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Effect of genetic background on growth of mice hemizygous for wild-type or dwarf mutated bovine growth hormone transgenes

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Abstract. The effects of a high-growth genetic background on the growth of mice hemizygous for one of two growth hormone transgenes were examined. Male mice hemizygous for wild-type (W) and dwarf mutant (M) bovine growth hormone (bGH) transgenes were crossed with females of a high-growth selected (S) and control (C) line as follows: W × S, W × C, M × S and M × C. Body weights of progeny were recorded weekly from 2 to 10 weeks of age. F_1 progeny were classified as carriers (P) or non-carriers (N) of the transgene by assaying tail DNA for bGH using the polymerase chain reaction and agarose gel electrophoresis. A deficiency in the number of F_1 progeny carrying the W ($P < 0.05$) and M ($P < 0.01$) bGH transgene was most likely due to differential prenatal and early postnatal mortality. Bodyweight means of wild-type transgenic mice were larger ($P < 0.05$) than those of non-transgenic littermates by 3 weeks of age in a C background in contrast to 5 weeks in S. The wild-type bGH transgene increased adult body weights more in the C (155%) than in the S (136%) background, indicating transgene expression by selection background interaction ($P < 0.05$). However, the growth response to the wild-type transgene in the S background was still large. The dwarf mutant transgene had a greater effect on growth reduction in the S (70%) than in the C (84%) background, thus causing transgene expression by selection background interaction ($P < 0.05$). Gender by wild-type transgene effect interactions ($P < 0.001$) for adult body weight were caused by the transgene reducing the gender difference for body weight in C and eliminating it in S. The dwarf mutant caused a larger negative effect on growth in males than in females, resulting in a gender by dwarf mutant transgene interaction ($P < 0.001$) for adult body weights. Results indicate that the effect of a GH transgene on growth can be affected both by a high-growth genetic background and the gender of progeny.

Key words: Growth hormone – Mice – Transgene – Growth

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Introduction

Hormonal regulation of growth is complex and involves many hormones, including growth hormone (GH), insulin-like growth factor I (IGF-I), insulin, thyroid hormones, glucocorticoids and androgens (Davis 1988). However, it is generally accepted that GH and its regulation are central to the control of growth either directly by binding to receptors on precursor bone, muscle or fat cells (Boyd and Bauman 1989) or indirectly by stimulating the secretion of IGF-I in the liver (Gluckman et al. 1987). Injections of porcine (p) GH into growing pigs increase growth rate and reduce carcass fat (Etherton et al. 1987).

An alternative approach to injecting GH to enhance growth of animals is to integrate the GH gene into the germ line using transgenic technology. A dramatic increase in growth was observed in transgenic mice containing the mouse metallothionein promoter attached to human (h) (Palmiter et al. 1982) or rat (r) (Palmiter et al. 1983) GH genes. Mice containing the bovine (b) (Kopchick et al. 1989) or ovine (o) (Pomp et al. 1992) GH transgenes also show accelerated growth rates. Incorporation of the transgene for hGH or pGH in the pig has not resulted in as large an increase in growth as was observed in mice (reviewed by Kopchick and Cioffi

1991). However, pGH transgenic pigs are leaner and have a higher feed efficiency than non-transgenic littermates (Pursel et al. 1989).

Pursel et al. (1989) made one suggestion why pigs have responded to the GH transgene so differently from mice. During domestication of the pig in Europe and North America, many generations of artificial selection for growth may have limited the pig's ability to respond to GH as effectively as the mouse. The objectives of the present study were to compare the effect of high-growth selected and unselected genetic backgrounds on the growth of mice carrying the wild-type bGH transgene, and to compare a dwarf mutant bGH transgene in these genetic backgrounds.

Materials and methods

Genetic stocks

Sires were hemizygous transgenic males from two different lines of transgenic mice. Each transgenic line was represented by two males descended from a single transgenic founder individual. One line expresses wild-type bGH. Hemizygous carriers of the bGH transgene are about 1.7 times the size of non-transgenic littermates originating from a C57BL/6J × SJL/J background (Chen et al. 1991). The second line expresses the bGH analog bGH-M8, which contains amino-acid substitutions at positions 117, 119 and 122 and has been shown to act as a GH antagonist resulting in dwarf transgenic mice (DTM) (Chen et al. 1991).

The recombinant DNA plasmids used to produce these mice are pBGH-10△6, which encodes wild-type bGH, and pBGH-M8, which encodes the bGH antagonist resulting in a dwarf transgenic mouse (Chen et al. 1990). The procedure for production of transgenic mice by microinjection of the DNA construct into the male pronucleus of fertilized mouse eggs has been described previously (Wagner et al. 1981). The recipient eggs used to found the transgenic lines were obtained from F_1 females of the cross C57BL/6J × SJL/J (B6SJLF1/J).

Female mice from two genetic lines were used as dams to be mated to males of the two transgenic lines. One line (M16) was selected for high 3–6-week weight gain for about 30 generations (Eisen 1975) and has been maintained without selection for over 60 generations. The other line (ICR) was the random-bred base population from which M16 was formed and serves as an unselected control.

Experimental design

The following F_1 crosses of sires × dams were made: wild-type bGH × M16; wild-type bGH × ICR; dwarf mutant bGH × M16; dwarf mutant bGH × ICR. Females were caged with males and checked daily for a copulatory plug. Upon detection of a copulatory plug, females were removed and caged individually. Beginning on day 18 after a plug was observed, females were checked daily for a litter. The range in litter size at birth was from 2 to 15. Mice were weaned at 3 weeks of age and housed in groups of four. Weekly body weight gains and the 3–6 week gain were calculated. Individual body weights were recorded weekly from 2 to 10 weeks of age. Mice had free access to food and water throughout the study. Purina Mouse Chow 5015 (17% minimum crude protein) and Purina Laboratory Chow 5001 (23% minimum crude protein) (Purina Mills, Richmond, Ind.) were fed to dams during gestation-lactation and to progeny during postweaning growth, respectively.

