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Growth hormone and insulin-like growth factor-I measurements in high growth (*hg*) mice

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Summary

Effects of a recessive gene causing high growth (*hg*) were studied on two major components of the growth axis in mice. Plasma and pituitary levels of growth hormone and plasma levels of insulin-like growth factor I (IGF-I) were measured in three lines homozygous for *hg*, each compared with a control line of alike genetic background but wild type for the *hg* locus (*Hg*). Line Gh (*hghg*) and line GH (*HgHg*) are from a line which had undergone long-term selection for high postweaning weight gain; line Ch (*hghg*) and line CH (*HgHg*) were extracted from the second backcross of Gh to C57BL/6J; line L54 (*hghg*) was from the sixth backcross to C57BL/6J (B6) (*HgHg*). Pituitary GH levels and plasma IGF-I levels were measured in both sexes at 3, 4·5, 6 and 9 wk of age. Plasma growth hormone was measured in 8- to 12-wk-old males at hourly intervals from 08.00 to 17.00. Body weight in lines homozygous for *hg* at 6 and 9 wk of age was 10–30% greater than in control lines. The ontogeny of this increased growth depended on genetic background. Pituitary growth hormone content was 52% lower in the two *hghg* lines measured (lines Ch and Gh) than in control lines at 4·5, 6 and 9 wk. Plasma growth hormone levels were also much lower in *hg* mice, with values only 20–30% of those in their respective controls. *hg* lines showed consistently low plasma growth hormone levels throughout the 9 hr sampling period, while control lines expressed the characteristic pulsatile hormone secretion. In contrast, plasma IGF-I levels were greater in line Ch (*hghg*) than in line CH (*HgHg*) at 3, 4·5 and 9 wk, and were also greater in line Gh (*hghg*) *vs.* line GH (*HgHg*) at 6 wk of age. The results suggest that the growth enhancing effect of the *hg* gene occurs through an IGF-I-mediated process. In addition, the genetic background itself is also a factor in the phenotypic expression of the gene.

1. Introduction

A significant contribution to our understanding of the mechanisms of metabolic control in mammals has come from the study of genetic variation resulting from genes with large effects. The high growth (*hg*) gene in mice, which dramatically increases post-weaning growth rate and mature body size (Bradford & Famula, 1984), represents a unique model to study the metabolic control of growth. Most other major gene mouse models related to endocrine regulation of growth produce dwarfism. The high growth gene seems to have a general influence on all chemical components of growth without dramatically altering overall body composition (Calvert *et al.* 1985). The gene alters energy metabolism by increasing efficiency of growth and/or decreasing maintenance energy requirements (Calvert *et al.* 1986).

In the study of the aetiology of disorders that are genetically transmitted in dwarf mouse mutants, it has been found that the entire growth hormone axis plays a central role in the regulation of growth competence in mammals. Factors such as hypothalamic releasing and inhibiting hormones, pituitary secretion of growth hormone itself, insulin-like growth factor I (IGF-I), plasma binding proteins of IGF-I, growth hormone (GH) and IGF-I cell surface receptors, and the interaction among these and other cellular components, present many possible sites where a genetic mutation could lead to significant differences in the size phenotype (Teller, 1985). At least five valuable experimental models of such autosomal recessive mutations leading to a small stature phenotype have been described in the mouse (Charlton, 1984).

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Genetic background affects the expression of single gene mutations. In *hg* lines different effects have been observed on a number of reproductive characters when the gene was expressed in high growth selected background compared to C57BL/6J background (Dilts, 1988). Also a larger percentage increase in net energy efficiency (47 *vs.* 25%) was observed by Calvert *et al.* (1986) when the *hg* gene was expressed in a high growth selected background than when expressed in a C57BL/6J background.

The objectives of the present investigation were to: (1) evaluate the circulating and pituitary levels of growth hormone and plasma levels of IGF-I in three lines of mice carrying the *hg* gene for rapid post-weaning growth, and (2) ascertain the effects of genetic background on the expression of the *hg* gene.

