Growth Hormone Response to Growth Hormone Releasing Hormone in Calves that Differ in Genetic Merit for Milk Yield

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ABSTRACT

Holstein heifer, steer, and bull calves from control (CL) and select (SL) lines of cows that differed by more than 4000 kg of milk during a 305-d lactation (SL > CL) were used to determine growth hormone (GH) response to 5 doses of GH releasing hormone (GHRH) and how this response was affected by gender, period (age), and genetic merit for milk yield. Doses (0, 2.5, 5, 10, and 20 μ g/100 kg of BW) of a GHRH analog were assigned randomly to each heifer (4 CL, 4 SL), steer (4 CL, 4 SL), and bull (3 CL, 3 SL) calf and administered on consecutive days at approximately 3, 6, and 10 mo of age (periods; P1, P2, and P3). Jugular blood samples ($n = 15$) collected between –30 and 240 min relative to GHRH administration were used to quantify area under the GH response curve (AUC) after subtracting mean prechallenge GH concentrations. Estimates of maximum response ($R_{\max}$) and sensitivity (ED_{50}) to GHRH were obtained from the hyperbolic dose response curves (AUC vs. dose). Data were analyzed for effects of dose, line, period, gender, and their interactions with period as the repeated effect. Prechallenge GH concentrations were not affected by genetic line, gender, or period. The AUC was not affected by line, but decreased with period and increased with GHRH dose. The $R_{\max}$ did not differ between lines or among genders, but decreased with period. The ED_{50} did not differ between lines or among periods, but heifers were more sensitive to GHRH than steers or bulls. Although GH response to GHRH has been identified as a potential indicator of genetic merit, it did not differ between these substantially different genetic lines.

(Key words: calf, genetic selection, milk yield, growth hormone)

Abbreviation key: AUC = area under the GH response curve, CL = control line, ED_{50} = effective dose required to elicit a 50% maximal response, GH = growth hormone, GHRH = growth hormone releasing hormone, P = period, $R_{\max}$ = maximum response, SL = select line.

INTRODUCTION

Several studies have attempted to determine relationships between genetic merit for milk yield and serum growth hormone (GH) response to growth hormone-releasing hormone (GHRH) administration, in part, to develop a method to identify young animals that have a greater potential for development of desirable traits such as milk yield. Alterations in GH response to administration of GHRH have been associated with improved genetic merit in mature bulls (Kazmer et al., 1992; Zinn et al., 1994) and calves (Lovendahl et al., 1991; Parchuri et al., 1993; Lovendahl et al., 1994), but others have detected no effects of genetic merit on GH response to a GHRH challenge (Kazmer et al., 1992; Zinn et al., 1994; Baumgard et al., 2002). In addition to other sources of variation, different doses and different molecules (bovine GHRH and various analogs) were used in these studies. Results of a dose response study might explain some of these differences and assist efforts to distinguish between inferior and superior animals before investing funds to raise them for a particular purpose.

Dose-response studies can characterize adaptations in the ability of animals to respond to physiological challenges by providing estimates of the maximum response obtainable ($R_{\max}$) and the effective dose required to elicit a half-maximal response (ED_{50}), which is an indication of sensitivity to the physiological challenge. Differences among animals or groups of animals in their response to a physiological challenge can be expressed as differences in either or both of these response characteristics. Administration of a single dose could provide either a maximal or submaximal response, but multiple doses are required to identify $R_{\max}$ and ED_{50} . Single doses cannot detect differences in either characteristic

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and might result in the failure to detect real treatment differences. For example, GH response to GHRH was greater in growing Hereford steers implanted with estradiol and trenbolone acetate than in nonimplanted steers, but the difference in response decreased as dose of GHRH increased (Hongerholt et al., 1992). In addition, differences in GH response between genetically superior and inferior mature bulls decreased as the amount of GHRH administered increased (Kazmer et al., 1992). Thus, identification of an optimum dose or range of doses of GHRH is necessary to enhance the ability to detect treatment effects and draw valid conclusions from studies designed to evaluate effects of treatments on GH response to GHRH. The primary objectives of this study were to determine $R_{\max}$ and ED_{50} for GH response to a GHRH analog in growing Holstein heifers and to determine whether these measures were affected by gender, age, and genetic merit for milk yield.