PCR assay for detection of transgene

The technique of DNA preparation for the polymerase chain reaction (PCR) was a modified version of the protocol described by Hogan et al. (1986). For each subject, 1–2 cm of tail was obtained and digested with 39 µl of a 10 mg/ml solution of proteinase K (Sigma, St. Louis, Mo.) in TE/SDS buffer for approximately 17 h in a 55 °C water bath. The digests were extracted twice with a 1:1 solution of phenol (equilibrated with Tris buffer) and chloroform:isoamyl alcohol (24:1) (Sigma, St. Louis, Mo.). Following each extraction, samples were vortexed and then centrifuged at 1,500 rpm for 4 min in serum separation tubes to separate aqueous and organic phases. DNA was precipitated with sodium acetate and ethanol. Samples were microcentrifuged for 30 s to pellet DNA, washed with 70% ethanol, and allowed to dry for approximately 4 h. Pellets were dissolved in 10 mM TE overnight. Genomic DNA concentration ranged from 0.5–4 mg/ml for each sample.

The polymerase chain reaction was conducted by amplification of a 1:80 dilution of the extracted DNA in a 25-µl reaction with Amplitaq® kit reagents and thermocycler (Perkin-Elmer Cetus, Norwalk, Conn.). Each primer (0.03125 µl) and 0.3 µl of Amplitaq® were added to the reaction. DMSO (Sigma, St. Louis, Mo.) was added to the reaction to enhance the amplification. The following temperature cycling program was used: 1 min, 20 s at 94 °C; 1 min at 45 °C; 3 min at 72 °C; 40 cycles; hold at 4 °C. Electrophoresis analysis was run by placing 20 µl of each PCR product on a horizontal agarose/TBE gel, containing ethidium bromide, in TBE buffer. Gels were run at approximately 90 v and 60 amp. Positive samples revealed an approximately 600 bp band under ultraviolet light.

Statistical analysis

Chi-square procedures were used to test the null hypothesis of a 1:1 segregation ratio of transgenic to non-transgenic progeny in each of the four F_1 crosses (Steel and Torrie 1980).

Each body weight or weight gain was analyzed using the following statistical model based on a split-plot design (Steel and Torrie 1980):

$$\begin{aligned} Y_{ijklmn} = {} & \mu + B_i + L_j + (BL)_{ij} + F_{k(ij)} + T_l + (BT)_{il} + (LT)_{jl} \\ & + (BLT)_{ijl} + (TF)_{lk(ij)} + S_m + (BS)_{im} + (LS)_{jm} \\ & + (BLS)_{ijm} + (TS)_{lm} + (BTS)_{ilm} + (LTS)_{jlm} + (BLTS)_{ijlm} \\ & + b(X_{ijklm} - \bar{X}) + e_{ijklmn} \end{aligned}$$

where

μ = overall mean,

B_i = fixed type of bovine growth hormone gene effect (W = wild-type M = dwarf mutant)

L_j = fixed line effect (S = selected, C = control),

$F_{k(ij)}$ = random litter effect (error term 1),

T_l = fixed transgene expression effect (P = positive, N = negative),

S_m = fixed gender effect (1 = male, 2 = female),

$(BL)_{ij}$, $(BT)_{il}$, $(LT)_{jl}$, $(BLT)_{ijl}$, $(TF)_{lk(ij)}$ (error term 2), $(BS)_{im}$, $(LS)_{jm}$, $(BLS)_{ijm}$, $(TS)_{lm}$, $(BTS)_{ilm}$, $(LTS)_{jlm}$ and $(BLTS)_{ijlm}$ are the respective interactions,

b = regression of the covariate number weaned on body weight,

$X_{ijklmn} - \bar{X}$ = deviation of number weaned for an individual from overall mean of number weaned,

e_{ijklmn} = residual (error term 3).

The mean square of the first three subscripted terms in the model were tested against the mean square for error term 1; subscripted terms five through eight were tested against error term 2 and the remaining terms were tested against error 3. Because the covariate term is constant for all individuals in a litter, the covariate was fitted before the litter effect.

The sample sizes for F_1 progeny subclasses are in Table 1. Let $\overline{WS,P}$; $\overline{WS,N}$; $\overline{WC,P}$; $\overline{WC,N}$; $\overline{MS,P}$; $\overline{MS,N}$; $\overline{MC,P}$; and $\overline{MC,N}$ represent the respective subclass means, averaged across gender. For example, $\overline{WS,P}$ and $\overline{WS,N}$ are the means of a trait, averaged over gender, for transgenic and non-transgenic progeny, respectively, from the F_1 cross of males hemizygous for wild-type bGH and females of the M16 line selected for high 3–6 week postweaning gain (Table 1). When the means were partitioned further by gender, 15 biologically meaningful orthogonal linear contrasts were developed to test differences in body weights and weight gains.

