower KP-10 dose increased the iAUC for plasma LH only during weeks 1 and 5 of lactation ($P = 0.024$ and 0.023, respectively)

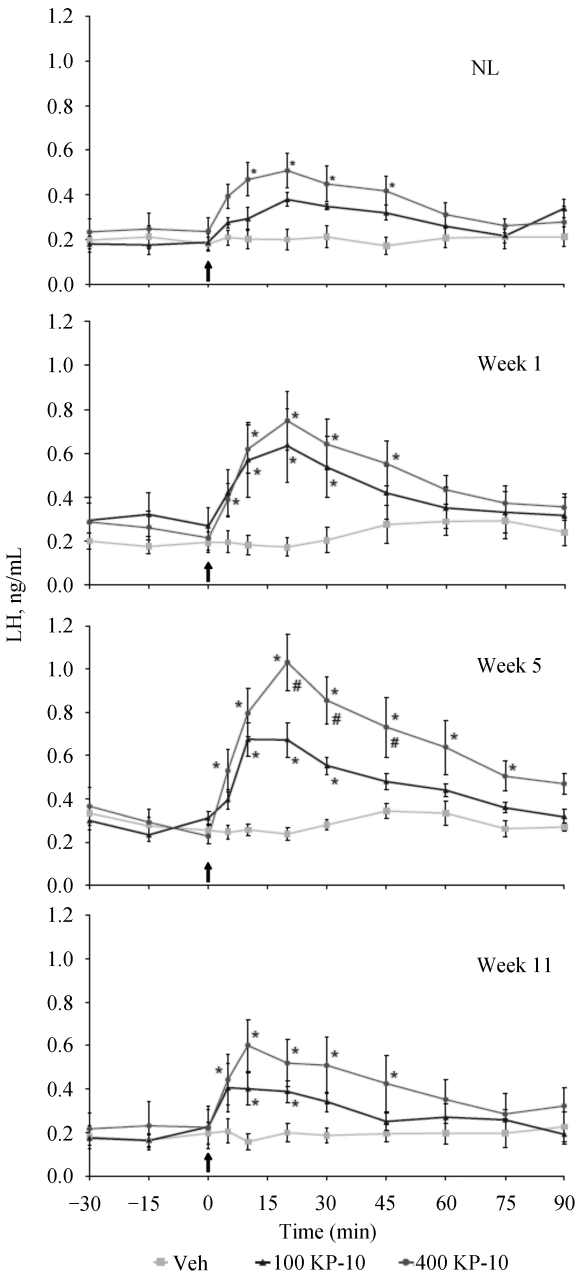


**Fig. 4.** Effect of lactational status and week of lactation on KP-10-induced changes in plasma LH concentrations in Holstein cows (n = 5/group; mean ± SEM). The arrows indicate administration of saline or KP. A treatment-time interaction was detected for LH ($P < 0.0001$). * Times at which effects of treatment were significantly different from saline-treated control cows ($P < 0.05$). Times at which effects of 400 KP-10 treated animal were significantly different from 100 KP-10 treated animals ($P < 0.05$).

compared with saline controls in the same group. Similarly, the iAUC for plasma LH following the high KP-10 dose was greater ($P = 0.035$) than the response to the lower dose in the lactating group, but only during week 5 of lactation. For all other groups (weeks 1 and 11 for lactating and non-lactating cows), the higher KP-10 dose increased the iAUC for plasma LH compared with saline treatment, but there was no difference ($P \geq 0.16$) between the two KP-10 doses (100 and 400 pmol/kg).

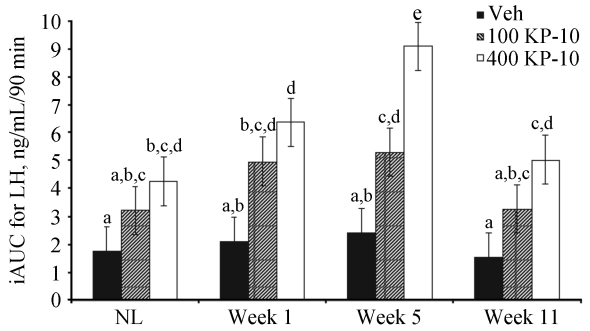


**Fig. 5.** Incremental areas under the curve (iAUCs) for plasma LH concentrations of non-lactating (NL) and lactating (weeks 1, 5 and 11) Holstein cows (n = 5/group; mean ± pooled SEM) from 0 to 90 min post-treatment with either physiological saline or KP-10. The iAUCs with different letters are significantly different ($P < 0.05$).