MATERIALS AND METHODS

Animals and Animal Management

A breeding project initiated in 1964 by Charles Young at the University of Minnesota as part of a regional research effort provided calves from static control (CL) and contemporary select (SL) lines of Holsteins (Hansen, 2000). Milk yield of multiparous SL cows exceeded that of multiparous CL cows by more than 4000 kg per 305-d lactation ($11,078 \pm 329$ vs. 6890 ± 403 kg) when the GHRH challenges were conducted (Baumgard et al., 2002). Each year, semen from 4 CL and 4 SL sires was used to inseminate CL and SL females, respectively, and coefficients of inbreeding were not allowed to exceed 6.25% in either line (Jones et al., 1994; Hansen, 2000). Calves used in this study were born within a 12-wk period and represented offspring from 4 CL sires and 11 CL dams or 6 SL sires and 12 SL dams. All heifers, and all but one steer, were born before any of the bulls. When the last bull was born, average age of the heifers (62.4 ± 5.7 d) was 18 d more than the steers (44.5 ± 9.0 d) and 52 d more than the bulls (10.5 ± 4.0 d). Calves were weaned at 5 wk of age. Bulls destined to become steers were castrated at least 2 wk before the study began. From about 2.5 to 3.5 mo of age, heifers (4 CL, 4 SL), steers (4 CL, 4 SL), and bulls (3 CL, 3 SL) and their diets were moved from group housing at Waseca, MN, to individual pens on the St. Paul campus of the University of Minnesota. Other than this temporary change in location, animal management and care were as described previously (Baumgard et al., 2002) and included daily observations for health abnormalities and treatment when appropriate. Steers were implanted with Ralgro (Schering-Plough, Kenilworth, NJ)

by 6 mo of age and steers and bulls were managed similarly throughout the study. Calf BW was determined and a catheter implanted in both jugular veins of each calf on d 0 of each of three 6-d (d 0 to 5) periods (P1, P2, and P3) when calves were approximately 3, 6, and 10 mo of age, respectively. Animal care and experimental procedures were approved by the University of Minnesota Institutional Animal Care and Use Committee.

GHRH Challenges

A human GHRH analog ([DesNH₂Tyr¹, D-Ala², Ala¹⁵]hGHRH(1-29)NH₂; compound RO 23-7863, Hoffmann-LaRoche, Nutley, NJ) stock solution (10 µg/mL in sterile physiological saline containing 0.1% BSA) was prepared and stored at -80°C (Baumgard et al., 2002). This analog is more resistant to biodegradation and has a greater biopotency than its native counterpart (Felix et al., 1988). The GHRH challenges (doses of 0, 2.5, 5, 10, and 20 µg per 100 kg of BW) were assigned randomly to each calf during each period and administered via jugular catheter on consecutive days (d 1 to 5) during P1, P2, and P3. To minimize age-related effects of the relatively rapid decrease in serum GH concentrations and response to GHRH by young calves (Baumgard et al., 2002), GHRH challenges during P1 and P2 were staggered. For P1 (July 30 to September 14), GHRH challenges for the 4 oldest heifers and 4 oldest steers (2 SL and 2 CL for each gender) were conducted during the same 5-d period. The GHRH challenges were conducted 2.5 wk later for the 4 youngest heifers and 4 youngest steers and 6 wk later for all 6 bulls. For P2 (November 3 to 20), GHRH challenges were conducted for all heifers and steers during the same 5-d period and 2 wk later for all bulls. For P3 (March 8 to 12), when effects of age were reduced (Baumgard et al., 2002), all calves were challenged during the same 5-d period. During each challenge, calves were haltered and tethered to facilitate blood sampling. Calves were allowed to lie down, had access to water, but were not allowed to eat during the 4.5 h of blood sampling.

Blood samples were collected via jugular catheter at -30, -20, -10, 0, 2.5, 5, 10, 20, 30, 45, 60, 90, 120, 180, and 240 min relative to GHRH administration. Blood samples collected during P1 remained at room temperature (21°C) for approximately 1 h and were then refrigerated (4°C) overnight. Serum was harvested (1200 × g, 15 min, 4°C) and stored at -20°C until assayed. Blood samples (10 mL) from P2 and P3 were immediately mixed with heparin (20 µL of 10,000 IU/mL) and placed on ice until plasma was harvested. Plasma was stored at -20°C until assayed. Samples were analyzed in duplicate for GH concentration by a validated double anti-

body radioimmunoassay (Kazmer et al., 1992; 2000). The minimal detectable concentration was 0.7 ng/mL; intra- and interassay CV were 8.0 and 8.3%, respectively.