(1) W vs M = ¼ [($\overline{WS,P}$ + $\overline{WS,N}$ + $\overline{WC,P}$ + $\overline{WC,N}$ − ($\overline{MS,P}$ + $\overline{MS,N}$ + $\overline{MC,P}$ + $\overline{MC,N}$)], an overall comparison of F_1 progeny involving sires hemizygous for WT bGH vs sires hemizygous for bGH-M8;
(2) (P vs N)/W = ½ [($\overline{WC,P}$ + $\overline{WS,P}$) − ($\overline{WS,N}$ + $\overline{WC,N}$)], transgenic WT bGH vs non-transgenic littermates averaged over selected and control lines and gender;
(3) (S vs C)/W = ½ [($\overline{WS,P}$ + $\overline{WS,N}$) − ($\overline{WC,P}$ + $\overline{WC,N}$)], selected vs control lines averaged over transgene WT bGH and non-transgenic littermates and gender;
(4) ((P vs N) * (S vs C))/W = ½ [($\overline{WS,P}$ − $\overline{WS,N}$) − ($\overline{WC,P}$ − $\overline{WC,N}$)], interaction of WT bGH transgene with genetic line background;
(5) (P vs N)/M, (6) (S vs C)/M and (7) ((P vs N) * (S vs C))/M are contrasts analogous to (2), (3) and (4), respectively, and involve the dwarf mutant transgene bGH-M8;
(8) Male vs Female (G) = overall gender comparison;
(9) through (15) are interactions of gender with contrasts (1) through (7), respectively. For example, (10) G * (P vs N)/W = ¼ [($\overline{WS,P♂}$ + $\overline{WC,P♂}$) − ($\overline{WS,N♂}$ + $\overline{WC,N♂}$)] − ¼ [($\overline{WS,P♀}$ + $\overline{WC,P♀}$) − ($\overline{WS,N♀}$ + $\overline{WC,N♀}$], interaction of gender by WT bGH transgenic vs non-transgenic littermates, averaged over selected and control lines.

The error terms used to test these linear contrasts were based on the statistical model described above: contrasts (1), (3) and (6) were tested using error term 1; contrasts (2), (4), (5) and (7) using error term 2; and contrasts (8) through (15) using error term 3. The statistical tests are approximations because of the unequal sample size distribution.

Several additional a priori non-orthogonal contrasts were made to test specific hypotheses. One pair of tests was used to determine if growth of non-transgenic F_1 mice whose sire was hemizygous for the WT bGH transgene differed from those whose sire was hemizygous for the bGH-M8 transgene. These tests were made within each genetic background: $D_1 = \overline{WC,N} - \overline{MC,N}$ and $D_2 = \overline{WS,N} - \overline{MS,N}$. The final set of contrasts simply tests the hypotheses of an effect of the transgenes in selected and control backgrounds, respectively: $D_3 = \overline{WC,P} - \overline{WC,N}$; $D_4 = \overline{WS,P} - \overline{WS,N}$; $D_5 = \overline{MC,P} - \overline{MC,N}$; and $D_6 = \overline{MS,P} - \overline{MS,N}$. Error term 1 was used to test D_1 through D_6.

Results

Ratio of transgenic to non-transgenic F_1 progeny

Chi-square tests showed that fewer transgenic than non-transgenic mice were present in the F_1 progeny of each cross (Table 1). A significant deviation from a 1:1 ratio was found for the WT bGH gene in crosses to M16 females ($P<0.025$) but not in crosses to ICR females ($P>0.10$). These ratios were not heterogeneous ($P>0.10$) between crosses, and the pooled ratio of 43 transgenic and 64 non-transgenic mice showed a deviation ($P<0.05$) from the expected 1:1 ratio. A deficit of bGH-M8 transgenic mice was also found in crosses involving M16 ($P<0.10$) and ICR ($P<0.05$) females, and the pooled ratio was significant ($P<0.01$) as well.

Table 1. Sample sizes for F_1 progeny subclasses and chi-square test for 1:1 segregation of the transgenic and non-transgenic progeny

F_1 cross sire × dam[a]	Litters	Progeny[b]		Chi-square	Probability
		P	N		
W × S (WS)	6	21	37	5.77	<0.025
W × C (WC)	8	22	27	0.51	>0.10
		43	64	4.12[c]	<0.05
M × S (MS)	5	10	20	3.33	<0.10
M × C (MC)	5	12	27	4.41	<0.05
		22	47	9.06[c]	<0.01

[a] W, hemizygous for wild-type bovine growth hormone transgene (WT bGH); M, hemizygous for dwarf mutant bovine growth hormone transgene; S, line selected for high 3 to 6 week postweaning gain (M16); C, randomly selected control line (ICR)
[b] P, N, transgenic and non-transgenic progeny, respectively, as determined by PCR assay
[c] Pooled chi-square test after preliminary chi-square test showed the two groups not to be heterogenous

Non-transgenic F_1 progeny in high-growth and control backgrounds

An initial concern in making comparisons between the WT bgH and bGH-M8 transgenes is that there may be major growth differences in the background genotypes of the lines carrying these transgenes since they were obtained from segregating F_2 embryos. Also, crosses of males of these respective lines to females from the M16 and ICR lines could yield different degrees of heterosis and/or maternal effects. Tests for these types of differences were made on non-transgenic F_1 progeny and are presented in Table 2 for weekly body weights. In no case were significant differences detected in body weight, and similar results were found for weekly weight gains (data not shown). The results indicate that WC,N and MC,N F_1 progeny grew at similar rates, as did WS,N and MS,N F_1s (Figs. 1 and 3).

Transgenic vs non-transgenic comparisons

Weekly body weight and weight gain least squares means, averaged over gender, of F_1 progeny carrying the wild-type bGH transgene in a selected or control line background and their respecitve non-transgenic littermates are given in Figs. 1 and 2, respectively. Body weight means of WT bGH transgenic mice were larger ($P<0.05$) than non-transgenic littermates by 3 weeks of age in the control line background, and by 5 weeks in the high-growth selected line background (Table 3). The WT bGH transgene had the effect of increasing growth more in the control than in the selected line background, both in absolute terms and as a percent of non-transgenic littermates (Tables 3 and 4). The percentage increase in body weight of the WT bGH transgene over non-transgenic mice increased through about 8 to 9 weeks in both backgrounds and then plateaued.