2. Materials and methods

(i) *Lines of mice*

Three lines of mice (Gh, Ch, L54) homozygous for the high growth gene (*hghg*), were included in this study. Each of these lines had a corresponding control (GH, CH, B6) of similar genetic background but wild type for the high growth locus (*HgHg*). The high growth gene (*hg*) was originally discovered in the 29th generation of selection in a strain of mice (line G) selected for rapid post-weaning weight gain (Bradford & Famula, 1984). The origin of line G was a 4-way cross of inbred lines C57BL/6, AKR, C3H and DBA/2 (Bradford, 1971; Barria & Bradford, 1981). Line Gh (*hghg*) and line GH (*HgHg*) have the genetic background of line G, described by Calvert *et al.* (1986). Line Ch (*hghg*) was established by crossing G (*hghg*) mice to a standard inbred line C57BL/6J (B6), followed by two successive backcrosses to line B6 (Famula *et al.* 1988). Intermating of the normal sibs from the last backcross gave origin to control line CH (*HgHg*). Therefore the genetic background of these lines was 87·5% C57BL/6J and 12·5% line G. Line 54 (L54) was the result of six backcrosses to C57BL/6J, therefore having a genetic background of 99·2% C57BL/6J and 0·8% line G genes. B6 inbred mice were used as controls for line 54. Animals from generation 21 of Gh and GH, generation 25 of Ch and CH, and generation 5 of line 54 were used in these studies. Information on the three pairs of lines is summarized in Table 1.

Table 1 *Genetic background of* hghg *and* HgHg *lines of mice*

Line	Genotype (*hg* locus)	Genetic background
Gh	hghg	Line G[a]
GH	HgHg	Line G
Ch	hghg	87·5% C57BL/6J, 12·5% line G
CH	HgHg	87·5% C57BL/6J, 12·5% line G
L54	hghg	99·2% C57BL/6J, 0·8% line G
B6	HgHg	C57BL/6J

[a] Line selected for rapid post-weaning gain from a 4-way cross base population of C57BL/6J, AKR, C3H and DBA/2.

Mice were weaned at 3 weeks of age and caged by sex (4–6 mice/cage). Mice were housed in the same room maintained at 23 ± 2 °C and illuminated 14 h (05.00–19.00) per day. Food (white diet, Simonsen Laboratories, Gilroy, CA, guaranteed analysis of a minimum of 24% crude protein and 6% fat and a maximum of 3·5% crude fibre) and water were supplied *ad libitum*.

(ii) *Sample collection*

For the serial plasma growth hormone and IGF-I measurements blood was obtained from the tip of the tail by cutting a small piece with a razor blade. Approximately 40 μl of blood were collected in hematocrit tubes previously dipped in a 15% solution of EDTA(Na_2) and vacuum dried. The samples were centrifuged and 15–25 μl of plasma separated and stored at −20 °C until assayed. Collection took approximately 1 min from the time the animals were removed from their cages. Measurements of plasma growth hormone in lines GH and Gh were made only at 6 and 9 wk of age. Male mice were rapidly decapitated and blood collected from the trunk in tubes containing EDTA(Na_2). For pituitary growth hormone measurements the animals were decapitated and the glands carefully dissected, placed in 50 μl plastic tubes, quick frozen in liquid nitrogen, and stored at −70 °C prior to extraction.

(iii) *Determination of growth hormone*

Plasma. In this experiment nine 8- to 12-wk-old male mice from each line were bled at different hours of the day from 08.00 to 17.00 h at intervals of 6 days. With this sampling procedure several measurements were made on each individual animal at different times. Hematocrit values remained constant throughout the period of repeated sampling and body weight was unaffected. To obtain enough plasma for assay at each time, plasma from 3 different animals within each line was combined into one composite sample. Three composite samples were analysed for each line and time.

Pituitary extraction. Anterior pituitary glands were homogenized in 0·5 ml 0·01 M-NaOH, then diluted with 2 ml 0·05 M phosphate buffered saline containing 1% BSA (Downs *et al.* 1990). The homogenate was centrifuged at 10000 *g* for 10 min at 4 °C and the supernatant stored at −20 °C for subsequent radioimmunoassay (RIA).

Growth hormone RIA. Plasma and pituitary mouse

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growth hormone levels were measured by double antibody RIA, using ^{125}I-rat growth hormone as radioligand and a monkey anti-rat growth hormone serum by a previously described technique (Frohman & Bernardis, 1968). Bound radioactivity was immunoprecipitated by goat anti-monkey IgG serum in the presence of 2·2% polyethylene glycol. Results were expressed in terms of NIDDK rGH-RP-1 reference standard, to which mouse growth hormone exhibits parallel displacement. The intra- and interassay coefficients of variation were 3·4 and 7·4%, respectively. Plasma growth hormone levels below the minimum detectable value (ranging from 37–50 pg/tube, or 3·7–5·0 ng/ml using a 10 μl sample volume) were assigned that value for statistical analyses.