Calculations and Statistical Analyses

The mean of the 4 samples obtained before each GHRH administration provided an estimate of the prechallenge GH concentration. Area under the GH response curve (AUC) was calculated by linear trapezoidal summation between successive pairs of GH concentration and time coordinates after subtracting prechallenge GH. The AUC was calculated from GHRH administration (0 min) through 45, 60, 90, 120, and 240 min postadministration. The GHRH dose and AUC data were fitted to the hyperbolic equation $AUC = B_{\max}(\text{dose}) / (ED_{50} + \text{dose})$ using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA) and solved for estimates of $B_{\max}$ and ED_{50} . Peak GH was defined as the greatest GH concentration within 60 min after administration of GHRH. Time to peak was calculated as the duration, in minutes, from GHRH administration to peak GH within this 60-min interval.

One SL heifer was removed due to health problems after P1 and was replaced with a SL heifer of similar age and BW. Data from these 2 SL heifers were identified as individual animals in the analyses. One SL steer was removed from study due to health problems after P2 and was not replaced.

Data from P1, P2, and P3 were analyzed as a completely randomized design with the mixed model procedure for repeated measures with period as the repeated effect (SAS Inst. Inc., Cary, NC). A compound symmetry covariance structure was specified. The initial model used to evaluate treatment effects included the variable mean, main effects (period, line, gender, and dose), all possible interactions, and error. The 4-way interaction was not significant for any of the measured values and was removed from the model for the final analyses. Exclusion of nonsignificant ($P > 0.20$) 2- and 3-way interactions from the model did not alter data interpretation so these interactions were retained in the final model. To reduce potential problems with heterogeneity of variances, prechallenge GH, and peak GH were determined from natural log-transformed GH concentrations and these transformed data analyzed with the same model. For all evaluations, statistical comparisons were considered different when $P < 0.05$.

RESULTS

At the start of P1, bulls were younger ($P < 0.05$) than heifers (87 vs. 107 ± 6 d of age) and age of the steers

(89 ± 6 d) was similar to the bulls and heifers. During P1, BW among heifers (107 ± 9 kg), steers (97 ± 9 kg), and bulls (86 ± 9 kg) did not differ and did not differ between lines. There was an interaction of line and period because final (P3) BW of SL calves was greater ($P < 0.01$) than CL calves for heifers (307 vs. 279 ± 9 kg), steers (402 vs. 357 ± 9 kg), and bulls (326 vs. 286 ± 9 kg). Over the entire study, BW differed ($P < 0.01$) among genders and there was an interaction of gender and period, but these effects were confounded by the staggered time of GHRH administration and are not reported.

Analyses of untransformed prechallenge GH indicated heterogeneity of variance so analyses of the $\ln(\text{prechallenge GH})$ are reported (Table 1). Overall prechallenge GH for bulls, steers, and heifers were 5.3, 4.5, and 3.7 ng/mL, respectively. The $\ln(\text{prechallenge GH})$ did not differ among days within an animal-period (data not reported), among P1, P2, and P3, or between CL and SL calves (Table 1). Bulls tended ($P = 0.07$) to have greater $\ln(\text{prechallenge GH})$ than heifers and steers were intermediate (Table 1). There was an interaction ($P < 0.001$) of gender and period as $\ln(\text{prechallenge GH})$ was greater for bulls than steers or heifers and greater for steers than heifers during P1, similar for bulls, steers, and heifers during P2, and greater for bulls than steers or heifers during P3, respectively (Table 1).

Effects of line, gender, period, and dose of GHRH on AUC was similar for all time intervals (0 to 45, 60, 90, 120, or 240 min postchallenge). Therefore, only the 60-min AUC responses are reported. There was no effect of genetic line ($P = 0.70$) on AUC and there were no interactions of line (Figure 1) with gender ($P = 0.72$), period ($P = 0.35$), or dose ($P = 0.85$). The AUC increased ($P < 0.001$) with dose (0.11^a , 1.64^b , 2.46^c , 2.91^d , $3.22^e \pm 0.16 \mu\text{g} \cdot \text{min}/\text{mL}$), decreased ($P < 0.001$) with period (2.39^a , 1.91^b , $1.90^b \pm 0.14 \mu\text{g} \cdot \text{min}/\text{mL}$), and was greater ($P < 0.05$) in heifers than steers and intermediate in bulls (2.42^a , 2.17^{ab} , $1.61^b \pm 0.22 \mu\text{g} \cdot \text{min}/\text{mL}$). There was an interaction of period with gender ($P < 0.001$) because bulls and steers had a greater response than heifers during P1 and heifers had a greater response than bulls and steers during P2 and P3 (Figure 2). There was an interaction of period with dose ($P = 0.05$) because AUC for doses greater than $5 \mu\text{g}/100$ kg of BW decreased more than responses to doses less than $10 \mu\text{g}/100$ kg of BW as age of the calves increased (Figure 2).