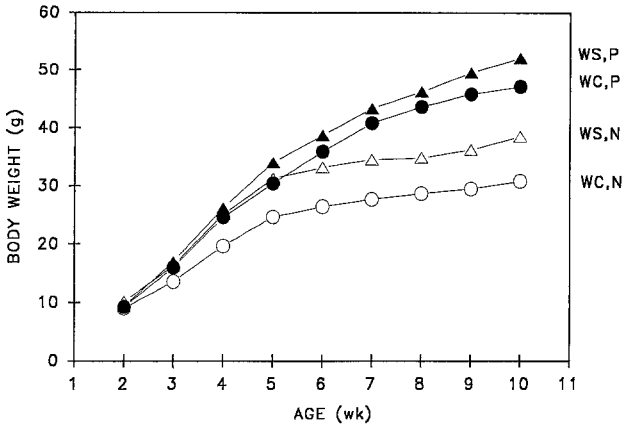

Fig. 1. Weekly body weight least squares means of F_1 progeny carrying the WT bGH transgene (*WS, P*; *WC, P*) and their respective non-transgenic littermates (*WS, N*; *WC, N*) averaged over gender

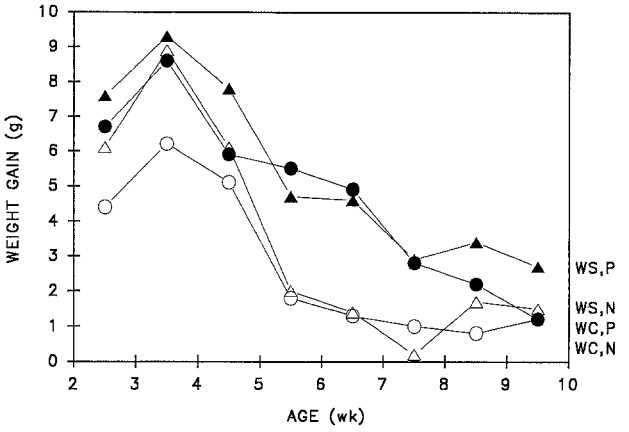

Fig. 2. Weight gain least squares mean of F_1 progeny carrying the WT bGH transgene (*WS, P*; *WC, P*) and their respective non-transgenic littermates (*WS, N*; *WC, N*) averaged over gender

Table 2. Mean difference in weekly body weights (g) between non-transgenic progeny whose sire was hemizygous for the WT bGH transgene and those whose sire was hemizygous for the bGH-M8 transgene, averaged over gender

Age (wks)	Genetic background[a]	
	C	S
2	−1.23[b]	−0.67[c]
3	−1.18	−1.31
4	−2.31	−0.28
5	−1.15	1.41
6	0.18	1.48
7	0.15	1.12
8	0.13	−1.03
9	−0.11	−0.47
10	0.59	0.02

[a] S and C refer to F_1 progeny from crosses involving females from either a line selected for high postweaning gain (M16) or a randomly selected control line (ICR)

[b] $D_1 = \overline{WC,N} - \overline{MC,N}$

[c] $D_2 = \overline{WS,N} - \overline{MS,N}$

Figures 3 and 4 show the mean growth curves and weight gains of F_1 mice carrying the dwarf mutant bGH transgene and non-carrier littermates in both the control and selected line backgrounds. By 3 weeks of age there was a reduction in growth in both the selected and control line backgrounds, but the reduction was greater and remained greater in the high-growth selected line background compared to the control line (Tables 3 and 4). While there was a gradual increase in the absolute body weight difference between transgenic and non-transgenic mice in both genetic backgrounds, the percentage decrease in growth remained stable across ages beginning at 3 weeks in the control background and 5 weeks in the selected background.

Orthogonal contrasts

Orthogonal linear contrasts were conducted to obtain independent estimates of the effects of the transgenes, selection back-

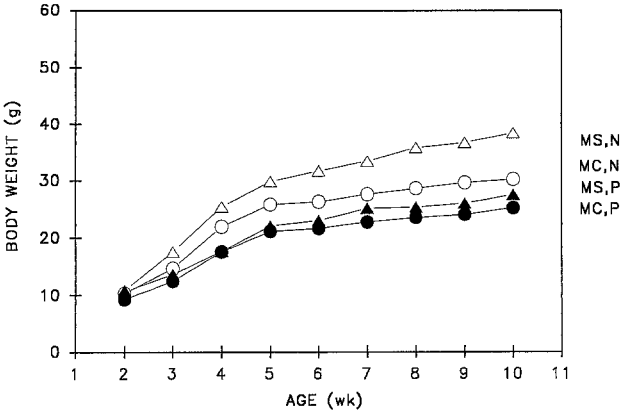

Fig. 3. Weekly body weight least squares mean of F_1 progeny carrying the bGH-M8 transgene (*MS, P*; *MC, P*) and their respective non-transgenic littermates (*MS, N*; *MC, N*) averaged over gender

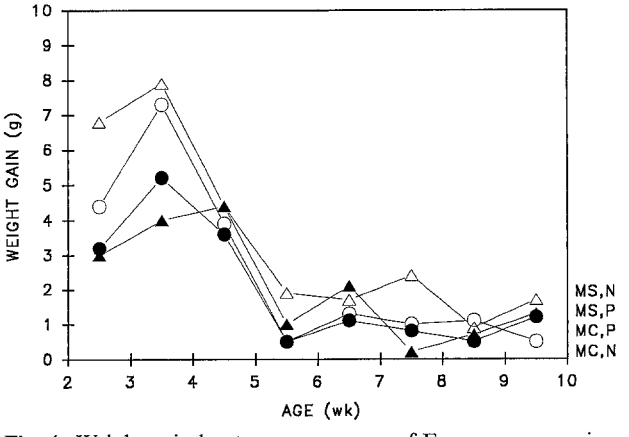

Fig. 4. Weight gain least squares means of F_1 progeny carrying the bGH-M8 transgene (*MS, P*; *MC, P*) and their respective non-transgenic littermates (*MS, N*; *MC, N*) averaged over gender