(iv) *Determination of IGF-I*

Plasma concentration of IGF-I was determined by radioimmunoassay (RIA) using labelled tracer, antiserum and protocol supplied by L. E. Underwood and J. J. Van Wyk through the National Hormone and Pituitary Program. ^{125}I human Somatomedin C/IGF-I was utilized as tracer and antiserum UB805C was used at a dilution of 1:10000. Binding protein interference was eliminated by acid extraction of plasma and immediate dilution before addition to the assay tubes (D'Ercole & Underwood, 1980).

Assay standards were prepared using recombinant human IGF-I (Amgen, Thousand Oaks, CA; IGF-I lot No. 404) as pure standard. All the plasma samples were assayed with the same batch of labelled IGF-I in 7 separate assays. The intra- and interassay coefficients of variation were 8 and 9%, respectively. The lower limit of detectable IGF-I was 15·6 pg/tube. Recovery of recombinant IGF-I was 96, 101, 116, 124 and 111% for 3·8, 7·5, 15·6, 31·3 and 62·5 pg added to assay tubes containing a standard quantity of acid-extracted and diluted plasma.

(v) *Statistical analyses*

All data were analysed by least-squares procedures for unequal subclass numbers (Harvey, 1979). Body weight, pituitary growth hormone levels and plasma IGF-I levels were initially analysed with a model incorporating an overall mean, fixed effects of line, sex and age, all 2- and 3-way interactions and a random residual error. For pituitary growth hormone and plasma IGF-I levels, effects of sex and interactions with sex were not significant, and data were pooled by sex prior to analysis within age groups. Single D.F. linear contrasts were used to test for differences between lines within each of the pairs with a common genetic background (Ch *vs.* CH, Gh *vs.* GH, L54 *vs.* B6). Analyses were conducted with and without adjustment by analysis of covariance for body weight at time of sample collection. Partial within line and age phenotypic correlation coefficients were calculated between body weight and each trait.

Data on plasma growth hormone levels were analysed with a model including effects of line, time of day and their interaction. A reduced model tested effects of line only. This model was used to include data on lines Gh and GH, and only data from ages 6 and 9 wk from those lines were utilized.

3. Results

(i) *Body weight*

Growth patterns for all pairs of lines (Ch, CH; Gh, GH; L54, B6) are presented in Fig. 1. Overall, line effects were highly significant ($P < 0·0001$). Lines Gh and GH with a growth-selected background (line G) were the largest, followed by lines Ch and CH (87·5% B6, 12·5% line G) and then lines L54 (99·2% B6, 0·8% line G) and B6. Males were larger than females ($P < 0·001$) and mice were heavier as they progressed in age ($P < 0·001$). Both line by age and sex by age interactions were important ($P < 0·05$), while other interactions were not significant. The sex by age interaction was the result of males being larger than females at 4·5, 6 and 9 wk but not at weaning (3 wk). None of the *hg* lines were significantly heavier than their controls at 3 wk. The expression of the *hg* gene was observed in post-weaning growth, all *hg* lines being significantly heavier than their respective controls at 6 and 9 wk ($P < 0·01$). However, the ontogeny of this increased growth differed depending on genetic background. The *hg* lines with a B6 background, Ch and L54, were on average 33% larger than controls at 6 and 9 wk, while line Gh was 10 and 23% larger at 6 and 9 wk, respectively. At 4·5 wk, line Ch was also heavier than controls ($P < 0·001$), while in line Gh this differentiation occurred only in males.

