Effects of Selection for Milk Yield on Growth Hormone Response to Growth Hormone Releasing Factor in Growing Holstein Calves¹

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ABSTRACT

Bull and heifer calves (n = 81) from genetic lines of Holstein cows that differed by more than 4000 kg milk/305-d lactation were used to determine effects of selection for milk yield on growth hormone (GH) response to a GH releasing factor (GRF) analog. Calves received GRF (4 µg/100 kg BW) on 10, 56, 140, 196, 252, and 364 ± 3 d of age. Jugular blood samples (n = 15) were obtained from –30 to 120 min relative to GRF administration. Area under the GH response curve (0 to 60 min, AUC60) was quantified after subtracting mean prechallenge GH concentrations. Data were analyzed for effects of line, age, gender, and their interactions with PROC MIXED of SAS for repeated measures and incorporated the spatial power law for unequally spaced data with age as the repeated effect. Means were considered different when *P* < 0.05. Prechallenge GH concentrations did not differ between lines, were greater in bulls than heifers (4.6 vs. 3.7 ng/ml), and decreased with age. The AUC60 decreased with age but did not differ between lines. Heifers responded more to GRF than bulls (1550 vs. 1336 ng × min/ml). Peak GH concentration decreased with age and was less in bulls than heifers (54.7 vs. 62.1 ng/ml) but did not differ between lines. Although plasma GH has been identified as an inheritable trait, we conclude the GH variables measured in this study were not useful in predicting genetic merit of calves from these substantially different lines of cows.

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

Abbreviation key: ADG = average daily gain, AUC = area under the curve, AUC60 = AUC from 0 to 60

min, CL = control line, GH = growth hormone, GHM = mean concentration of GH in samples collected at 7.5, 10, and 20 min after GRF administration, GRF = growth hormone releasing factor, PREC = prechallenge, SL = select line.

INTRODUCTION

Identification of genetically superior animals at an early age would provide several benefits to the livestock industry and could accelerate the rate of genetic improvement. Circulating concentrations or changes in circulating concentrations of hormones and metabolites associated with homeostatic and homeorhetic regulation of lactation and growth have been proposed as potential identifiers of superior animals (Bauman and Currie, 1980). Growth hormone (GH) plays an integral role in growth and lactation by coordinating nutrient distribution among various tissues (Bauman and Vernon, 1993). Several studies have suggested that GH concentrations are greater in superior animals (Barnes et al., 1985; Bonczek et al., 1988; Beerepoot et al., 1991). As a result, GH has received considerable attention as a potential identifier of genetic merit for production traits (Woolliams and Lovendahl, 1991).

Episodic release of GH from the anterior pituitary results in fluctuations in the circulating concentration of GH. This fluctuation necessitates frequent blood sampling over an extended interval to obtain an accurate estimate of mean GH concentration or to quantify the pattern of release. Administration of a GH secretagogue, such as growth hormone releasing factor (GRF), can cause a large release of GH which can overwhelm the episodic release of GH. Although this technique does not provide an estimate of the mean circulating GH concentration, it can reduce the number of samples required to obtain an accurate estimate of GH status (Woolliams and Lovendahl, 1991). Some results suggest GH response to GRF is associated with genetic potential for milk yield (Lovendahl et al., 1991; Zinn et al., 1994). Our objective was to use growing Holstein calves from two genetically different lines to determine whether selection for milk yield has altered GH response to GRF.

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MATERIALS AND METHODS

Animal Management

All calves were from genetic lines of Holstein cows maintained under identical environment and management practices at the University of Minnesota Southern Research and Outreach Center in Waseca, MN. Development of the static, unselected control line (**CL**) and the contemporary, select line (**SL**) was initiated in 1964 by Dr. Charles Young as a component of a multi-state, north central regional project [NC-2 (Hansen, 2000)]. The CL cows and their female descendants have been artificially inseminated with semen from sires (4 sires/yr in a 5-yr rotation) that were breed average for predicted transmitting ability for milk (PTA-milk) in 1964. The SL cows and their female descendants have been bred with semen from the highest PTA-milk sires available each year. The herd of 90 lactating cows was composed of approximately one third CL and two thirds SL cows. Milk yield from the two lines differed by more than 4000 kg of milk/305-d lactation (6890 ± 403 vs. $11,078 \pm 329$ kg for multiparous CL and SL cows) when the GRF challenges were conducted.

Animal care and experimental procedures were approved by the University of Minnesota Institutional Animal Care and Use Committee. Animals were observed daily for health abnormalities and treated when appropriate. For the first 6 wk of life, calves born in the spring, summer, or fall were housed outside in individual hutches, while calves born in the winter were kept inside in individual crates in a room maintained at 7 to 10°C. At 6 wk of age, all calves were comingled outside in larger hutches. At 4 mo of age, heifers were transferred to a free-stall, slatted floor barn and bulls to a loose-housing, manure-pack barn. Bull ($n = 6/\text{group}$) and heifer ($n = 8/\text{group}$) calves were kept in groups of similar age and BW in their respective barns until completion of the study. Calf BW was determined at birth, every 28 ± 3 d thereafter, and on the day of GRF challenge.