Table 3. Mean difference in weekly body weights (g) between transgenic and non-transgenic mice in control and selected line backgrounds, averaged over gender

Age (wks)	WT bGH transgene				bGH-M8 transgene			
	C[a]		S[a]		C[a]		S[a]	
2	0.22[b]	(102)[f]	−0.77[c]	(92)	−1.08[d]	(89)	−0.16[e]	(99)
3	2.49*	(119)	0.66	(104)	−2.22*	(85)	−3.96*	(78)
4	4.96**	(125)	1.07	(104)	−4.40*	(80)	−7.80**	(69)
5	5.78**	(123)	2.81*	(109)	−4.66**	(82)	−7.79**	(74)
6	9.44***	(136)	5.50***	(117)	−4.69**	(82)	−8.74***	(72)
7	13.10***	(147)	8.74***	(125)	−4.85**	(82)	−8.34***	(75)
8	14.93***	(152)	11.42***	(133)	−5.06**	(82)	−10.49***	(70)
9	16.26***	(155)	13.31***	(136)	−5.62**	(81)	−10.68***	(71)
10	16.28***	(153)	13.62***	(135)	−4.95**	(84)	−10.98***	(71)

* $P<0.05$, ** $P<0.01$, *** $P<0.001$

[a] S and C refer to F_1 progeny from crosses involving females from the line selected for high postweaning gain (M16) and the randomly selected control line (ICR), respectively

[b] $D_3 = \overline{WC,P} - \overline{WC,N}$

[c] $D_4 = \overline{WS,P} - \overline{WS,N}$

[d] $D_5 = \overline{MC,P} - \overline{MC,N}$

[e] $D_6 = \overline{MS,P} - \overline{MS,N}$

[f] Values in parentheses are means of transgenic mice as a percent of the mean of non-transgenic littermates

Table 4. Mean difference in weight gain (g) between transgenic and non-transgenic mice in control and selected line backgrounds, averaged over gender

Age interval (wks)[a]	WT bGH transgene		bGH-M8 transgene	
	C[b]	S[b]	C[b]	S[b]
G23	2.27*[c]	1.43[d]	−1.14[e]	−3.75**[f]
G34	2.47*	0.41	−2.11*	−3.89**
G45	0.82	1.75**	−0.30	0.02
G56	3.66**	2.68**	−0.03	−0.95
G67	3.65***	3.23***	−0.16	0.40
G78	1.82	2.68*	−0.21	−2.19
G89	1.33	1.71*	−0.56	−0.17
G910	0.03	1.15*	0.66	−0.32
G36	6.96***	4.84***	−2.47*	−4.81***

* $P<0.05$, ** $P<0.01$, *** $P<0.001$

[a] Weight gain between week i and j

[b] S and C refer to F_1 progeny from crosses involving females from the line selected for high postweaning gain (M16) and the randomly selected control line (ICR), respectively

[c] $D_3 = \overline{WC,P} - \overline{WC,N}$

[d] $D_4 = \overline{WS,P} - \overline{WS,N}$

[e] $D_5 = \overline{MC,P} - \overline{MC,N}$

[f] $D_6 = \overline{MS,P} - \overline{MS,N}$

ground, gender, and their respective interactions on body weights (Table 5) and weight gains (Table 6). Note that the effect of selection is halved in the F_1 because both selected and control line females were mated to the transgene-carrying males from a third line.

As expected, mice sired by males hemizygous for WT bGH were larger and gained weight more rapidly than mice sired by males hemizygous for the dwarf mutant (W vs M). The effect of the wild-type bGH transgene was significant ($P<0.01$) for body weight at 3 weeks of age and for 2–3-week weight gain [(P vs N)/W]. The WT bGH transgenic effect on body weight continued to be significant ($P<0.001$) from 4 to 10 weeks and increased with age. The effect of selection was also apparent ($P<0.05$) for 3-week body weight and for 2–3-week weight gain [(S vs C)/W]. The effect of selection on body weight increased to about 5 weeks and then plateaued, although a slight increase occurred at 10 weeks.

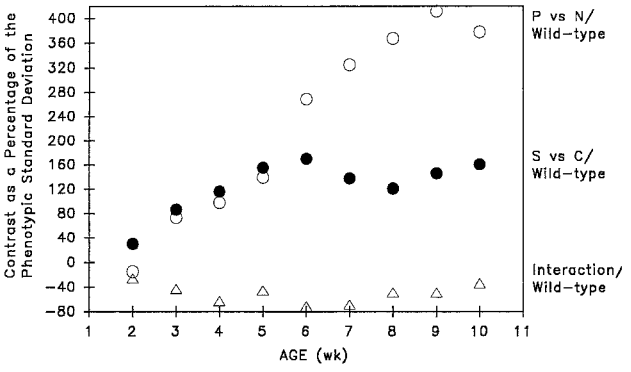

Fig. 5. Plot of linear contrasts of body weight within wild-type crosses as a percentage of phenotypic standard deviation

The effect of the WT bGH transgene on body weight between 6 and 10 weeks of age was two-to-three times the effect of selection. The significant ($P<0.05$) interaction effects [(P vs N)*(S vs C)/W] found for body weights from 3 to 9 weeks of age were due to the earlier observation that the wild-type bGH transgene increased growth more in the control than in the selected line background.