## DISCUSSION

Our study examined the effect of lactation on KP-10-stimulated GH and LH secretion in dairy cows and demonstrated that IV KP-10 (doses of 100 and 400 pmol/kg BW) had no effect on GH. Neither lactational status, stage of lactation (weeks 1, 5 or 11), nor degree of fat mobilization enhanced the GH response to KP-10. Conversely, there was an effect of lactational status and stage of lactation on KP-stimulated LH in dairy cows, and this response was the opposite to that from KP-10 in lactating rats (Roa et al., 2006; Yamada et al., 2007).

Recent evidence points to a possible role of KP-10 in the regulation of GH secretion in rodents and cattle (Gutierrez-Pascual et al., 2007; Kadokawa et al., 2008b; Whitlock et al., 2008). Initial studies found that *Kiss*1 and *Kiss*1*r* are expressed in the pituitary cells of peripubertal male and female rats. Both gonadotropes and somatotropes are activated (assessed by influx of ionized calcium) *in vitro* by KP-10 to secrete LH and GH, respectively (Gutierrez-Pascual et al., 2007). In addition, KP-10 stimulates GH and prolactin release from cultured bovine anterior pituitary cells (Kadokawa et al., 2008b). Administration of KP at a very high dose (3,653 pmol/kg BW) to prepubertal heifers (Kadokawa et al., 2008a) and at

a low dose (100 pmol/kg BW) to postpubertal heifers (unpublished observations) also increases palsma GH concentrations. More evidence that KP may regulate GH secretion is provided from studies in the ewe indicating that the ovine hypothalamus expresses *Kiss1r*, as well as lactotropes, gonadotropes, and somatotrope cells in the anterior pituitary gland. In addition, low but detectable amounts of KP are found in hypophyseal portal blood (Smith et al., 2008). Collectively, the following results support the concept that KP plays a role in the regulation of GH secretion: 1) KP neurons are associated with hypothalamic nuclei integral to the control of GH synthesis and/or secretion; 2) KP is released into hypophyseal portal blood; 3) somatotropes express *Kiss1r*; and 4) KP induces secretion of GH by cultured pituitary cells.

However, conflicting results of other experiments have questioned the role of KP in the regulation of GH secretion. While central and peripheral KP administration stimulates an increase in secretion of gonadotropins in prepubertal female pigs, it fails to stimulate GH secretion (Lents et al., 2008). Similar results were reported for ovariectomized adult cows following peripheral KP-10 treatment (Whitlock et al., 2008). Interestingly, circulating GH concentrations were increased following peripheral KP-10 administration when the same ovariectomized adult cows were treated with estradiol and/or progesterone (Whitlock et al., 2008). It is possible the absence of or low concentrations of reproductive steroids in prepubertal female pigs and ovariectomized cows blunt or prevent KP-10-stimulated GH secretion, and sex steroids enhance the sensitivity of the somatotropic-axis to KP-10. GH release is enhanced by high concentrations of estradiol (Meinhardt and Ho, 2007; Hudmon et al., 2009) and this increase is accompanied by an estradiol-induced LH surge (Scanlan and Skinner, 2002). The mechanism(s) regulating the effects of reproductive steroids on GH have not been completely elucidated, but available results suggest that KP has a role.

In lactating dairy cows, there is a differential response of cows to the GH regulatory system following treatment with glucose, propionate, GH releasing hormone, and somatostatin due to the stage of lactation and degree of fat mobilization (Sartin et al., 1985; Sartin et al., 1989). Since lactational status and stage of lactation may enhance the sensitivity of the GH regulatory system to stimulation, the present study aimed to determine whether lactation affects the sensitivity of the GH regulatory system to KP-10. Our results showed that KP-10 did not affect plasma GH concentrations of lactating dairy cows as neither lactational status nor stage of lactation affected the GH response to KP-10. Perhaps the stimulatory effect of KP on the somatotropic-axis in ruminants is more central (at the level of the hypothalamus rather than the pituitary). For example, IV administration of KP had no effect on serum GH concentrations in ovariectomized ewes (Whitlock et al., 2010), but it increased circulating GH concentrations after central (intracerebroventricular) administration of KP in the same ewes (Whitlock et al., 2010). Although peak lactation in dairy cattle is associated with elevated basal GH concentrations in blood and an increased responsiveness to GH stimulation (Sartin et al., 1985; Sartin et al., 1986), the results of the present experiment do not indicate that lactational status alters the sensitivity of the GH regulatory system to IV KP in dairy cows.