(ii) *Pituitary growth hormone levels*

Least-squares means of pituitary growth hormone levels for lines Ch, CH and Gh, GH at 3, 4·5, 6 and 9 wk of age are presented in Fig. 2. Overall, levels were different among lines and age groups ($P < 0·0001$) but not between sexes, and line by age interaction was important ($P < 0·001$). In general, pituitary growth hormone levels were significantly lower for *hg* mice regardless of genetic background. Collectively, *hg* lines averaged 52% lower levels than control lines. At 3 wk, levels were lower in line Ch than in line CH ($P < 0·01$), but were similar for lines Gh and GH. At all other ages, levels were lower in both *hg* lines relative to their controls. Adjustment of pituitary growth hormone levels for body weight by analysis of covariance magnified these results, with both *hg* lines having significantly lower levels at all ages. At 6 and 9 wk of age there was a noticeable

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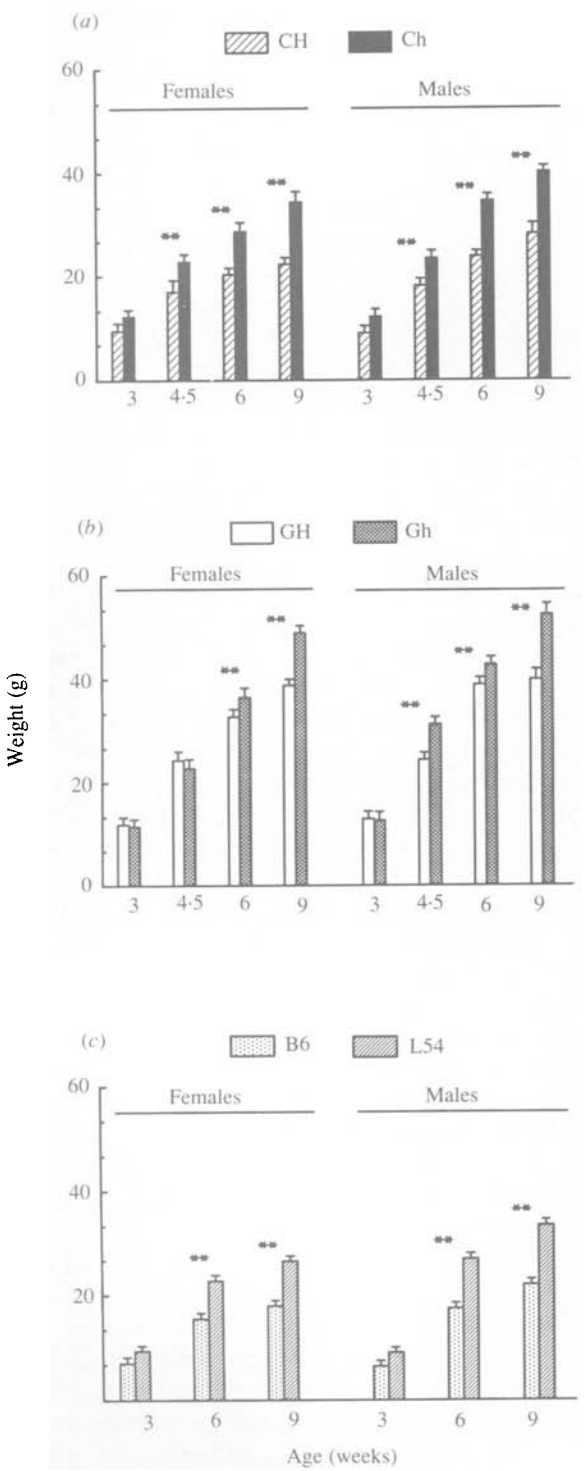

Fig. 1. Body-weight profiles of mouse lines possessing a high growth gene (*hghg*) and their control lines (*HgHg*). (*a*) Least-squares means (±S.E) for lines CH (*HgHg*) and Ch (*hghg*). (*b*) Least-squares means (±S.E.) for lines GH (*HgHg*) and Gh (*hghg*). (*c*) Least-squares means (±S.E.) for lines B6 (*HgHg*) and 54 (*hghg*). Numbers of animals ranged from 10 to 15 in each line by sex by age subclass. ** Lines differed ($P < 0.01$).

difference in pituitary growth hormone level between control lines GH and CH. Line GH had 18 and 76% higher levels than line CH, whereas the *hg* lines, Gh and Ch, had similar levels of pituitary growth hormone at both ages.