There were interactions (Figure 3, Table 2) between gender and dose ($P < 0.01$) and among gender, dose, and period ($P < 0.01$) for AUC. The AUC for steers was less ($P < 0.01$) than that of heifers when 2.5, 5.0, and $10 \mu\text{g}$ of GHRH/100 kg of BW were administered and less ($P < 0.05$) than that of bulls when 5.0 or $20.0 \mu\text{g}$

Table 1. Transformed (natural logarithm) prechallenge concentrations of growth hormone¹ (GH) in control (CL) and select (SL) line² calves.

Gender ³	Line ⁴	Period			Mean	SEM
		1	2	3		
Heifers	CL	1.11	1.50	1.23	1.28	0.14
	SL	1.12	1.52	1.39	1.34	0.13
	Mean	1.12 ^a	1.51	1.31 ^a	1.31	0.11
Steers	CL	1.66	1.80	1.40	1.62	0.14
	SL	1.20	1.74	1.28	1.41	0.14
	Mean	1.43 ^b	1.77	1.34 ^a	1.51	0.11
Bulls	CL	1.80	1.44	1.92	1.72	0.16
	SL	1.78	1.46	1.62	1.62	0.16
	Mean	1.79 ^c	1.45	1.77 ^b	1.67	0.13
Overall	Mean	1.45	1.58	1.47	1.50	0.07

^{a,b,c}Gender means within a column with different superscripts differ ($P < 0.05$).

¹Least squares means of ln transformed GH concentrations (ng/mL) in 4 samples collected during the 30 min before GHRH administration on 5 successive days during 3 periods (P1, P2, P3) when calves were approximately 3, 6, and 10 mo of age, respectively. There were 8 heifers, 8 steers, and 6 bulls with equal representation of CL and SL within each gender except during P3 when there were only 3 SL steers. Steers were implanted with Ralgro during P2 and P3.

²Milk yield of multiparous SL cows exceeded that of multiparous CL cows ($11,078 \pm 329$ vs. 6890 ± 403 kg per 305-d lactation) when the GHRH challenges were conducted.

³Interaction ($P < 0.001$) of gender and period.

⁴No effect of genetic line ($P = 0.49$).

of GHRH/100 kg of BW were administered. The AUC for heifers was greater ($P < 0.05$) than bulls for the 2.5 and 10 $\mu\text{g}/100$ kg BW dose, equal ($P > 0.19$) to bulls for the 5 $\mu\text{g}/100$ kg BW dose, and less ($P < 0.05$) than bulls for the 20 $\mu\text{g}/100$ kg BW dose. Magnitude of these responses varied among periods, but the general trend was for AUC in steers and bulls to be greater ($P < 0.05$) in P1 than in P2 and P3. In contrast, response in heifers increased ($P < 0.05$) for all nonzero doses, except the 20 $\mu\text{g}/100$ kg of BW dose from P1 to P2 and did not differ between P2 and P3 except for the decreased ($P < 0.05$) AUC for the 10 $\mu\text{g}/100$ kg of BW dose during P3.

A plateau in AUC was not obtained for 1 SL bull during P1 and 1 SL and 1 CL steer during P2 resulting in extremely large and unreasonable estimates of R_{max} and ED_{50} . These data were not included in the analyses. Data from 1 CL bull during P3 did not converge and thus estimates of R_{max} and ED_{50} were not obtained. The R^2 of the fit for the 61 remaining individual animal-period GHRH dose and AUC data to the hyperbolic curve ranged from 0.29 to 1.00 and averaged 0.88 ± 0.03 . Eleven of these 61 data sets had an R^2 between 0.40 and 0.69, and 49 data sets had an R^2 greater than 0.74. The R^2 indicated goodness of fit did not differ between lines ($P = 0.32$) or among periods ($P = 0.18$) or genders ($P = 0.68$).

The R_{max} for AUC response (Table 2) decreased ($P < 0.05$) with period (4.78^a , 3.90^b , $3.73^b \pm 0.35$ $\mu\text{g} \cdot \text{min}/\text{mL}$), did not differ between lines ($P = 0.71$) or among genders ($P = 0.34$), and was not affected by interactions ($P >$

0.44) of period, line, and gender. Genetic line did not affect ED_{50} for AUC response ($P = 0.32$). Bulls and steers had similar ED_{50} for AUC response and both were greater ($P < 0.01$) than that of heifers (Table 2). A trend was detected for ED_{50} to be greater ($P = 0.07$) during P2 (3.8 , 6.9 , 3.6 ± 0.9 $\mu\text{g}/100$ kg of BW). There was a trend for an interaction ($P = 0.07$) of gender and period as ED_{50} for bulls and steers increased from P1 to P2 and decreased from P2 to P3, and ED_{50} for heifers decreased from P1 to P2 and remained unchanged from P2 to P3 (Table 2).