Because the linear contrasts across age are affected by scale, it is instructive to make comparisons across age by taking the contrasts as a percentage of the phenotypic standard deviation or a percentage of the mean. In the present study the two scales gave similar results for body weight so that only the former is presented (Fig. 5). The effect of the transgene increased continuously through 9 weeks of age and then plateaued, whereas the

Table 5. Orthogonal linear contrasts of body weight means (g) from 2 to 10 wk of age

Contrast[a]	Age (wks)								
	2	3	4	5	6	7	8	9	10
W vs M	−0.78	1.08*	3.26*	5.39***	7.92***	9.35***	10.02***	11.17***	11.76***
(P vs N)/W	−0.28	1.57**	3.01***	4.29***	7.47***	10.92***	13.17***	14.78***	14.95***
(S vs C)/W	0.57	1.85*	3.57*	5.07**	4.72***	4.62**	4.33**	5.23***	6.35***
(P vs N)*(S vs C)/W	−0.49	−0.92*	−1.94**	−1.48*	−1.98*	−2.31*	−1.75*	−1.76*	−1.33
(P vs N)/M	−0.62	−3.06***	−6.10***	−6.23***	−6.72***	−6.59***	−7.78***	−8.14***	7.97***
(S vs C)/M	0.97	2.06*	1.80	2.43*	3.37*	4.09*	4.53*	4.54**	5.24**
(P vs N)*(S vs C)/M	0.46	−0.84	1.70	−1.56	−2.02*	−1.75	−2.72*	−2.53*	−3.02*
Male vs Female (G)	−0.03	0.26	1.37***	2.55***	3.90***	4.67***	4.71***	5.64***	5.45***
G*(W vs M)	−0.18	−0.54**	−1.24***	−1.35**	−1.48**	−1.72***	−1.84***	−1.54***	−1.47**
G*[(P vs N)/W]	−0.79	−0.48*	−2.43***	−2.66***	−2.05***	−2.24***	−1.92***	−2.22***	−2.29***
G*[(S vs C)/W]	−0.35	−0.55*	−1.03**	−1.12**	0.11	0.13	−1.35*	−1.14*	−1.51*
G*[(P vs N)*(S vs C)W]	−0.02	0.22	0.26	−0.39	−0.08	−0.15	0.35	−0.05	−0.30
G*[(P vs N)/M]	0.05	−0.46	−1.27**	−0.85	1.35*	−1.34*	−1.34*	−1.35*	−1.20
G*[(S vs C)/M]	0.72	0.94**	0.64	1.09	1.82**	1.71*	1.50*	2.31**	2.01*
G*[(P vs N)*(S vs C)/M]	0.10	−0.39	−0.29	−0.36	−0.66	−0.54	−0.56	−0.62	−0.68

* $P<0.05$; ** $P<0.01$; *** $P<0.001$

[a] Adjusted for number of pups weaned; W and M refer to F_1 progeny from crosses involving male mice hemizygous for the wild-type (W) and dwarf mutant (M) bovine growth hormone transgene, respectively; P and N refer to positive and negative PCR reaction, respectively, for the bovine growth hormone transgene in F_1 progeny; S and C refer to F_1 progeny; from crosses involving females from a line selected for high postweaning growth (M16) and a randomly selected control line (ICR), respectively; G denotes gender, male vs female

Table 6. Orthogonal linear contrasts of mean weight gains (g)

Contrast[b]	Age interval (wks)[a]								
	G23	G34	G45	G56	G67	G78	G89	G910	G36
W vs M	1.85**	2.17**	2.14***	2.52**	1.49**	0.62	1.21***	0.48*	6.85***
(P vs N)/W	1.85***	1.44***	1.28*	3.17***	3.45***	2.25**	1.52***	0.59*	5.90***
(S vs C)/W	1.28*	1.72*	1.49*	−0.35	−0.11	−0.28	1.02**	0.89**	2.86**
(P vs N)*(S vs C)/W	−0.42	−1.03**	0.45	−0.49	−0.21	0.43	0.09	0.56*	−1.06*
(P vs N)/M	−2.44***	−3.00***	−0.13	−0.49	0.06	−0.59	−0.36	0.17	−3.64***
(S vs C)/M	1.09*	−0.30	0.65	0.93	0.72	0.22	0.02	0.69	1.29*
(P vs N)*(S vs C)/M	−1.30**	−0.89	0.16	−0.46	0.28	−0.48	0.19	−0.49	−1.17
Male vs Female (G)	0.29	1.10***	1.18***	1.35***	0.77**	0.04	0.89**	−0.02	3.62***
G*(W vs M)	−0.36	−0.67**	−0.12	−0.14	−0.22	−0.26	0.25	0.25	−0.93**
G*[(P vs N)/W]	0.31	−1.94***	−0.22	0.61*	−0.19	0.31	−0.14	−0.18	−1.57***
G*[(S vs C)/W]	−0.40	−0.47	−0.15	1.29***	0.02	−1.49***	0.14	−0.02	0.67
G*[(P vs N)*(S vs C)/W]	0.48	0.05	−0.66	0.31	−0.06	0.49	−0.32	−0.27	−0.30
G*[(P vs N)/M]	−0.51	−0.84*	0.44	−0.51	0.01	0.00	−0.02	0.16	−0.88
G*[(S vs C)/M]	0.21	−0.25	0.42	0.73*	−0.11	−0.21	0.81	−0.31	0.87
G*[(P vs N)*(S vs C)/M]	−0.48	0.12	−0.08	−0.30	0.11	−0.01	−0.05	−0.07	0.27

* $P<0.05$; ** $P<0.01$; *** $P<0.001$

[a] Weight gain between week i and j

[b] See Table 5 for definition of terms

effect of selection increased only through 6 weeks after which there was a slight drop but essentially little change. The interaction showed a gradually greater negative value through 6 weeks followed by a trend toward a smaller negative value.