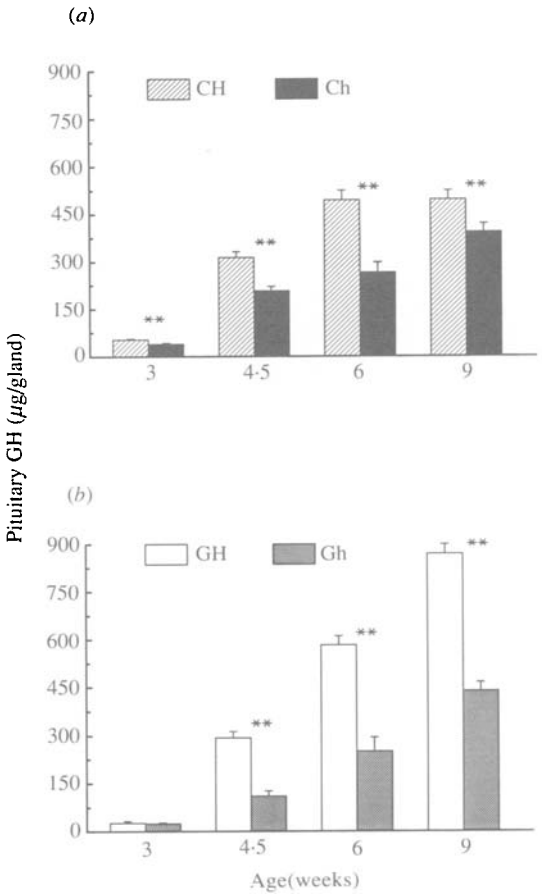

Fig. 2. Pituitary growth hormone (GH) profiles of mouse lines possessing a high growth gene (*hghg*) and their control lines (*HgHg*). (*a*) Least-squares means (±S.E.) for lines CH (*HgHg*) and Ch (*hghg*). (*b*) Least-squares means (±S.E.) for lines GH (*HgHg*) and Gh (*hghg*). Data are pooled by sex within age groups. Numbers of animals assayed ranged from 7 to 17 within line by age subclass, with a mean of 11. ** Lines differed ($P < 0.01$).

Within-line phenotypic correlations between pituitary growth hormone level and body weight were high at all age periods (range from 0·51 to 0·68; $P < 0.001$). While measurements of growth hormone were for whole pituitaries, a separate study has shown that pituitary weights are similar among *hg* and control lines (data not shown).

(iii) *Plasma growth hormone levels*

Profiles of least-squares means of plasma growth hormone levels for pairs of lines Ch, CH and L54, B6 at hourly intervals are presented in Fig. 3(*a*, *b*). Figure 3*c* shows least-squares means pooled across time intervals for these lines, as well as least-squares means for lines Gh and GH pooled for 6 and 9 wk. Line effects were highly significant ($P < 0.001$), while overall effects of time of day and line by time of day interaction were not significant. In general, results of plasma growth hormone are even more striking than those for pituitary growth hormone levels. Plasma growth hormone levels were only 28% as high on average in *hghg* as in *HgHg* control mice, and this

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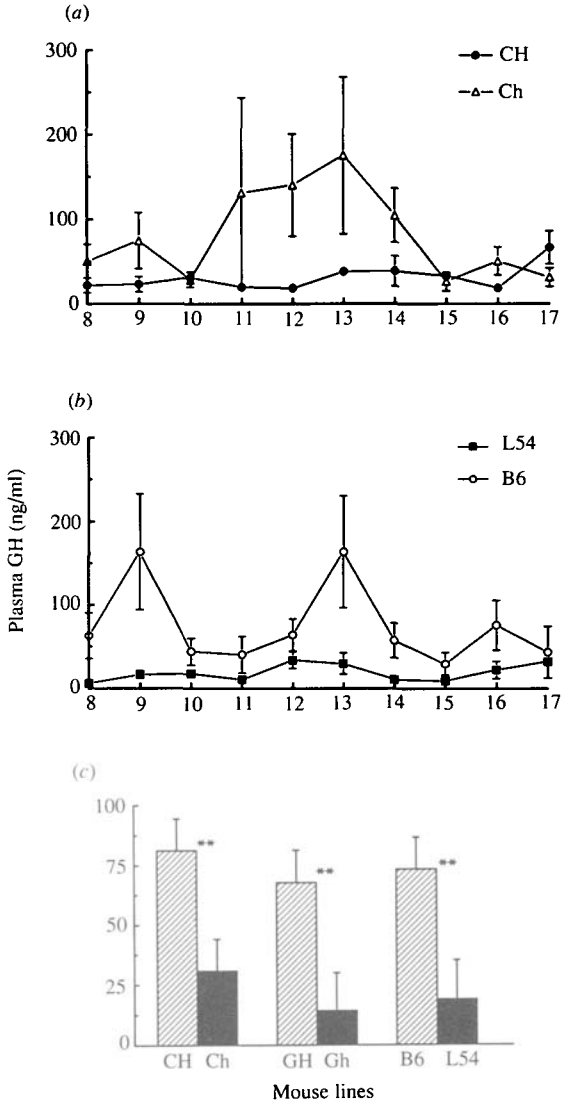