Heterogeneity of variance was evident when GH response was determined as peak height, thus these data were transformed to natural logarithms for analyses. Peak GH increased ($P < 0.001$) with dose, but no effects of period ($P = 0.14$), line ($P = 0.77$), or gender ($P = 0.19$) were detected (least squares means not reported). There were interactions between period and gender ($P < 0.001$) and between gender and dose ($P < 0.05$), and these interactions were consistent with those detected with the AUC response. There were no interactions of line with gender, dose, or period. There was a trend ($P = 0.06$) for an interaction of line, gender, and dose and this was primarily due to the interaction of gender and dose. Peak height values were numerically greater for SL steers and heifers and numerically less for SL bulls than their CL counterparts at all doses except the 10 $\mu\text{g}/100$ kg of BW dose. However, none of the comparable gender by dose means differed between lines. Time to peak GH for the nonzero doses of GHRH averaged 16.8

Figure 1. Effect of selection for milk yield on growth hormone (GH) response in growing Holstein calves from control (CL) and select (SL) lines to dose of a human growth hormone releasing hormone (GHRH) analog. The GHRH challenges were conducted during 3 periods (P1, P2, and P3) when calves were approximately 3, 6, and 10 mo of age, respectively. Steers were implanted with Ralgro during P2 and P3. The GH response was measured as area under the GH response curve (AUC) from 0 to 60 min after GHRH administration. Each observation represents the least squares means from 4 heifers, 4 steers, and 3 bulls, except during P3 when there were only 3 SL steers. Milk yield of the multiparous SL cows exceeded that of the multiparous CL cows ($11,078 \pm 329$ vs. 6890 ± 403 kg per 305-d lactation) when the GHRH challenges were conducted. There was no effect of genetic line ($P = 0.70$) and no interaction of line with GHRH dose ($P = 0.85$; top panel), period ($P = 0.35$; middle panel), or gender ($P = 0.72$; bottom panel).

Figure 2. Effect of gender (top panel) or dose (bottom panel) of a human growth hormone releasing hormone (GHRH) analog on growth hormone (GH) response in Holstein calves from control (CL) and select (SL) lines at approximately 3, 6, and 10 (P1, P2, and P3, respectively) mo of age. Steers were implanted with Ralgro during P2 and P3. The GH response was measured as area under the GH response curve (AUC) from 0 to 60 min after GHRH administration. Each gender mean represents the least squares means from 4 heifers, 4 steers, and 3 bulls from each of the CL and SL, except during P3 when only 3 SL steers were tested. Milk yield of the multiparous SL cows exceeded that of the multiparous CL cows ($11,078 \pm 329$ vs. 6890 ± 403 kg per 305-d lactation) when the GHRH challenges were conducted. Each dose mean represents the least squares means of 22 calves during P1 and P2, and 21 calves during P3. Lines did not differ. Interactions were detected between period (age) and gender ($P < 0.001$) and between period and dose ($P = 0.05$).

± 1.3 min and was not affected by line, gender, dose, period, or their interactions (results not reported).

DISCUSSION

The primary objectives of this study were to determine $R_{\max}$ and ED_{50} for GH response to a GHRH analog and to determine whether these measures were affected by gender, age, and genetic merit for milk yield. A secondary objective was to determine if a dose or range of doses of this GHRH analog would elicit a GH response

Figure 3. Effect of gender and age of Holstein calves from control (CL) and select (SL) lines and dose of a human growth hormone releasing hormone (GHRH) analog on growth hormone (GH) response. The GHRH challenges were conducted during 3 periods (P1, P2, and P3) when calves were approximately 3, 6, and 10 mo of age, respectively. Steers were implanted with Ralgro during P2 and P3. The GH response was measured as area under the GH response curve (AUC) from 0 to 60 min after GHRH administration. Each observation represents the least squares means from 4 heifers, 4 steers, and 3 bulls from each of the CL and SL, except during P3 when only 3 SL steers were tested. Milk yield of the multiparous SL cows exceeded that of the multiparous CL cows ($11,078 \pm 329$ vs. 6890 ± 403 kg per 305-d lactation) when the GHRH challenges were conducted. Genetic lines did not differ. Interactions were detected between gender and dose ($P < 0.01$) and among gender, dose, and age ($P < 0.01$) on GH response.

suitable to assess effects of genetic merit, gender, and age on pituitary function in growing Holstein calves.