Diets

All calves were fed 2 to 4 L of colostrum within 2 to 6 h after birth. They were fed milk replacer at a rate of 0.45 kg/d per calf during wk 1 through 4 and at 0.22 kg/d per calf during wk 5. Calves were weaned at 5 wk of age. Starter diet (14.2% CP, 2.01 Mcal NE_m/kg, 1.39 Mcal NE_g/kg) primarily contained ground corn, oats, and soybean meal and was offered free choice from 3 d to 6 wk of age. From 6 wk to 4 mo of age, calves were offered 2.3 to 3.2 kg starter diet/d and fed reed canary grass *ad libitum*. From 4 to 8 mo of age, heifer calves were fed a mixed diet composed of corn silage, alfalfa

haylage, high moisture corn, and a vitamin-mineral premix (16.5% CP, 1.63 Mcal of NE_m/kg, 1.02 Mcal of NE_g/kg) and reed canary grass (1.4 to 1.8 kg/d). From 8 mo of age until the completion of study, heifers were fed a diet composed of corn silage, alfalfa haylage, reed canary grass, high moisture corn, and a mineral-vitamin premix (15.5% CP, 1.55 Mcal NE_m/kg, 0.95 Mcal NE_g/kg) and reed canary grass (1.4 to 1.8 kg/d). Diets for heifers were formulated to support gains of 0.77 to 0.82 kg/d (NRC, 1989). Diet shifts for bulls were based on average BW/pen. Bulls consumed the starter diet until their average BW/pen was 180 kg (5 to 6 mo of age). From 180 to 300 kg BW, bulls consumed a grower diet composed of corn, oats, soybean meal, dehydrated alfalfa, tallow, vitamins, and minerals (13.0% CP, 2.02 Mcal NE_m/kg, 1.40 Mcal NE_g/kg) and reed canary grass (1.4 to 1.8 kg/d). From an average BW/pen of 300 kg (8 to 9 mo of age) until completion of the study, bulls were fed a finishing diet composed of corn, oats, dehydrated alfalfa, tallow, and vitamins and minerals (11.1% CP, 2.01 Mcal NE_m/kg, 1.39 Mcal NE_g/kg). Diets for bulls were formulated to support gains of 1.14 to 1.60 kg/d (NRC, 1989). Water was available *ad libitum* throughout the study.

GRF Challenge

A human GRF analog ([DesNH₂Tyr¹,D-Ala²,Ala¹⁵]hGRF(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 on three occasions during the study, aliquoted (3 ml/tube), and stored at -80°C. This analog is more resistant to biodegradation and has a greater biopotency than its native counterpart (Felix et al., 1988). The GRF challenges were conducted when calves were 10, 56, 140, 196, 252, and 364 ± 3 d of age. On the day of challenge, calves were separated from their feed and fitted with an indwelling jugular catheter. After catheterization, calves were haltered and tethered to facilitate ease of blood sampling. Calves were allowed to lie down and drink but not allowed to eat during the challenge. Pre-challenge blood sampling was initiated approximately 2 h after calves were catheterized and at least 3 h after they were denied access to feed. Based on a preliminary dose study (Weber et al., 1997), calves were infused intravenously with 4 µg GRF/100 kg BW at time zero and blood samples collected at -30, -20, -10, -5, 0, 2.5, 5, 7.5, 10, 20, 30, 45, 60, 90, and 120 min relative to GRF administration. Blood samples from d 10 and 56 were allowed to sit at room temperature for approximately 1 h and then refrigerated overnight. Serum was harvested ($1200 \times g$, 15 min) and stored at -20°C until assayed. Blood samples from the remaining

challenges were immediately mixed with heparin (20 μ l of 10,000 IU/ml) and placed on ice until plasma was harvested (1200 $\times$ g, 15 min). Plasma was stored at -20°C until assayed.

Sample Analyses

Plasma and serum GH concentrations were quantified using a validated homologous double antibody radioimmunoassay (Kazmer et al., 2000). Recombinantly derived bovine GH (SV-3001-B, Pharmacia & Upjohn, Kalamazoo, MI) was used as the standard and as the iodinated tracer. Before use, the first antibody (rabbit anti-oGH2; AFP-C0123080; NIDDK) was diluted 1:20,000 and the second antibody (goat anti-rabbit; lot # 35318; Pel-Freez, Rogers, AK) diluted 1:75. Samples were analyzed in triplicate. The minimal detectable concentration of GH was 0.7 ng/ml, and intra- and interassay CV were 6.0 and 8.6%, respectively.

Calculations and Statistical Analysis

All calves born between August of 1995 and August of 1996 were considered potential candidates for the study and were initially included to guard against calf loss due to health problems and mortality. After 56 d of age and before any GH assays were conducted, calves were blocked ($n = 23$) by birth date (<41-d intervals) into groups of four (line $\times$ gender). A total of 84 calves [39 CL (18 heifers, 21 bulls) and 45 SL (22 heifers, 23 bulls)] were initially assigned to the study. Three bull calves were subsequently excluded from the study because of illness or death. Therefore, 38 CL (18 heifers, 20 bulls) and 43 SL (22 heifers, 21 bulls) calves completed the study. The unequal representation of CL and SL cows in the herd (33:67), an uneven calving pattern, and removal of the three bull calves resulted in nine incomplete (two blocks with two calves, seven blocks with three calves) and 14 complete blocks. Of the 486 possible challenges in this study, two were missed when a CL bull was removed after 196 d of age because of illness and seven were removed from the dataset and not analyzed due to abnormal response patterns.

The mean pre-challenge (**PREC**) GH concentration was estimated from the five samples obtained before GRF was administered. After GRF administration, area under the GH response curve (**AUC**) was calculated by linear trapezoidal summation between successive pairs of GH concentration and time coordinates after subtracting PREC GH. The AUC for GH response was summarized for 45-, 60-, and 90-min intervals. Interpretation of results was similar for each time interval, so only the 60 min of AUC (**AUC60**) data are reported. Peak GH was defined as the greatest concentra-

tion of GH within 60 min after administration of GRF and was not adjusted for PREC GH. A third estimate of GH response (peak **GHM**) was calculated as the mean GH concentration in the 7.5-, 10-, and 20-min samples and was not adjusted for PREC GH. The GH response to GRF was computed in three formats (AUC, peak GH, peak GHM) to allow comparison with published estimates. Time to peak GH was calculated as the duration in minutes from administration of GRF to peak GH. Individual average daily gain (**ADG**) was calculated as the ratio of the differences between initial and final BW and age during each BW interval.