Fig. 3. Plasma growth hormone (GH) profiles of mouse lines possessing a high growth gene (*hghg*) and their control lines (*HgHg*). (*a*) Least-squares means (±S.E.) for lines CH (*HgHg*) and Ch (*hghg*) from 08.00 to 17.00 h. For each line at each hour, means are for three samples consisting of plasma pooled from three 8–12 wk males. (*b*) Least-squares means (±S.E.) for lines B6 (*HgHg*) and 54 (*hghg*) from 08.00 to 17.00 h, as in A. (*c*) Least-squares means (S.E.) for lines CH, Ch, and B6, 54 pooled across time periods, and for lines GH (*HgHg*) (n = 14) and Gh (*hghg*) (n = 10) pooled from 6 and 9 wk male mice. ** Lines differed ($P < 0{\cdot}01$).

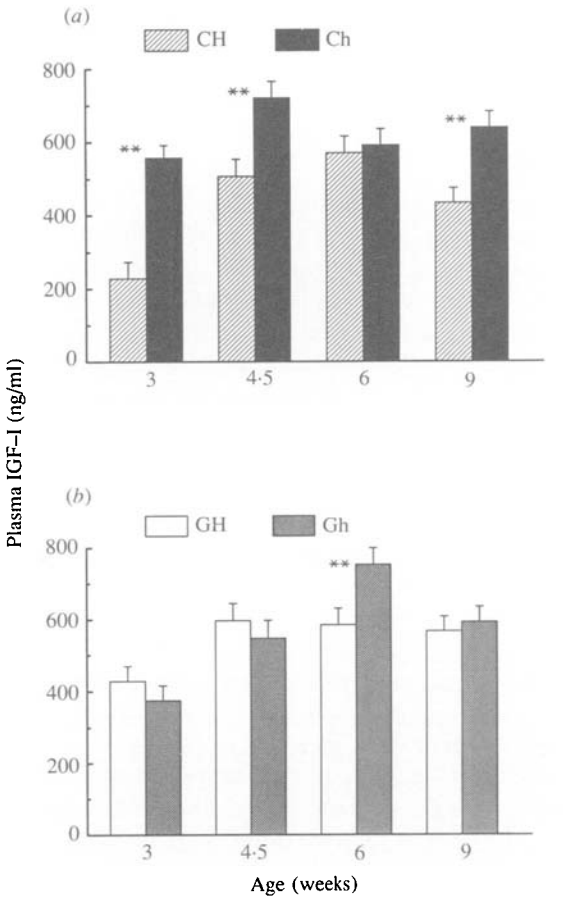

Fig. 4. Plasma insulin-like growth factor I (IGF-I) profiles of mouse lines possessing a high growth gene (*hghg*) and their control lines (*HgHg*). (*a*) Least-squares means (±S.E.) for lines CH (*HgHg*) and Ch (*hghg*). (*b*) Least-squares means (±S.E.) for lines GH (*HgHg*) and Gh (*hghg*). Data are pooled by sex within age groups. Numbers of animals assayed ranged from 18 to 31 within line by age subclass, with a mean of 27. ** Lines differed within age groups ($P < 0{\cdot}01$).

difference was consistent for each pair of comparisons (Ch *vs.* CH, Gh *vs.* GH, L54 *vs.* B6). While line by time of day interaction was not statistically significant, it is apparent that the *hg* lines lack any form of temporal pattern in growth hormone secretion, with continuously low levels. In contrast, control lines expressed peaks in secretion, most notably during midday hours (11.00–13.00).