Dose-response plots (Figures 2 and 3) revealed a nearly maximal GH response to 10 and 20 μ g of this GHRH analog for both lines and all genders and periods (age), except for 3-mo-old bulls. The $R_{\max}$ analyses confirmed the graphical representation of the data and indicated that actual $R_{\max}$ varied between 3.0 and 5.5 μ g·min/mL (mean of 4.1 ± 0.5 μ g·min/mL) and did not differ between lines or among genders or periods (ages). Thus, only the 2.5 μ g of GHRH/100 kg of BW provided a less than maximal response for gender, period, and line combinations in these 3- to 10-mo old Holstein calves. Regardless, whether analyzed with all doses or only the 2.5 μ g/100 kg BW dose (results not reported), there was no effect of line ($P > 0.72$) and no interaction ($P > 0.24$) of line with any combination of gender and/or period. Thus, our results indicate that genetic merit for milk yield had no effect on submaximal or maximal stimulation of GH release from the pituitaries of young Holstein calves or on pituitary sensitivity to this GHRH analog. However, additional evaluation of GH response to submaximal doses of GHRH in a greater number of CL and SL calves is warranted to further evaluate effects of genetic merit and to confirm whether the pituitaries of heifers are indeed more sensitive to this GHRH analog than pituitaries of steers or bulls.

Hongerholt et al. (1992) used this same GHRH analog and determined that AUC was greater in growing Hereford steers implanted with estradiol and trenbolone acetate than in nonimplanted steers and that the magnitude of difference in response decreased as dose of GHRH increased from 7 to 28 μ g/100 kg of BW. When the same analog was administered to mature Holstein bulls that differed in genetic merit, AUC did not differ among doses of 11, 22, or 33 μ g/100 kg of BW and was not affected by genetic merit (Kazmer et al., 1992). In contrast, maximum GH concentration after a dose of 11 μ g/100 kg of BW was greater in the genetically superior bulls. These authors subsequently determined that a submaximal dose of 4 μ g/100 kg of BW of this same GHRH analog was more appropriate for mature bulls (Zinn et al., 1994). Results from these studies suggest that $R_{\max}$ was not affected by treatment and that increased genetic merit had a greater effect on pituitary sensitivity (on the ED_{50}) than the maximal response to GHRH. Results from the current dose study support those results and indicate that a dose of less than 5.0 μ g/100 kg of BW also provides a submaximal GH response in growing Holstein heifers.

Others have evaluated doses of less than 5 μ g/100 kg of BW of this analog (Connor et al., 1999) or of bovine GHRH (Auchtung et al., 2001b). Connor et al. (1999) administered 1.5 μ g/100 kg of BW of the same analog

Table 2. Effect of gender, period,¹ and dose of growth hormone releasing hormone (GHRH) analog² on growth hormone (GH) response.³

GH response	Heifers			Steers			Bulls			Gender means			SEM
	P1	P2	P3	P1	P2	P3	P1	P2	P3	Heifers	Steers	Bulls	
AUC ⁴ to Dose ²													
0.0	0.04 ^a	0.08 ^a	0.27 ^a	0.10 ^a	0 ^a	0.05 ^a	0.33 ^a	0.23 ^a	0 ^a	0.13 ^a	0.04 ^a	0.16 ^a	0.27
2.5	1.45 ^{b,w}	2.83 ^{b,x}	2.36 ^{b,x}	1.53 ^b	0.98 ^b	1.02 ^b	2.86 ^{b,w}	0.80 ^{a,x}	0.87 ^{a,x}	2.22 ^{b,z}	1.18 ^{b,y}	1.51 ^{b,yz}	0.27
5.0	1.92 ^{b,w}	3.67 ^{cd,x}	3.66 ^{c,x}	2.51 ^{c,w}	0.91 ^{b,x}	1.76 ^{bc,w}	3.16 ^{bc}	2.34 ^b	2.19 ^b	3.08 ^{c,z}	1.73 ^{c,y}	2.56 ^{c,z}	0.27
10.0	2.91 ^{c,w}	4.17 ^{cd,x}	3.09 ^{bc,w}	3.25 ^{cd,w}	1.81 ^{c,x}	2.25 ^{c,x}	3.96 ^{c,w}	2.05 ^{b,x}	2.73 ^{bc,x}	3.39 ^{c,z}	2.44 ^{d,y}	2.91 ^{c,yz}	0.27
20.0	3.18 ^c	3.31 ^{bd}	3.40 ^c	3.62 ^{d,w}	2.53 ^{c,x}	1.82 ^{bc,x}	5.03 ^{d,w}	2.90 ^{b,x}	3.14 ^{c,x}	3.30 ^{c,yz}	2.66 ^{d,y}	3.69 ^{d,z}	0.27
R _{max} ⁵	4.12	4.06	3.78	4.69	3.38	2.99	5.53	4.26	4.42	3.99	3.69	4.74	0.48
ED ₅₀ ⁶	4.37	1.36	1.32	4.53	9.20	4.23	2.62	10.00	5.37	2.35 ^y	5.98 ^z	6.00 ^z	0.71