All statistical analyses were conducted with the SAS System (SAS, 2001). Data from the 81 individual calves were analyzed as a completely randomized, incomplete block design with the mixed model procedure for repeated measures and incorporated the spatial power law for unequally spaced data with age as the repeated effect. The model used to evaluate treatment effects was

$$Y = \mu + \text{block} + \text{line} + \text{age} + \text{gender} + (\text{line} \times \text{age}) \\ + (\text{line} \times \text{gender}) + (\text{age} \times \text{gender}) + (\text{line} \times \text{age} \\ \times \text{gender}) + \text{error}.$$

To reduce potential problems with heterogeneity of variances, PREC GH, peak GH, and peak GHM were also determined from ln-transformed GH concentrations. These transformed data were analyzed with the same model. Results were consistent whether geometric or transformed data were evaluated, so only the results of geometric data are reported. Results of the repeated measures ADG analyses indicated periods of similarity (birth to 112 d of age) and dissimilarity (112 to 364 d of age) among the gender $\times$ line combinations. Individual ADG for these intervals and from birth to 364 d of age were calculated and treatment effects evaluated as a completely randomized, incomplete block design for effects of gender, line, and their interaction with the general linear models procedure.

Spearman's rank correlation (PROC CORR of SAS) was used to evaluate relationships among PREC GH, AUC60, peak GH, and peak GHM. Correlations were classified as weak ($|r| < 0.5$), moderate ($0.5 \leq |r| \leq 0.8$), or strong ($|r| > 0.8$). The PTA-milk value for each calf was calculated as the mean of its sire and dam PTA-milk (August 2000 base) which were obtained from the Animal Improvement Program Laboratory (Beltsville, MD). Linear regression (PROC REG of SAS) was used to evaluate the ability of the PREC GH and GH response (AUC60, peak GH, peak GHM) to predict PTA-milk and subsequent ADG. For all evaluations, statistical comparisons were considered different when $P < 0.05$.

Figure 1. Mean BW of control (CL) and select (SL) bull and heifer calves from birth through 364 d of age. Birth weights (42.6 , 43.1 , 36.2 , and 42.2 ± 5.0 kg) for CL-bulls, SL-bulls, CL-heifers, and SL-heifers did not differ. Mean BW first differed at 140 d of age between bulls and heifers (147 vs. 137 ± 3.5 kg) and between SL and CL calves (147 vs. 137 ± 3.5 kg). Genetic merit was calculated as the mean of the sire and dam PTA-milk (Animal Improvement Programs Laboratory, August 2000 base) for each calf and averaged -3154 ± 53 kg for CL and 649 ± 60 kg for SL calves.

RESULTS

Body Weight and Average Daily Gain

Birth weights were not affected by line (CL 39.4 vs. SL 42.6 ± 3.5 kg) or gender (heifers 39.2 vs. bulls 42.8 ± 3.5 kg). Calves were healthy and increased their BW (Figure 1) throughout the trial. Calf BW was affected by interactions of age and line ($P = 0.002$), age and gender ($P < 0.0001$), and age, line, and gender ($P = 0.02$). Gender and line had no effect on calf BW from birth through 112 d of age. By 140 d of age, bull calves weighed more than heifer calves (147 vs. 137 ± 3.5 kg, $P = 0.048$) and SL calves weighed more than CL calves (147 vs. 137 ± 3.5 kg, $P = 0.038$). By 168 d of age, the SL heifer calves weighed more than CL heifer calves (165 vs. 149 ± 5.0 kg, $P = 0.027$) and by 224 d of age, SL bulls weighed more than CL bulls (278 vs. 255 ± 4.9 kg, $P = 0.0009$).

Calf ADG during these 28-d intervals was affected by the interactions of age and gender ($P = 0.0001$) and age, line, and gender ($P = 0.017$) and tended to be affected by the interaction of age and line ($P = 0.064$). During the first 112 d of age, calf ADG did not differ between genders or lines and averaged 0.63 ± 0.02 kg/d. By d 140 of age, ADG of bulls exceeded that of heifers (1.18 vs. 0.94 ± 0.06 kg/d, $P = 0.004$) and this effect persisted throughout the remainder of the study. As indicated by the above P -values, the effect of line was less than, and not as persistent as, the effect of gender on calf ADG during these 28-d intervals. During the interval of 112 to 364 d of age, ADG was greater for SL

than CL calves (Table 1). Effects of gender and line on ADG during the entire study (1 to 364 d of age) were similar to those of the interval between 112 to 364 d of age.

Prechallenge GH Concentration

The PREC GH concentrations decreased ($P < 0.0001$) with age (Figure 2). Overall PREC GH concentration averaged 4.1 ± 0.20 ng/ml and did not differ between lines. Bulls had an overall greater PREC GH concentration than heifers (4.6 vs. 3.7 ± 0.20 ng/ml).

GH Response to GRF

Administration of GRF increased circulating concentrations of GH (Figure 3). The overall AUC60 response averaged 1443 ± 70 ng $\times$ min/ml and did not differ between lines (Table 2). Overall, heifers had a greater AUC60 response than bulls (1550 vs. 1336 ± 70 ng $\times$ min/ml). The age-associated decrease in AUC60 was similar to that for PREC GH, as younger (10 and 56 d of age) calves responded more than older (> 56 d of age) calves (Table 2). When response to GRF was evaluated as peak GH (Table 3), heifers responded more than bulls (62.1 vs. 54.7 ± 2.75 ng/ml) and young calves responded more than older calves. There was also a trend ($P = 0.09$) for peak GH to be greater in CL than SL calves (61.7 vs. 55.1 ± 2.73 ng/ml). Similar results (data not presented) were obtained for peak GHM. Calf age affected ($P < 0.05$) the time required to reach peak GH (13.5^{ab} , 14.6^a , 11.7^{bc} , 11.4^{bc} , 10.4^c , $13.9^{ab} \pm 1.0$ min, presented according to increasing age with unlike superscripts denoting differences). Gender had no effect on time to peak GH, but there was a trend ($P = 0.09$) for SL calves to achieve peak GH later than CL calves (13.3 vs. 11.9 ± 0.59 min).