(iv) *Plasma IGF-I levels*

Least-squares means of plasma IGF-I levels in lines Ch, CH and Gh, GH are presented in Fig. 4. Line and age effects were highly significant ($P < 0{\cdot}0001$), and line by age interaction was important ($P < 0{\cdot}01$). Age effects were due to lower levels at 3 wk. IGF-I levels were similar for males and females at all ages. Pooled across age groups, the *hg* gene in the B6 background (line Ch) was associated with significantly higher levels of IGF-I relative to its control line, while in the high growth background no significant difference was observed. Line Ch expressed significantly higher IGF-I levels than line CH at 3 ($P < 0{\cdot}0001$), 4·5 ($P < 0{\cdot}01$) and 9 wk ($P < 0{\cdot}001$) and showed a non-significant increase at 6 wk. In contrast, line Gh had higher levels than line GH only at 6 wk of age ($P < 0{\cdot}01$). After adjustment of plasma IGF-I levels by analysis of covariance for body weight, IGF-I in line Ch at 3 wk was no longer significant ($P = 0{\cdot}1$), while line Gh had significantly higher IGF-I levels than line GH at 9 wk as well as at 6 wk of age.

The within line phenotypic relationship between plasma IGF-I levels and body weight was highly dependent upon age at sample collection. The correlation was highest at 3 wk ($r = 0{\cdot}44, P < 0{\cdot}0001$) and

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then decreased steadily with age (4·5 wk; $r = 0{\cdot}22$, $P < 0{\cdot}05$; 6 wk: $r = 0{\cdot}04$, N.S.) until it became negative at 9 wk ($r = -0{\cdot}24$, $P < 0{\cdot}01$).

Discussion

This study has characterized effects of a gene causing rapid postweaning weight gain on IGF-I and growth hormone levels, two important determinants of growth. In general, it was found that the high growth gene (*hg*) causes elevated circulating IGF-I levels, while growth hormone levels in both the pituitary gland and plasma are decreased. Additionally, mice homozygous for *hg* lack characteristic pulsatile patterns of growth hormone secretion, with levels consistently low throughout the day. These are the first results which illustrate endocrine mechanisms which may underlie the significantly increased growth caused by the mouse high growth gene.

While *hg* has been previously characterized as a single locus autosomal mutation (Bradford & Famula, 1984), the actual gene affected has yet to be identified. It is very intriguing to compare mice possessing the high growth gene with mice that have been engineered to overproduce human IGF-I (Mathews *et al.* 1988). There are striking similarities between the *hg* and transgenic animals. Both exhibit modest increases in growth relative to their controls, due at least in part to similarly elevated levels of circulating IGF-I. Additionally, in both types of mice there is severe inhibition of growth hormone production. The present results, therefore, raise the possibility that elevation of IGF-I levels is a direct consequence of the *hg* gene, due to a molecular alteration of the IGF-I locus which would lead to increased expression of the gene or increased stability of the gene product. This hypothesis is further supported by mapping studies, which place *hg* and IGF-I loci in the same region of mouse chromosome 10 (J. F. Medrano, D. Pomp and B. A. Taylor, manuscript in preparation).

Decreased levels of growth hormone production and secretion are likely an indirect consequence of *hg*. Although the major growth promoting effects of IGF-I are autocrine and/or paracrine, plasma IGF-I levels were also elevated in *hg* mice, reflecting the increased tissue (primarily hepatic) production. Elevated plasma IGF-I levels would be expected to exert negative feedback on growth hormone production, either indirectly via a negative influence on hypothalamic GHRH secretion (Shibasaki *et al.* 1986) or stimulation of hypothalamic somatostatin secretion (Berelowitz *et al.* 1981), or directly at the pituitary level (Berelowitz *et al.* 1981; Yamashita & Melmed, 1987). Depression of growth hormone pulsatility would also be expected due to negative feedback mechanisms (Tannenbaum *et al.* 1983).

The use of pooled sera from 3 mice was necessary because the size of this animal is insufficient to permit the use of an implanted intraatrial cannula for repetitive sampling (as can be done in the rat) or for the collection of sufficient plasma in an acceptable time period (1 min cage removal and under conditions of minimal stress) for hormone assay. Overall, there is considerable indirect evidence in the literature to indicate that the control of GH secretion in the mouse is very similar to that in the rat, where secretion is pulsatile (with a 3–4 h interval between pulses) and also that, in unstressed animals housed in the same environment, the pulses are synchronized (Tannenbaum & Martin, 1976; Tannenbaum & Ling, 1984; Katakami *et al.* 1984). A single study in the mouse, using plasma collected at the time of decapitation, suggests a similar pattern (Sinha *et al.* 1977). Our results are also consistent with a 3–4 h interpulse interval.