The effect of the mutant transgene [(P vs N)/M] on body weight within the dwarf mutant crosses was not significant at 2 weeks of age, but was negative ($P<0.001$) at all subsequent ages (Table 5). The dwarf mutant effect had its major impact on weight gain between 2 and 3 weeks and 3 and 4 weeks of age, respectively (Table 6). Per unit phenotypic standard deviation, the effect of the transgene became more negative from 2 to 4 weeks and then showed little change (Fig. 6). The effect of selection [S vs C)/M] on body weight was positive and increased with age (Table 5), but there was little change per unit phenotypic standard deviation after 6 weeks of age (Fig. 6). Significant ($P<0.05$) interaction effects [(P vs N)*(S vs C)/M] on body weight at 6, 8, 9 and 10 weeks of age can be explained by the observation that the presence of the bGH-M8 gene reduced growth more in the high-growth than in the control line background.

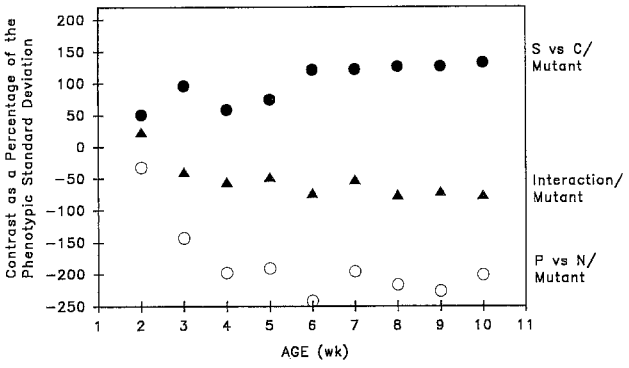

Fig. 6. Plot of linear contrasts of body weight within mutant bGH crosses as a percentage of phenotypic standard deviation

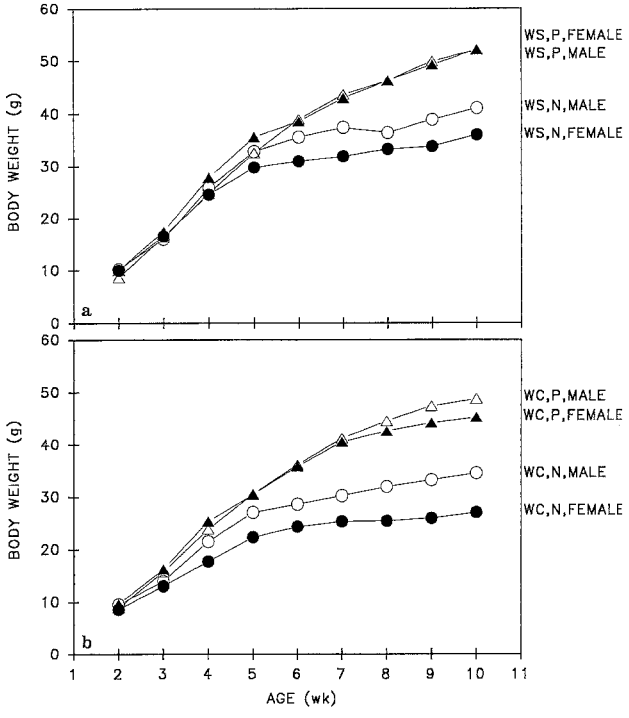

Fig. 7A, B. Weekly body weight least squares means by gender of F_1 progeny carrying the WT bGH transgene and their respective non-transgenic littermates. **A** Selected background; **B** control background

Males were larger ($P<0.001$) than females from 4 to 10 weeks of age (Table 5). Growth of males exceeded that of females primarily between 3 and 7 weeks of age (Table 6). Interactions of gender with transgenic, and gender with selection, effects were significant in many cases for body weight (Table 5) but not for weight gain (Table 6). The presence of the WT bGH transgene has reduced the gender difference in body weight in the control line background and completely eliminated it in the selected background (Fig. 7a, b), thus causing a significant negative gender by transgene interaction in the wild-type crosses [G*[/P vs N)/W]] for body weight at 4 ($P<0.05$) and 5 through 10 ($P<0.001$) weeks of age. The significant negative gender by selected vs control line interaction in the wild-type crosses {G*[(S vs C)/W]} for body weights at 3 through 5 and 8 through

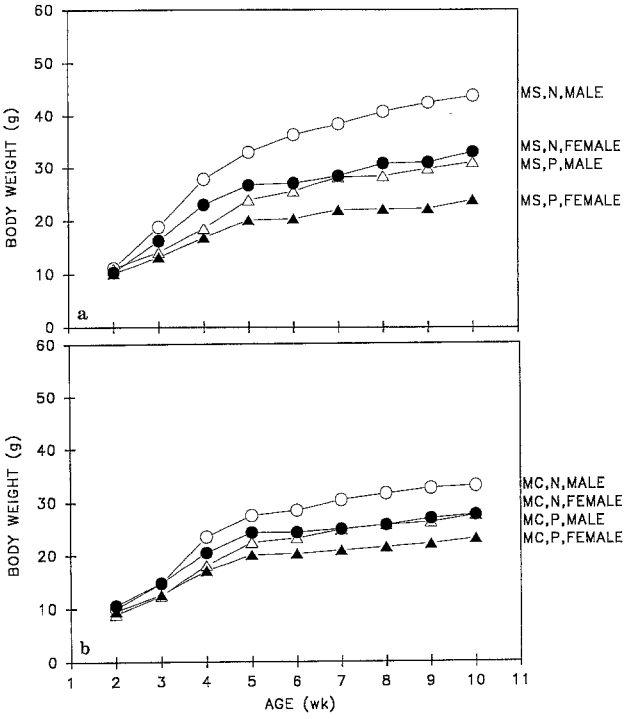

Fig. 8. Weekly body weight least squares means by gender of F_1 progeny carrying the bGH-M8 transgene and their respective non-transgenic littermates. **A** Selected background; **B** control background

10 weeks was due to the selection effect being smaller in male than in female F_1 progeny (Fig. 7a, b).