The hypothalamus plays a crucial role in regulation of secretion of hormones affecting fertility in all mammals; therefore, it is the focus of most research on the integration of metabolism and reproduction. Neurons that contain receptors for metabolic hormones and those that send afferent inputs to GnRH neurons are likely responsible for sensing the metabolic milieu and controlling GnRH secretion as a function of nutrient availability and adipose reserves. The neuropeptide KP may function to integrate energy balance, metabolism, and the endocrinology of reproduction (Crown et al., 2007). The present study provides the first evidence that the stage of lactation affects KP-stimulated LH secretion in dairy cows, and therefore, changes in LH responsiveness and sensitivity to KP during early lactation were investigated. Our results showed that the highest dose of KP-10 (400 pmol/kg BW) elicited significant LH responses in both lactating and non-lactating cows, while the lower dose (100 pmol/kg BW) stimulated an increase in plasma LH only in lactating cows. Notably, the LH response (as assessed by iAUC) to the higher dose of KP was greatest for cows during week 5 of lactation. These results suggest that the sensitivity of the gonadotropic axis to KP is greatest during early lactation in dairy cows.

Interestingly, the reported effects of lactation on KP-stimulated LH in rats are inconsistent (Roa et al., 2006; Yamada et al., 2007). Roa et al. (2006) suggest that hypothalamic sensitivity to KP is

reduced during lactation because no LH response was detected after central injection of various doses of KP, which were fully effective in diestrus rats. Conversely, Yamada et al. (2007) reported that a dose of KP comparable to that used by Roa et al. (2006) increases plasma LH in both lactating and non-lactating rats, which suggests that lactation may not affect the LH response to KP. Ovariectomized rats were used in the study that found no effect of lactation on KP-induced plasma LH, while gonad-intact rats were used in the earlier lactating rat experiment and the current study on cows. Effects of gonadal steroids on the KP system (Smith, 2008) may explain differences in results from the rat studies mentioned above. In either event, the current study found that the lactating dairy cow responded differently than the rat to KP-10. An understanding of this difference in KP-10 responsiveness may be important in understanding the effects of suckling and lactation on reproduction.

The transition from pregnancy to early lactation in rats is associated with a decrease in total hypothalamic *Kiss*1 mRNA. Total hypothalamic expression of *Kiss*1*r* mRNA is not affected by the stage of pregnancy or lactation; however, in early lactation, total hypothalamic *Kiss*1 mRNA expression is not different from that for cyclic female rats in diestrus. These findings lead to uncertainty on the potential contribution of decreased hypothalamic expression of *Kiss*1 mRNA to suppression of the gonadotropic axis during lactation (Roa et al., 2006). In contrast, *Kiss*1 and *Kiss*1*r* gene expression in specific hypothalamic nuclei of non-lactating and lactating rats is different (Yamada et al., 2007). With regard to lactating rats, hypothalamic *Kiss*1 mRNA expression is reduced in fasted and insulin-induced hypoglycemic (IIH) rats (Castellano et al., 2005; Kinsey-Jones et al., 2009). However, *Kiss*1*r* mRNA expression in the hypothalamus of rats subjected to metabolic stress (fasting and IIH) is different from that in lactating rats. In contrast to results from studies of lactating rats, hypothalamic *Kiss*1*r* mRNA expression is elevated in fasted prepubertal and adult IIH rats (Castellano et al., 2005; Kinsey-Jones et al., 2009). Castellano et al. (2005) hypothesized that metabolic stress-induced decreases in *Kiss*1 expression might result in a compensatory increase in expression of *Kiss*1*r* to enhance sensitivity of the gonadotropic axis to KP. Indeed, KP-induced release of LH in prepubertal rats is enhanced during food deprivation, which supports this hypothesis (Castellano et al., 2005). Although the mechanism responsible for the results observed for dairy cows in the present study are unknown, it is possible that there is an effect of negative energy balance during early lactation on the sensitivity of the hypothalamic-pituitary axis to KP-10 similar to that observed in rats subjected to metabolic stress. An increase in *Kiss*1*r* expression in the hypothalamus, specifically GnRH neurons, of high-producing dairy cows under conditions of negative energy balance is hypothesized to be important. It is possible that another factor(s) also contributed to the enhanced LH response to KP-10. For example, KP-neurons are central mediators of the effects of sex steroids and season on reproduction (Smith et al., 2007). Our experiments were conducted over a period of 6 months, and therefore, cows were exposed to variations in ambient temperature and photoperiod. Further, there was an effect of lactation on plasma progesterone concentrations, and sex steroids could affect KP-neurons as well as storage and release of LH by gonadotropes (Colazo et al., 2008). Although the mechanism(s) for enhanced responsiveness of LH to KP-10 in dairy cattle is not apparent from our study, the stage of lactation post-partum can affect the release of LH from cultured cells isolated from the anterior pituitary gland of beef cattle (Foster et al., 1980; Rutter and Randel, 1984; Leung et al., 1986). Previous studies showed that there was a linear increase in pituitary LH stores after parturition, although acyclic cows and cyclic cows after week 4 of lactation did not differ in LH content. There was also a linear increase in LH release from cultured pituitary cells to day 42 (week 6), but there was no difference between day 28 acyclic cows and lactating cows at weeks 6 and 7 postpartum. Moreover, there were no differences in GnRH receptor characteristics at any day postpartum in beef cows (Foster et al., 1980; Rutter and Randel, 1984; Leung et al., 1986). These results suggest that changes in the pituitary do not account for increases in LH release in response to KP-10 during the period of peak lactation in dairy cows.