Relationships Among Variables

Overall peak GH and AUC60 ($r = 0.92$, $P < 0.0001$) and peak GHM and AUC60 ($r = 0.96$, $P < 0.0001$) were highly correlated and similar correlations (not reported) occurred between these variables when evaluated within or among main effects of gender, line, or age. The overall correlations between PREC GH and AUC60 ($r = 0.38$, $P < 0.0001$), between PREC GH and peak GH ($r = 0.48$, $P < 0.0001$), and between PREC GH and peak GHM ($r = 0.52$, $P < 0.0001$, data not shown) were weak (Table 4). Similarly weak to moderate relationships (r between 0.34 and 0.60 , all $P < 0.0001$) existed between these variables within each line, each gender, and each line $\times$ gender combination. Within individual days of age, correlation coefficients for these

Table 1. Effect of age, gender, and genetic line¹ of Holstein calves on average daily gain (ADG, kg/d).²

Age (days)	Bulls			Heifers			Line			SEM
	CL	SL	Mean	CL	SL	Mean	CL	SL	Mean	
1–112	0.62	0.64	0.63	0.59	0.67	0.63	0.61 ^c	0.65 ^d	0.63	0.02
112–364	1.29	1.34	1.32 ^a	0.90	0.98	0.94 ^b	1.09 ^a	1.16 ^b	1.14	0.02
1–364	1.08	1.14	1.11 ^a	0.81	0.88	0.85 ^b	0.95 ^a	1.01 ^b	0.98	0.02

^{a,b}Gender or line means within a row with different superscripts differ ($P < 0.01$).

^{c,d}ADG of select line (SL) calves tended to exceed ADG of control line (CL) calves ($P = 0.08$).

¹Genetic merit was calculated as the mean of the sire and dam PTA-milk (Animal Improvement Programs Laboratory, August 2000 base) for each calf and averaged -3154 ± 53 kg for CL and 649 ± 60 kg for SL calves.

²Least squares means ($n = 20$ CL, 21 SL bulls and 18 CL, 22 SL heifers).

relationships tended to be even weaker (r from 0.08 to 0.59) with 14 of the 18 correlation coefficients less than 0.50.

Mean PTA-milk values were greater for SL (649 ± 60 kg) than CL (-3154 ± 53 kg) calves and individual PTA-milk values for SL (range: -211 to 1535 kg) and CL (range: -3880 to -2617 kg) calves did not overlap. Overall relationships between PTA-milk and PREC GH or any measure of GH response were negative and nonsignificant. No consistent or strong relationships were detected between PTA-milk and PREC GH or any measure of GH response within line, gender, age, or line $\times$ gender grouping. Although it was weak, the only significant relationship was between PTA-milk and PREC GH for the SL heifers ($R^2 = 0.05$, $P = 0.01$).

When evaluated by age, relationships between PTA-milk and PREC GH and between PTA-milk and AUC60 (Table 5 for 140- and 196-d-old calves), peak GH, or peak GHM were weak and nonsignificant for most of the 24 possible line $\times$ gender $\times$ age groupings. There were several trends ($0.05 < P < 0.10$) that accounted for less than 20% of the variation in PTA-milk, but the only significant relationships were between PTA-milk and PREC GH for 56-d old SL heifers ($R^2 = 0.18$, $P = 0.05$), between PTA-milk and AUC60 (Table 5, Figure 4) for 140-d old SL heifers ($R^2 = 0.27$, $P = 0.02$), and between PTA-milk and peak GHM for 140-d old SL heifers ($R^2 = 0.22$, $P = 0.03$). These significant relationships and about one half of the PTA-milk and GH relationships for individual line $\times$ gender $\times$ age groupings

Figure 2. Mean GH (ng/ml) in five samples collected during the 30 min before administration of a human growth hormone releasing factor analog ($4 \mu\text{g}/100$ kg BW) to control (CL) and select (SL) bull and heifer calves at 10, 56, 140, 196, 252, and 364 d of age. These pre-challenge (PREC) GH concentrations did not differ between CL and SL calves, bulls had greater PREC GH than heifers (4.6 vs. 3.7 ± 0.21 ng/ml) and PREC GH decreased with age (7.3^w , 4.9^x , 3.7^y , 3.4^y , 3.3^y , and $2.1^z \pm 0.34$ ng/ml). Genetic merit was calculated as the mean of the sire and dam PTA-milk (Animal Improvement Programs Laboratory, August 2000 base) for each calf and averaged -3154 ± 53 kg for CL and 649 ± 60 kg for SL calves.

Figure 3. Mean GH response (ng/ml) to a human growth hormone releasing factor (GRF) analog ($4 \mu\text{g}/100$ kg BW) administered at time zero at 10, 56, 140, 196, 252, and 364 d of age to control (CL) and select (SL) bulls and heifers. Area under the response curve from 0 to 60 min post GRF challenge did not differ between CL and SL (1472 vs. 1414 ± 70 ng $\times$ min/ml) was less in bulls than heifers (1336 vs. 1550 ± 70 ng $\times$ min/ml), and decreased with age (2746^x , 1704^y , 957^z , 1168^z , 1078^z , and $1004^z \pm 114$ ng $\times$ min/ml). Genetic merit was calculated as the mean of the sire and dam PTA-milk (Animal Improvement Programs Laboratory, August 2000 base) for each calf and averaged -3154 ± 53 kg for CL and 649 ± 60 kg for SL calves.