The present results should add considerably to the growing information base regarding genetic control of components of the somatotropic axis and their effects on growth in mice and other mammalian species. McKnight & Goddard (1989) found that selection for high lean tissue in mice resulted in higher IGF-I levels at 10 wk, while selection for high or low fat content did not alter IGF-I levels. In pigs, a line selected for high growth rate expressed lower levels of growth hormone relative to a line selected for low growth rate (Norton *et al.* 1989). This result is particularly interesting as it parallels that found for the high growth mice in the present study, even though the genetic mode of increased growth is different in the two cases (selection *vs.* mutation).

IGF-I has also been shown to be genetically related to increased growth through direct selection studies (Blair *et al.* 1990). Positive response to selection for IGF-I was observed, although realized heritability ($0{\cdot}15 \pm 0{\cdot}12$; Blair *et al.* 1989) was considerably lower than that estimated from covariance of full sibs ($0{\cdot}40 \pm 0{\cdot}27$; Blair *et al.* 1987). Significantly, a positive correlated response in body weight was observed when selection was for high IGF-I levels.

Interestingly, an endocrine pattern similar to that presently described for high growth mice has been observed in chickens selected for growth. Decreased growth hormone levels were found in fast growing chickens relative to control lines (Burke & Marks, 1982; Lauterio *et al.* 1986; Goddard *et al.* 1988). Additionally, temporal patterns of growth hormone secretion were altered due to selection for growth (Burke & Marks, 1982), when point measurements were taken from 0 to 8 wk of age. In terms of IGF-I, Huybrechts *et al.* (1984) indicated that heavy strains may have increased levels once they reach 6 wk of age, but Goddard *et al.* (1988) concluded that there were no differences among growth selected and non-selected chickens in IGF-I levels. Goddard *et al.* (1988) also drew some general conclusions from a review of a number of related studies. Among these was that heavy lines always have lower plasma growth hormone levels regardless of the mode of increased growth (i.e.

selection, introduction of a dwarfing gene, or natural genotype differences). This general relationship does not appear to hold in the present study with mice. Despite large differences in body weight among the three genetic backgrounds, there were no significant differences in plasma growth hormone levels between them. The observed changes in plasma growth hormone level were clearly due to the presence of the high growth gene, and the decrease due to the gene was present even in the growth selected background.

The influence of genetic background on expression of *hg* was noted in this study. The two genetic backgrounds, either selected (line G) or non-selected (C57BL/6J) for high growth, differ significantly from one another on their effects on growth and growth hormone levels in the presence of *hg*. Interestingly, pituitary growth hormone levels that were markedly different at 6 and 9 wk in the control lines with different genetic backgrounds, GH and CH, were very similar in the *hg* lines, Gh and Ch. The lines with *hg* exhibit a marked reduction in pituitary and plasma growth hormone levels that negate the effects of the genetic background genes. The response in IGF-I levels when the *hg* gene was expressed in the two genetic backgrounds showed important differences. In a C57BL/6J background, *hg* led to higher levels at 3, 4·5 and 9 wk lending support to a direct feedback inhibition effect on growth hormone levels. In contrast, in the growth selected background IGF-I was only increased at 6 wk. It is likely that the high growth background is masking effects of the *hg* gene in terms of IGF-I. This is supported by the analysis of IGF-I levels when all lines are adjusted to a common body weight. In this case, IGF-I levels are significantly higher for *hg* mice in the high growth background at 9 wk as well as at 6 wk, suggesting that the *hg* gene in the high growth background may also produce a similar feedback inhibition effect on growth hormone mediated through elevated levels of IGF-I. Alternatively other genetic mechanisms may have evolved in this line as a result of a strong genetic selection that interacts with the *hg* gene in regulating the levels of growth hormone.

The results of this study have characterized important physiological differences in the expression of the high growth gene. The effects of the *hg* gene on growth hormone and IGF-I levels, accompanied by a markedly enhanced growth response represent a unique model for the study of growth regulation. The results suggest that further work is necessary to investigate the genetic and physiological nature of growth hormone and IGF-I regulation, with particular consideration to the interactions of the expression of the *hg* gene in different genetic backgrounds.

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