^{a,b,c,d}Means within column with different superscript letters differ ($P < 0.05$).

^{w,x}Period means within gender-dose row with different superscript letters differ ($P < 0.05$).

^{y,z}Overall gender means within with different superscript letters differ ($P < 0.05$).

¹Periods (P) 1, 2, and 3 were conducted when calves were about 3, 6, and 10 mo of age. Steers were implanted with Ralgro during P2 and P3.

²Dose of a human GHRH analog ((DesNH₂Tyr¹, D-Ala², Ala¹⁵)hGHRH(1-29)NH₂; compound RO 23-7863, Hoffmann-LaRoche, Nutley, NJ) administered as 0, 2.5, 5.0, 10, and 20 $\mu\text{g}/100$ kg of BW.

³Least squares means ($n = 8$ heifers, 8 steers, and 6 bulls, except during P3 when there were only 3 SL steers) with equal representation of control (CL) and select (SL) genetic lines within each gender. Milk yield of multiparous SL cows exceeded that of multiparous CL cows ($11,078 \pm 329$ vs. 6890 ± 403 kg/305-d lactation) when the GHRH challenges were conducted.

⁴Area under the GH response curve (AUC, $\mu\text{g}\cdot\text{min}/\text{mL}$) from 0 to 60 min after administration of GHRH. Genetic line did not affect AUC ($P = 0.70$) and no interactions were detected for line with gender ($P = 0.72$), period ($P = 0.35$), or dose of GHRH ($P = 0.85$).

⁵Maximum AUC response ($\mu\text{g}\cdot\text{min}/\text{mL}$) estimated as the plateau of the hyperbolic plot of GHRH dose and GH AUC. Genetic line did not affect R_{max} ($P = 0.71$) and no interactions were detected for line with gender ($P = 0.34$) or period ($P = 0.60$). Mean for P1 was greater ($P < 0.05$) than means for P2 and P3 ($4.78, 3.90, 3.73 \pm 0.35$ $\mu\text{g}\cdot\text{min}/\text{mL}$, respectively).

⁶Dose of GHRH ($\mu\text{g}/100$ kg of BW) required to produce 50% of R_{max}. Genetic line did not affect ED₅₀ ($P = 0.32$) and no interactions were detected for line with gender ($P = 0.12$) or period ($P = 0.58$).

and demonstrated a significant ($P < 0.05$) but weak ($R^2 = 0.07$) positive relationship between AUC and subsequent average daily gain of 7- to 8-mo old Angus bulls. In a subsequent study with bovine GHRH, Connor et al. (2000) administered 4.5 $\mu\text{g}/100$ kg of BW and a second dose of 1.5 $\mu\text{g}/100$ kg of BW 3 h later. The GH response was measured as the increase in GH concentration 10 min after GHRH administration. A somewhat stronger relationship ($R^2 = 0.18$; $P < 0.01$) between average daily gain and GH response to the second administration of GHRH was detected (Connor et al., 2000). In another study, this group used the same technique (one sample before and after GHRH administration) and detected a weak negative relationship ($R^2 = 0.05$; $P = 0.06$) between GH response and subsequent average daily gain of 7- to 8-mo-old beef heifers (Auchtung et al., 2001b). Using the same technique, they classified beef cows by expected progeny difference for milk and detected a weak positive relationship ($R^2 = 0.12$; $P < 0.05$) between GH response and milk yield in beef cows challenged with 4.5 $\mu\text{g}/100$ kg of BW of bovine GHRH at 218 d postpartum (Auchtung et al., 2001a). In each of these studies (Auchtung et al., 2001a,b), the relationships were influenced by 3 to 4 animals with large GH responses.