Table 2. Effect of age, gender, and genetic line¹ of Holstein calves on growth hormone (GH) response (AUC60²) to GRF.^{3,4}

Age (day)	Bulls			Heifers			Line			SEM
	CL	SL	Mean	CL	SL	Mean	CL	SL	Mean	
10	2729	2760	2745	2950	2544	2747	2840	2652	2746 ^x	112
56	1677	1687	1682	1769	1685	1727	1723	1686	1704 ^y	114
140	914	901	907	1062	951	1006	988	926	957 ^z	113
196	826	817	822	1626	1403	1515	1226	1110	1168 ^z	114
252	947	897	922	1237	1229	1233	1092	1063	1078 ^z	115
364	900	973	936	1022	1120	1071	961	1047	1004 ^z	115
Mean	1332	1339	1336 ^a	1611	1489	1550 ^b	1472	1414	1443	70

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

^{x,y,z}Means within column with different superscripts differ ($P < 0.0001$).

¹Genetic merit was calculated as the mean of the sire and dam PTA-milk (Animal Improvement Programs Laboratory, August 2000 base) for each calf and averaged -3154 ± 53 kg for control line (CL) and 649 ± 60 kg for select line (SL) calves.

²Area under the GH response curve (AUC, ng $\times$ min/ml) within 60 min after a GRF challenge (4 μ g/100 kg BW).

³An analog of human growth hormone releasing factor (GRF).

⁴Least squares means (n = 20 CL, 21 SL bulls and 18 CL, 22 SL heifers).

were negative. Multiple regression (PREC GH and AUC60) did not improve the ability to predict PTA-milk.

No consistent relationships were detected when AUC60 was regressed on subsequent ADG. None of the regression equations for any of the heifer groupings (SL, CL, or overall) were significant (all $R^2 < 0.12$, all $P > 0.4$). The AUC60 results from 10-d-old bull calves were not related to their ADG during 1 to 112 d of age for the SL ($R^2 = 0.14$, $P = 0.1$), CL ($R^2 = 0.07$, $P = 0.3$), or overall ($R^2 < 0.01$, $P = 0.9$) groupings. Similar nonsignificant relationships ($R^2 = 0.13$, $P = 0.13$) were obtained for SL and CL bulls when AUC60 for 56-d old bulls was regressed on their ADG during 1 to 112 d of

age. This relationship was significant when the SL and CL bulls were combined ($R^2 = 0.12$, $P = 0.03$). Regression equation coefficients for the 140- and 196-d of age AUC60 data (Table 6) indicate only the 196-d of age data for SL bulls provided a significant relationship. Inclusion of PREC GH did not improve the ability to predict ADG.

DISCUSSION

The response of circulating GH to a GRF challenge has been hypothesized as a potential predictor of domestic animal performance (Lovendahl et al., 1991; Suttie

Table 3. Effect of age, gender, and genetic line¹ of Holstein calves on growth hormone (GH) response (peak GH²) to GRF.^{3,4}

Age (day)	Bulls			Heifers			Line			SEM
	CL	SL	Mean	CL	SL	Mean	CL	SL	Mean	
10	93.3	101.4	97.4	108.7	87.4	98.0	101.0	94.4	97.7 ^x	4.55
56	60.9	62.1	61.5	69.9	56.8	63.4	65.4	59.4	62.4 ^y	4.64
140	40.3	33.0	36.6	48.3	43.2	45.7	44.3	38.1	41.2 ^z	4.58
196	46.3	45.8	46.1	72.2	56.1	64.1	59.3	50.9	55.1 ^y	4.62
252	54.3	39.6	46.9	59.6	53.5	56.6	57.0	46.5	51.8 ^{yz}	4.63
364	40.0	39.1	39.6	47.0	42.9	44.9	43.5	41.0	42.2 ^z	4.66
Mean	55.8	53.5	54.7 ^a	67.6	56.6	62.1 ^b	61.7 ^c	55.1 ^d	58.2	2.74

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

^{c,d}Peak GH of control line (CL) calves tended to exceed that of select line (SL) calves ($P = 0.09$).

^{x,y,z}Means within column with different superscripts differ ($P < 0.0001$).

¹Genetic merit was calculated as the mean of the sire and dam PTA-milk (Animal Improvement Programs Laboratory, August 2000 base) for each calf and averaged -3154 ± 53 kg for CL and 649 ± 60 kg for SL calves.

²Greatest concentration of GH (ng/ml) within 60 min after the GRF challenge (4 μ g/100 kg BW).

³An analog of human growth hormone releasing factor (GRF).

⁴Least squares means (n = 20 CL, 21 SL bulls and 18 CL, 22 SL heifers).

Table 4. Effect of gender and genetic line¹ of Holstein calves on the correlation of pre-challenge growth hormone (GH²) concentration with AUC60³ and peak GH.^{4,5}

Group	AUC60 ^a			r	Peak GH ^a		
	CL	SL	Overall		CL	SL	Overall
Heifers	0.429	0.455	0.439		0.483	0.537	0.500
Bulls	0.365	0.439	0.406		0.519	0.530	0.527
Overall	0.340	0.414	0.381		0.463	0.504	0.484

^aAll correlation coefficients differed from zero ($P < 0.0001$).

¹Genetic merit was calculated as the mean of the sire and dam PTA-milk (Animal Improvement Programs Laboratory, August 2000 base) for each calf and averaged -3154 ± 53 kg for control line (CL) and 649 ± 60 kg for select line (SL) calves.

²Prechallenge GH was calculated as the mean of five samples obtained during the 30 min prior to administration of a human growth hormone releasing factor (GRF) analog.

³Area under the GH response curve (AUC, ng $\times$ min/ml) within 60 min after a GRF challenge ($4 \mu\text{g}/100$ kg BW).