Interactions with gender were also present in the dwarf mutant crosses. The significant negative gender by transgene interaction effects {G*[(P vs N))/M} for body weight at 4 and 6 through 9 weeks were caused by the larger negative effect of the dwarf mutant transgene in males than in females (Fig. 8a, b). The significant positive gender by selection interaction effects {G*[(S vs C)/M]} for body weight at 3 and 6 through 10 weeks were due to the selection effect being larger in males than females (Fig. 8b).

The gender by transgene by selection interaction was not significant for any body weights or weight gains in either wild-type or dwarf mutant crosses.

Discussion

In progeny of male parents hemizygous for the WT bGH transgene or a bGH antagonist (bGH-M8)-encoding transgene mated to female parent lines lacking the transgene, there was a significant deficiency of progeny carrying the transgene from that expected for Mendelian segregation. Similar results have been reported for bGH transgenic mice (Naar et al. 1991; Sabour et al. 1991; Nagai et al. 1992). Abnormal transmission of a transgene may be due to germline mosaics (Wilkie et al. 1986) or differential embryo and neonatal survival. The latter phenomenon is a more likely cause for the abnormal segregation, as segregation-ratio deviation is expected to be much higher with germline mosaics.

The present study confirms earlier findings that the wild-type growth hormone transgene from several species enhances growth in mice (Palmiter et al. 1982; 1983; Nagai et al. 1985; Shanahan et al. 1989; Sabour et al. 1991; Pomp et al. 1992). The main objective of our study was to determine if the genetic background in which the WT bGH transgene is expressed affects the growth response. The results clearly demonstrate that the bGH transgene has a larger absolute and relative effect on growth in an F_1 of a cross using female parents from a randomly selected line than in an F_1 of a cross using female parents from a high-growth selected line. However, although the maximum relative response of the transgene was not as great in crosses with high-growth line females (136% of non-transgenics) compared with control line females (155% of controls), the response was still sizable. Therefore, the results do not support the hypothesis proposed by Pursel et al. (1989) that selection for growth in the pig may have limited its response to the GH transgene relative to what has been observed in the mouse. Prior selection may have contributed some effect, however, since a genetic background by transgene interaction was observed with mice in the present study. Other factors which may account for species differences in response to the GH transgene include physiological differences between species in normal response to the growth hormone cascade, differential response to foreign hormones across species, and detrimental effects due to overexpression of the GH transgene (Kirkpatrick and Rutledge 1991).

An additional differential response of the WT bGH transgene was the age at which the gene was first seen to affect body weight significantly: 3 weeks in the control background vs 5 weeks in the high-growth background (Table 3). These results suggest that genes affecting growth normally being expressed in the selection line background through 4 weeks of age have a greater effect than the WT bGH transgene.

The dwarf mutant animals, resulting from expression of a bGH antagonist, developed by Chen et al. (1990), also exhibited a differential magnitude of absolute and relative expression on body weight dependent on genetic background; but in this case the greater effect was found in terms of reducing growth in the high-growth line background compared to the control background. Chen et al. (1991) have reported that the dwarf transgenic mouse possesses low IGF-I and high pituitary GH levels; the decrease in IGF-I, resulting from the interaction between the bGH antagonist and endogenous mouse GH and GH receptors, leads to the dwarf phenotype. Enhancement of the dwarf phenotype in the high-growth background may be the result of phenotypic changes in the endogenous GH secretion rate and the quantity of GH receptors and a possible cascade effect on other hormones, which have occurred as a result of selection for high growth rate.

Interaction of wild-type and dwarf mutant transgene expression with gender may be the result of interactions of GH expression with effects of the male and female sex hormones and requires further investigation. Pomp et al. (1992) also reported a genotype × sex interaction effect on postweaning growth in mice whereby transgenic females with the oGH transgene responded with greater relative growth than did males. This phenomenon has been reported in pigs treated with exogenous GH (Campbell et al. 1989). In contrast, Koops and Grossman (1991) did not find a significant interaction of bGH expression by gender for body weights at 7 and 12 weeks of age; they did find a significant interaction at 26 weeks which was opposite to that found in the present study since male response to the transgene was greater than that in females.

It was noted earlier that the effect of selection is halved in the F_1 since both selected and control line females were mated to unselected males carrying the transgene and that the transgene effect on body weight was two-to-three times the selection efffect. With continued backcrossing to the respective selected and control lines, the effect of selection for growth should be enhanced relative to the transgene effect. Other assumptions which if not valid could bias the coefficient of one-half for the selection effect in the F_1 include absence of differences in heterosis between the crosses involving the selected and control lines with the transgenic lines and differential line maternal effects. Statistical tests indicated that these effects were either not important or else cancelled each other.

The present results indicate that phenotypic expression of the WT bGH and bGH antagonist transgenes on growth is dependent on genetic background. Because GH transgenes can effect feed efficiency and body composition (Pomp et al. 1992), two traits genetically correlated with growth, it will be important to determine the effect genetic background has on the expression of these and other growth related traits.

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