In summary, the results of the present study provide insight into interactions of lactation and fat mobilization on KP-induced stimulation of the somatotropic and gonadotropic axes in lactating dairy cows. Both lactational status and fat mobilization associated with early lactation and a negative energy balance in dairy cows failed to sensitize the somatotropic axis to physi-

ologically relevant doses of KP. However, an effect of stage of lactation on KP-stimulated plasma LH concentrations in the dairy cow was observed. These results differed from those reported for other species, which may reflect uncharacterized differences in responses due to lactational status among species. These findings increase our current knowledge of the reproductive physiology of dairy cows and may prove useful in efforts to define the potential therapeutic uses of KP or synthetic *Kiss1r* agonists (Tomita et al., 2008) useful for pharmacological manipulation of the gonadotropic axis to enhance fertility in dairy cows.

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## LITERATURE CITED

Accorsi, P. A., N. Govoni, R. Gaiani, C. Pezzi, E. Seren, and C. Tamanini. 2005. Leptin, GH, PRL, insulin and metabolic parameters throughout the dry period and lactation in dairy cows. Reprod. Domest. Anim. 40(3): 217-223.

Beam, S. W., and W. R. Butler. 1999. Effects of energy balance on follicular development and first ovulation in postpartum dairy cows. J. Reprod. Fertil. 54(Suppl): 411-424.

Caraty, A., J. T. Smith, D. Lomet, S. Ben Said, A. Morrissey, J. Cognie, B. Doughton, G. Baril, C. Briant, and I. J. Clarke. 2007. Kisspeptin synchronizes preovulatory surges in cyclical ewes and causes ovulation in seasonally acyclic ewes. Endocrinology. 148(11): 5258-5267.

Castellano, J. M., V. M. Navarro, R. Fernandez-Fernandez, R. Nogueiras, S. Tovar, J. Roa, M. J. Vazquez, E. Vigo, F. F. Casanueva, E. Aguilar, L. Pinilla, C. Dieguez, and M. Tena-Sempere. 2005. Changes in hypothalamic KiSS-1 system and restoration of pubertal activation of the reproductive axis by kisspeptin in undernutrition. Endocrinology. 146(9): 3917-3925.

Colazo, M. G., J. P. Kastelic, H. Davis, M. D. Rutledge, M. F. Martinez, J. A. Small, and R. J. Mapletoft. 2008. Effects of plasma progesterone concentrations on LH release and ovulation in beef cattle given GnRH. Domest. Anim. Endocrinol. 34(1): 109-117.

Coleman, E. S., T. H. Elsasser, R. J. Kemppainen, D. A. Coleman, and J. L. Sartin. 1993. Effect of endotoxin on pituitary hormone secretion in sheep. Neuroendocrinology. 58(1): 111-122.

Crown, A., D. K. Clifton, and R. A. Steiner. 2007. Neuropeptide signaling in the integration of metabolism and reproduction. Neuroendocrinology. 86(3): 175-182.