⁴Greatest concentration of GH (ng/ml) within 60 min after a GRF challenge ($4 \mu\text{g}/100$ kg BW).

⁵Correlations from 20 CL, 21 SL bulls and 18 CL, 22 SL heifers.

et al., 1991; Zinn et al., 1994; Connor et al., 1999). Refinement of the challenge technique may allow identification of superior animals at a young age and could reduce the time required for trait evaluation. The SL and CL Holsteins in our study differ in their ability to produce milk (primiparous SL cows produce nearly twice as much as primiparous CL cows; Hansen, 2000) and in their rate of growth [ADG of SL calves is about 6% greater than CL calves, Figure 1, Table 1 (Murphy, 1992; Weber et al., unpublished)]. Thus, the calves used in this study offer a unique opportunity to evaluate the hypothesis that physiological characteristics can be

used to distinguish between animals of inferior and superior merit.

PREC GH Concentrations

Although not an extensive evaluation of baseline GH concentrations, the PREC GH samples provide an estimate of basal circulating concentrations of GH. Consistent with the general pattern observed in previous evaluations of cattle (Plouzek and Trenkle, 1991; Lapierre et al., 1992; McAndrews et al., 1993) and most animal species (Harvey et al., 1995), circulating GH concentra-

Table 5. Effect of gender and genetic line¹ of Holstein calves on the regression of AUC60² with PTA-milk.

Group	n	Day of age at AUC60 ³ measurement	Regression Equation Coefficients			
			Slope	Intercept	R ²	P
Bulls						
CL	20	140	0.036	-3216	0.003	0.83
	19	196	0.091	-3286	0.023	0.54
SL	20	140	-0.050	695	0.006	0.74
	20	196	-0.131	762	0.040	0.40
Overall	40	140	-0.067	-1206	0.0004	0.90
	39	196	-0.157	-1098	0.002	0.77
Heifers						
CL	18	140	0.101	-3228	0.042	0.42
	17	196	-0.035	-3058	0.015	0.64
SL	21	140	-0.279	919	0.266	0.02
	21	196	-0.008	668	0.0003	0.94
Overall	39	140	-0.411	-678	0.184	0.41
	38	196	-0.229	-686	0.013	0.49

¹Genetic merit was calculated as the mean of the sire and dam PTA-milk (Animal Improvement Programs Laboratory, August 2000 base) for each calf and averaged -3154 ± 53 kg for control line (CL) and 649 ± 60 kg for select line (SL) calves.

²Area under the GH response curve (AUC, ng $\times$ min/ml) within 60 min after administration of a human growth hormone releasing factor analog challenge ($4 \mu\text{g}/100$ kg BW).

³No significant relationships were detected when PTA-milk was regressed on AUC60 measurements obtained at 10, 56, 252, or 364 d of age.

Figure 4. Relationship of GH response (AUC60) at 140 d of age and genetic merit (PTA-milk) of control (CL) and select (SL) bulls (dashed lines) and heifers (solid lines). Area under the GH response curve (AUC60) was determined from 0 to 60 min post administration of a human growth hormone releasing factor analog ($4 \mu\text{g}/100 \text{ kg BW}$). Genetic merit was calculated as the mean of the sire and dam PTA-milk (Animal Improvement Programs Laboratory, August 2000 base) for each calf and averaged $-3154 \pm 53 \text{ kg}$ for CL and $649 \pm 60 \text{ kg}$ for SL calves. The only significant relationship between AUC60 and PTA-milk was for SL heifers ($\text{PTA-milk} = -0.279 \times (\text{AUC60}) + 919$, $R^2 = 0.27$, $P = 0.02$). Similar relationships at 10, 56, 196, 252, and 364 d of age were nonsignificant ($P > 0.4$).

tions decreased as calves aged. Our results indicate intensive selection for milk yield has not altered PREC GH in Holstein calves during their first year of life.

This agrees with results from other studies that also used pre-challenge GH concentrations to compare effects of selection on basal GH (Lovendahl et al., 1991; Lovendahl et al., 1994; Weber et al., 1997).

Previous studies with these two genetic lines have reported conflicting results regarding the effect of selection for milk yield on circulating GH concentrations. A trend was detected for SL heifers to have greater prechallenge concentrations of GH than CL heifers at $208 \pm 2 \text{ d}$ of age (Massri et al., 1985), but these results were influenced greatly by one SL heifer. An evaluation of samples from bull calves (12/line) collected every 30 min over a 2.5 h interval determined that GH concentrations were greater in SL calves during the first 9 mo of age whether the calves were fed or deprived of feed and water for 24 h (Parchuri et al., 1993). Weber et al. (1997) used a sampling scheme similar to that of the current study and detected no effect of selection on PREC GH in heifers, bulls, or steers (4/line-gender combination) at 3, 6, or 10 mo of age. In a separate study with multiparous cows (15/line), we demonstrated that selection for milk yield had no effect on prepartum PREC GH (Weber et al., 1999). However, even though the magnitude and duration of postpartum negative energy balance was similar in SL and CL cows, postpartum PREC GH was greater in SL than in CL cows (Weber et al., 1999).

As summarized by Woolliams and Lovendahl (1991), considerable variation in technique, physiological stage

Table 6. Effect of gender and genetic line¹ of Holstein calves on the regression of AUC60² with subsequent average daily gain (ADG).