Evaluation of serum GH concentrations and of GH response to GHRH between genetically superior and

inferior animals has provided variable results and these inconsistencies and potential reasons for this variation have been described (Baumgard et al., 2002). Method of assessing the GH response and dose of GHRH administered certainly affect the ability to detect treatment differences. When we used peak GH concentration during our sampling interval to assess GH response, no effects of period, gender, period $\times$ gender, or period $\times$ dose $\times$ gender were detected. However, effects of these treatments and treatment interactions were detected when AUC was used to assess GH response. In addition, the interaction of gender and dose was stronger when evaluated as AUC than as peak GH. These results indicate that variation associated with peak GH concentration during a sampling interval makes this approach a less sensitive method than use of AUC. This is not surprising given that AUC incorporates more data and should be less subject to spurious results than reliance on results obtained from 1 or 2 samples. Because of the variation in time to peak GH concentration after GHRH administration, collection of a single sample at a specific time point might introduce more variation and would likely miss the actual peak GH concentration. These factors minimize the likelihood that this single sample approach would provide an accurate assessment of treatment effects.

In our previous efforts with this analog (Baumgard et al., 2002), we used 4 $\mu\text{g}/100\text{ kg}$ of BW to assess effects of genetic merit for milk yield on components of the somatotrophic axis. Although this dose study had less statistical power than our previous study to assess specific effects of genetic merit, gender, and age on AUC, results from both studies were similar. Genetic merit did not affect GH response to dose of GHRH in either study. In addition, results from both studies indicated that serum GH concentrations did not differ between these substantially different genetic lines of Holstein calves. Our previous study (Baumgard et al., 2002) evaluated calves from 10 to 364 d of age and detected the well-defined (Harvey and Daughaday, 1995) age-associated decrease in serum GH concentrations. The failure to detect such an effect in the current study is not surprising because serum GH concentrations are relatively stable in calves between 3 and 10 mo of age (Baumgard et al., 2002). Although not reported, BW gains of heifers and bulls in this study were similar to those reported previously (Baumgard et al., 2002). In addition, the age at which BW differences between lines and between heifers and bulls were detected in the current study were similar to those detected previously (Baumgard et al., 2002). Steers were implanted with Ralgro between P1 and P2 and again between P2 and P3. We and others have demonstrated that steroid (Hongerholt et al., 1992) and Ralgro (Williams et al., 1991; Hufstedler et al., 1996; Thomas et al., 2000) implants can increase serum GH and GH response to GHRH by growing ruminants. Our study design does not provide a direct test of Ralgro, but the implant may have contributed to the numerical increase in serum GH for steers during P2. However, in contrast to results from wethers (Thomas et al., 2000), there was no apparent effect of Ralgro implant on GH concentrations or GH AUC values in our steers.

The decrease in GH response to GHRH with age is also well defined (Harvey and Daughaday, 1995). Although variable results have been reported in cattle (Plouzek and Trenkle, 1991; Parchuri et al., 1993), we detected this effect previously (Baumgard et al., 2002) and in the current study. Both of our studies indicate that the greatest decreases occurred when calves were young and that age effects diminished after calves were 6 mo old. Overall, GH response was greater in heifers than steers (bulls were similar to heifers and steers) in the current study. This similarity between heifers and bulls was influenced greatly by the larger response of bulls than heifers at 3 mo of age (during P1) and the greater response of heifers than bulls at 6 and 10 mo of age (during P2 and P3). In our previous study (Baumgard et al., 2002), overall GH response was greater in heifers than bulls primarily due to numeri-

cally greater responses of heifers near the onset of puberty. These results are consistent with a sexual dimorphism in GH response (Gluckman et al., 1987; Harvey and Daughaday, 1995).

CONCLUSIONS

This dose-response study indicated that a submaximal GH response was obtained when less than 5 $\mu\text{g}/100\text{ kg}$ of BW of the GHRH analog was administered to 3- to 10-mo-old Holstein calves. Maximum GH response (R_{max}) to the GHRH analog was not affected by genetic merit, age, or gender. Overall sensitivity (ED_{50}) to GHRH was greater in heifers than in steers or bulls, but was not affected by age or genetic merit. Serum GH concentrations and GH response (AUC) to GHRH were not affected by genetic merit for milk yield. The GH response variables AUC, R_{max} , and ED_{50} were not useful predictors of genetic merit for milk yield of growing calves from these 2 substantially different lines of Holstein cows, but results indicate that evaluation of submaximal doses of GHRH in a larger number of calves is needed to confirm that genetic merit has no effect on GH response.

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