Foster, J. P., G. E. Lamming, and A. R. Peters. 1980. Short-term relationships between plasma-Lh, Fsh and progesterone concentrations in postpartum dairy-cows and the effect of Gn-Rh injection. J. Reprod. Fertil. 59(2): 321-327.

Gutierrez-Pascual, E., A. J. Martinez-Fuentes, L. Pinilla, M. Tena-Sempere, M. M. Malagon, and J. P. Castano. 2007. Direct pituitary effects of kisspeptin: activation of gonadotrophs and somatotrophs and stimulation of luteinising hormone and growth hormone secretion. J. Neuroendocrinol. 19(7): 521-530.

Gutierrez, C. G., J. G. Gong, T. A. Bramley, and R. Webb. 2006. Selection on predicted breeding value for milk production delays ovulation independently of changes in follicular development, milk production and body weight. Anim. Reprod. Sci. 95(3-4): 193-205.

Hansen, L. B. 2000. Consequences of selection for milk yield from a geneticist's viewpoint. J. Dairy. Sci. 83(5): 1145-1150.

Hudmon, A., G. Davenmport, E. S. Coleman, and J. L. Sartin. 2009. Low doses of estradiol partly inhibit release of GH in sheep without affecting basal levels. Domest. Anim. Endocrinol. 37: 181-187.

Kadokawa, H., M. Matsui, K. Hayashi, N. Matsunaga, C. Kawashima, T. Shimizu, K. Kida, and A. MiyaMot. 2008a. Peripheral administration of kisspeptin-10 increases plasma concentrations of GH as well as LH in prepubertal Holstein heifers. J. Endocrinology. 196(2): 331-334.

Kadokawa, H., S. Suzuki, and T. Hashizume. 2008b. Kisspeptin-10 stimulates the secretion of growth hormone and prolactin directly from cultured bovine anterior pituitary cells. Anim. Reprod. Sci. 105(3-4): 404-408.

Kinsey-Jones, J. S., X. F. Li, A. M. Knox, E. S. Wilkinson, X. L. Zhu, A. A. Chaudhary, S. R. Milligan, S. L. Lightman, and K. T. O'Byrne. 2009. Down-regulation of hypothalamic kisspeptin and its receptor, *Kiss1r*, mRNA expression is associated with stress-induced suppression of luteinising hormone secretion in the female rat. J. Neuroendocrinol. 21(1): 20-29.

Lents, C. A., N. L. Heidorn, C. R. Barb, and J. J. Ford. 2008. Central and peripheral administration of kisspeptin activates gonadotropin but not somatotropin secretion in prepubertal gilts. Reprod. 135(6): 879-887.

Leung, K., V. Padmanabhan, L. J. Spicer, H. A. Tucker, R. E. Short, and E. M. Convey. 1986. Relationship between Pituitary Gnrh-Binding Sites and Pituitary Release of Gonadotropins in Postpartum Beef-Cows. J. Reprod. Fertil. 76(1): 53-63.

Lucy, M. C. 2001. Reproductive loss in high-producing dairy cattle: where will it end? J. Dairy Sci. 84(6): 1277-1293.

Lucy, M. C. 2003. Mechanisms linking nutrition and reproduction in postpartum cows. Reprod. 61(Suppl): 415-427.

Meinhardt, U. J., and K. K. Ho. 2007. Regulation of growth hormone action by gonadal steroids. Endocrinol. Metab. Clin. North. Am. 36(1): 57-73.

NRC. 2001. Nutrient Requirment of Dairy Cattle. 7th. ed. National Academy Press, Washington, DC.

Reimers, T. J., S. V. Lamb, S. A. Bartlett, R. A. Matamoros, R. G. Cowan, and J. S. Engle. 1991. Effects of hemolysis and storage on quantification of hormones in blood samples from dogs, cattle, and horses. Am. J. Vet. Res. 52(7): 1075-1080.

Roa, J., E. Vigo, J. M. Castellano, V. M. Navarro, R. Fernandez-Fernandez, F. F. Casanueva, C. Dieguez, E. Aguilar, L. Pinilla, and M. Tena-Sempere. 2006. Hypothalamic expression of KiSS-1 system and gonadotropin-releasing effects of kisspeptin in different reproductive states of the female Rat. Endocrinology. 147(6): 2864-2878.