		Day of Age		Regression Equation Coefficient			
Group	n	At AUC60 ³	ADG	Slope	Intercept	R ²	P
		(x) measurement	Interval (y)	(× 10 ⁻⁵)			
Bulls							
CL	20	140	140–364	−2.96	1.341	0.012	0.65
	19	196	196–364	2.45	1.228	0.008	0.72
SL	20	140	140–364	−1.78	1.378	0.008	0.70
	20	196	196–364	−14.00	1.433	0.240	0.03
Overall	40	140	140–364	−2.26	1.359	0.010	0.54
	39	196	196–364	−6.97	1.343	0.058	0.14
Heifers							
CL	17	140	140–364	2.07	0.882	0.017	0.62
	17	196	196–264	1.24	0.934	0.028	0.52
SL	22	140	140–364	−0.96	0.997	0.007	0.71
	22	196	196–364	0.46	1.010	0.002	0.85
Overall	39	140	140–364	−0.99	0.962	0.005	0.68
	39	196	196–364	0.48	0.982	0.003	0.76

¹Genetic merit was calculated as the mean of the sire and dam PTA-milk (Animal Improvement Programs Laboratory, August 2000 base) for each calf and averaged $-3154 \pm 53 \text{ kg}$ for control line (CL) and $649 \pm 60 \text{ kg}$ for select line (SL) calves.

²Area under the growth hormone response curve (AUC, $\text{ng} \times \text{min}/\text{ml}$) within 60 min after administration of a human growth hormone releasing factor analog challenge ($4 \mu\text{g}/100 \text{ kg BW}$).

³No significant relationships were detected when ADG was regressed on AUC60 measurements obtained at 10, 56, 252, or 364 d of age.

of the animals used, and study design contribute to the variation observed among published studies designed to identify effects of selection on GH and GH response to GRF. Differences in the number of animals evaluated, the number and frequency of samples obtained prior to GRF administration, and study designs contribute to the discrepancies observed among the GRF challenge studies (Massri et al., 1985; Parchuri et al., 1993; Weber et al., 1997; this study) conducted with calves from these two genetic lines. Because the typical sampling regimen before GRF administration only captures a small portion (< 30 min) of the episodic nature of GH release, it is possible that a true representation of basal GH concentration is not obtained by this method. However, our observation that selection for milk yield has not altered basal GH concentration in growing calves is supported by results from a more comprehensive evaluation (blood sampled every 15 min for 7 h) of multiparous SL and CL cows that demonstrated basal GH concentrations differed only when the SL and CL cows were lactating (Weber et al., 1998).

Bull calves had numerically greater PREC GH concentrations than heifer calves whether all calves consumed the same diet (GH data through 56-d of age) or bulls consumed a more energy dense diet (GH data after 120-d of age) than heifers. Others have also determined that GH concentrations are greater in growing bulls than in heifers (Plouzek and Trenkle, 1991). Our results and those of Lovendahl et al. (1991) and Woolliams et al. (1993) did not identify an interaction between gender and age. Although effects of gender in our study are confounded with possible diet effects after calves were 120-d of age, these effects are likely small as our calves were in positive energy and nutrient balance throughout the study. In addition, slight reductions in intake typically either increase or have no effect on serum GH in heifers (Lapierre et al., 1992). Thus, intake-associated increases in circulating concentrations of GH in our heifers would be expected to reduce our ability to detect the real effects of gender. Severe restrictions in intake are required to reduce circulating GH concentrations (Elsasser et al., 1988).

GH Response to GRF

As expected, administration of the GRF analog increased circulating GH concentration in the calves. Although some studies (Achthung et al., 2001b; Lovendahl et al., 1991; Lovendahl and Sejrsen, 1993; Woolliams et al., 1993; Zinn et al., 1994) have suggested that the magnitude of the GH response to GRF is associated with genetic merit for milk yield, GH response (AUC60, peak GH, peak GHM) did not differ between our two distinctly different genetic lines. This lack of a differ-

ence between lines was consistent for all of the ages tested and in agreement with previous results from these two genetic lines (Massri et al., 1985; Parchuri et al., 1993; Weber et al., 1997). There were suggestions that time to peak was affected by age and genetic line. However, most of the mean values were between 10 and 15 min, which were between two consecutive (10 and 20 min) sampling times. These results indicate more frequent sampling (especially between 10 and 20 min) is required for a thorough evaluation of the effects of age and genetic line on time to peak GH.

Although there were some inconsistencies among the three measurements of response (AUC60, peak GH, peak GHM), each method of measurement indicated the GRF induced GH response decreased as age of the calves increased. This effect of age on GH response was primarily due to the greater response at 10- and 56-d of age, as response did not differ among calves more than 56 d old (Table 2). This is consistent with results from other studies (Plouzek and Trenkle, 1991; Lapierre et al., 1992; Parchuri et al., 1993). There was no age $\times$ line interaction for any of the GH response variables.

The reduction in GH response (AUC60, peak GH, peak GHM) with age closely paralleled the decrease in basal (PREC) GH, but the correlation between PREC GH and GH response was weak. These significant but weak relationships are consistent with results from other studies (Lovendahl et al., 1994; Woolliams et al., 1993) and illustrate that GH responses are only partially dependent on basal GH concentration. This is not surprising given the multiple neurotransmitters, neurohormones, and pathways that regulate secretion of GH (McMahon et al., 2001). Mechanisms that mediate the age-dependent decrease in basal GH and GH response are ill defined but at least include increased plasma volume and increased metabolic clearance rate (Lapierre et al., 1992) and may include increased secretion of somatostatin (Harvey et al., 1995).

Bulls had greater PREC GH concentrations, but heifers had a greater overall GH response to GRF than bulls. This effect of gender was primarily due to heifers having greater GH responses at 140-, 196-, and 252-d of age (Table 2, 3). At all other ages, mean response was very similar between genders. There was no gender $\times$ age interaction. Our dose study detected a greater response in heifers than bulls at 6 and 10 mo of age (Weber et al., 1997) and others (Lovendahl et al., 1991) have reported similar results with 124-d-old calves. The reason for this larger response by heifers is unclear. However, both genders are becoming sexually mature at this age and the influence of sex steroid hormones on sexually dimorphic GH response has been proposed (Gluckman et al., 1987; Harvey et al., 1995). Although effects of gender in our study are confounded with possi-

ble diet effects after calves were 140-d of age, effects of this confounding on GH response are likely small, as all calves were growing and in positive energy and nutrient balance throughout the study. Elsasser et al. (1989) detected no effect of diet on GH response to GRF in beef steers that gained 0.6 or 1.0 kg BW/d.