Royal, M. D., A. P. Flint, and J. A. Woolliams. 2002. Genetic and phenotypic relationships among endocrine and traditional fertility traits and production traits in Holstein-Friesian dairy cows. J. Dairy Sci. 85(4): 958-967.

Rutter, L. M., and R. D. Randel. 1984. Postpartum Nutrient Intake and Body Condition - Effect on Pituitary-Function and Onset of Estrus in Beef-Cattle. J. Anim. Sci. 58(2): 265-274.

Sartin, J. L., K. A. Cummins, R. J. Kemppainen, D. N. Marple, C. H. Rahe, and J. C. Williams. 1985. Glucagon, Insulin, and Growth-Hormone Responses to Glucose-Infusion in Lactating Dairy-Cows. Am. J. Physiol. 248 (1): E108-E114.

Sartin, J. L., R. J. Kemppainen, and K. A. Cummins. 1986. Effects of Growth Hormone-Releasing Factor (Grf), Somatostatin (Srif) and Glucose on Growth-Hormone (Gh) Secretion in Lactating Dairy-Cows. Fed Proc. 45 (3): 280-280.

Sartin, J. L., R. J. Kemppainen, K. A. Cummins, and J. C. Williams. 1988. Plasma concentrations of metabolic hormones in high and low producing dairy cows. J. Dairy Sci. 71(3): 650-657.

Sartin, J. L., A. C. Pierce, R. J. Kemppainen, F. F. Bartol, K. A. Cummins, D. N. Marple, and J. C. Williams. 1989. Differential growth hormone responses to glucose and growth hormone releasing hormone in lactating dairy cows. Acta Endocrinol. (Copenh). 120(3): 319-325.

Scanlan, N., and D. C. Skinner. 2002. Estradiol modulation of growth hormone secretion in the ewe: no growth hormone-releasing hormone neurons and few somatotropes express estradiol receptor alpha. Biol. Reprod. 66(5): 1267-1273.

Smith, J. T. 2008. Kisspeptin signalling in the brain: steroid regulation in the rodent and ewe. Brain Res. Rev. 57(2): 288-298.

Smith, J. T., B. V. Acohido, D. K. Clifton, and R. A. Steiner. 2006. KiSS-1 neurones are direct targets for leptin in the ob/ob mouse. J. Neuroendocrinol. 18(4): 298-303.

Smith, J. T., C. M. Clay, A. Caraty, and I. J. Clarke. 2007. KiSS-1 messenger ribonucleic acid expression in the hypothalamus of the ewe is regulated by sex steroids and season. Endocrinology. 148(3): 1150-1157.

Smith, J. T., A. Rao, A. Pereira, A. Caraty, R. P. Millar, and I. J. Clarke. 2008. Kisspeptin is present in ovine hypophysial portal blood but does not increase during the preovulatory luteinizing hormone surge: evidence that gonadotropes are not direct targets of kisspeptin *in vivo*. Endocrinology. 149(4): 1951-1959.

Tena-Sempere, M. 2006. GPR54 and kisspeptin in reproduction. Hum. Reprod. Update. 12(5): 631-639.

Tomita, K., S. Oishi, H. Ohno, S. C. Peiper, and N. Fujii. 2008. Development of novel G-protein-coupled receptor 54 agonists with resistance to degradation by matrix metalloproteinase. J. Med. Chem. 51(23): 7645-7649.

Whitlock, B. K., J. A. Daniel, R. R. Wilborn, H. S. Maxwell, B. P. Steele, and J. L. Sartin. 2010. Interaction of kisspeptin and the somatotropic axis. Neuroendocrinology. 92(3): 178-188.

Whitlock, B. K., J. A. Daniel, R. R. Wilborn, S. P. Rodning, H. S. Maxwell, B. P. Steele, and J. L. Sartin. 2008. Interaction of estrogen and progesterone on kisspeptin-10-stimulated luteinizing hormone and growth hormone in ovariectomized cows. Neuroendocrinology. 88(3): 212-215.

Yamada, S., Y. Uenoyama, M. Kinoshita, K. Iwata, K. Takase, H. Matsui, S. Adachi, K. Inoue, K. I. Maeda, and H. Tsukamura. 2007. Inhibition of metastin (kisspeptin-54)-GPR54 signaling in the arcuate nucleus-median eminence region during lactation in rats. Endocrinology. 148 (5): 2226-2232.