Relationship Between GH Response and Genetic Potential

If GH response to GRF was associated with genetic potential for milk yield, the association should be apparent when examined in widely different populations such as the SL (649 ± 60 kg PTA-milk) and CL (-3154 ± 53 kg of PTA-milk) calves used in this study. No such relationships were detected either between or within these distinct populations. There were some suggestions of a relationship, but none of these suggestions were consistent and the variability did not appear to be related to any specific physiological changes associated with calf development. Although there appeared to be more trends for a relationship between PTA-milk and GH response in SL than in CL calves, more of these trends also suggested an inverse relationship (Figure 4) which is inconsistent with the hypothesis that GH response would increase in the superior animal. This lack of a consistent relationship between GH response and PTA-milk indicates either no difference exists between our two lines or sensitivity of the methodology utilized was insufficient to detect the difference.

Positive relationships between GH response and genetic merit for milk yield have been detected when calves were fed at maintenance (Woolliams et al., 1993) or ad libitum (Lovendahl et al., 1994), when challenges were administered at 0.5 and 3.5 h (Lovendahl et al., 1991) or 24 h (Lovendahl et al., 1994) after feeding, and when single challenges of GRF were administered (Lovendahl et al., 1994; Woolliams et al., 1994). Our methods are consistent with those used in these studies. In addition, the GRF used in our study was used to establish a relationship between GH response and genetic merit for milk yield (Zinn et al., 1994). We also established that GH response to our dose was within the linear dose response range (Weber et al., 1997).

No consistent relationship between AUC60 and subsequent ADG was detected in this study. Thus, GH response to GRF did not to provide a reliable estimate of subsequent growth. Heifers were not fed ad libitum, and although SL heifers grew 6% more rapidly than CL heifers, it is possible that their restricted intakes diminished the opportunity to detect any relationship between AUC60 and ADG. However, feed intake of bull calves was not restricted and SL bulls had a similar 6% advantage in their ADG relative to CL bulls. As with

the heifer data, there were no consistent relationships between AUC60 and ADG for the bulls. These results suggest the restricted intake of the dairy heifers had little effect on the ability to detect potential relationships between AUC60 and ADG. This is consistent with the similar GH response to GRF by beef steers fed to achieve an ADG of 0.6 or 1.1 kg/d (Elsasser et al., 1989). Although the interval between food consumption and administration of the GRF challenge can affect the GH response (Moseley et al., 1988), GH response appears to be unaffected by intake sufficient to achieve an ADG of at least 0.6 kg/d. However, when intake restriction is severe (33% of ad libitum), GH response to GRF is increased (Lapierre et al., 1992).

Dahl and colleagues have also identified considerable variation in estimates of the relationship between GH response to a single GRF challenge and subsequent ADG. They examined this relationship in beef bull calves (220 to 260 d of age) that received 1.5 or 4.5 μ g GRF/100 kg BW. They detected no relationship for the 4.5- μ g dose (Connor et al., 1999, 2000) and either no relationship (Connor et al., 2000) or a weak ($R^2 = 0.07$, $P = 0.048$) positive relationship for the 1.5- μ g dose (Connor et al., 1999). Use of a PREC clearance dose of GRF reduced the variation in GH response, but the detected relationships still varied from negative to positive (Connor et al., 2000; Auchtung et al., 2001a,c). Our results and those Auchtung et al. (2001a) indicate a clear delineation of the influences of (at least) GRF dose and animal age and gender are required before GH response to GRF can be used to predict subsequent ADG.

Summary

The lack of a consistent relationship between GH response and PTA-milk or ADG for any of the various line, gender, or line $\times$ gender groupings and the fact that there was a relatively even distribution of positive and negative relationships, cast considerable doubt on the ability of GH response to a single GRF challenge to accurately estimate PTA-milk or subsequent ADG of an animal. This lack of a relationship between GH (circulating GH or response to GRF) and genetic merit of the calves might be explained by the overall importance of GH to animal performance (Bauman and Vernon, 1993). As an integral component of animal growth, development, and function, a complex set of regulatory factors (McMahon et al., 2001) integrate to provide sufficient GH for an effective coordination of tissue metabolism. Therefore, it may be expected that particular set points of GH are required for normal function. The lack of a difference in GH concentrations between our CL and SL calves suggests that these set points for GH are essentially equal across a wide range of genetic

potential. In this light, it seems unlikely that biologically meaningful relationships between GH concentration and genetic merit will be detected within groups of animals with minimal differences in their genetic merit when these animals are well fed, receive adequate care, and experience normal growth. However, superior animals should be better equipped to adapt (return to normal set points) more quickly or respond (greater response to a second administration of GRF) more strongly than inferior animals. It remains to be determined if endocrine profiles or response to a particular stimulus can distinguish between superior and inferior animals when they are subjected to conditions that alter their metabolism (such as a shift in nutrient availability) or when they experience a change in their physiological status.

CONCLUSIONS

The GH response to GRF decreased with age, was greater in heifers than bulls, and was partially dependent on GH concentration prior to GRF administration. Although GH has been identified as an inheritable trait, no consistent relationships were detected between either PTA-milk or ADG of the calves and estimates of mean circulating GH concentration or GH response to GRF. We conclude that the GH variables measured in this study are not useful predictors of genetic merit for milk yield or subsequent growth performance of Holstein calves raised according to industry standards.

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