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Role of growth hormone in the genetic change of mice divergently selected for body weight and fatness

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Summary

To elucidate the involvement of growth hormone (GH) in the genetic change produced by long-term selection in growth and fatness, a 'GH knock-out study' on over 900 mice was undertaken. Lines used had been selected for more than 50 generations for high (PH) and low (PL) body weight (initially protein mass) at 70 d(ays) and for high (F) and low fat content (L) at 98 d, producing a 3-fold difference in body weight and a 5-fold difference in fat content. GH deficiency was achieved by repeated backcrossing into each line a recessive mutant gene (*lit*) which has a defective GH releasing factor receptor. In the absence of GH, the P lines still differ in body weight (21 d to 98 d): e.g. at 98 d homozygous *lit*/*lit*: PH = 24·2 g, PL = 10·0 g; wild-type (*wt*): PH = 57·4 g, PL = 18·7 g. The effect of the GH deficiency on body weight (untransformed) was very much larger in the PH than in the PL line, but the interaction was much smaller, although still significant, on the log scale. This indicates that changes in the GH system contribute only a small part of the selection response in growth. GH deficiency increased fat percentage in all lines (including P), especially in males (99 d, males *lit*/*lit*: $F = 26{\cdot}4\,\%$, $L = 6{\cdot}9\,\%$; *wt*: $F = 22{\cdot}0\,\%$, $L = 4{\cdot}8\,\%$; females: 20·2 %, 5·2 %, 20·7 %, 3·0 %) with significant genotype × line and genotype × sex interactions. The interactions between the effects of the *lit* gene and the genetic background were, however, relatively small compared with these main effects and again indicate that other systems contributed most of the selection response.

1. Introduction

Selected lines of mice provide a unique model for the analysis of the genetic basis of quantitative traits in animals. Although the responses in traits such as body size and fatness are likely to be due to many loci, particular candidate loci or metabolic or hormonal pathways can be investigated to establish whether they are responsible for a substantial part of the genetic change. Their contribution to genetic variation in the trait in the segregating base population from which the selected lines were taken can then be assessed, with the aim of understanding the basis of quantitative genetic variation.

This paper is dedicated to Professor Douglas Falconer, as long time teacher, colleague and friend, for his outstanding contributions to quantitative genetics.

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In this laboratory we have lines of mice which have been selected divergently from the same base population for body weight and for fatness for more than 50 generations. At the age of selection, 70 d(ays), the high body weight line is 3 times as heavy as the low body weight line, but these lines differ little in proportion of fat (Hastings *et al.*, 1993; Bünger & Hill, 1999). The fat line has 5- to 6-fold higher fat percentage of body weight than the lean line at 98 d, this ratio increasing with age, but the lines differ little in fat-free body weight (Hastings *et al.*, 1991). These lines therefore provide very suitable material to investigate the role of candidate genes and pathways.

Growth hormone (GH) deprivation leads to reduced growth, partly due to its deficiency and partly due to consequential deficiency of insulin-like growth factor-I (IGF-I) (e.g. Nantosalonen *et al.*, 1993). GH also has profound effects on body composition, with higher levels promoting leaner animals and vice versa: in mice (Oberbauer *et al.*, 1997), rats (Bates *et al.*, 1993),

man (Fisker *et al*., 1997) and pigs (Pursel & Solomon, 1993). It is therefore likely that some of the selection response in body weight and/or fatness obtained is associated with this axis: animals either releasing 'less/more' or being 'less/more' responsive to GH or other hormones in the pathway.

The first analysis of the contribution of GH to the divergence created by body weight selection was undertaken over 20 years ago by Pidduck & Falconer (1978). They used 'genetic hypophysectomy', introgressing the hypopituitary dwarf gene (*dw*) by repeated backcrossing into their growth-selected high, low and unselected lines. Although this work gave the first insight into the contribution of specific hormones to the selection response, *dw*/*dw* animals also lack other anterior pituitary hormones (Cheng *et al*., 1983). The action of the autosomal recessive *lit* gene (not then available), however, seems to be restricted to GH. It causes GH deprivation due to a defect in the growth hormone releasing factor (GHRF) receptor gene (Lin *et al*., 1993; Chua *et al*., 1993). Homozygotes fail to release significant levels of GH in response to GHRF and are smaller than normal from about 14 d of age, with adult weights about 50–65% of controls.

The *lit* gene was introduced into our selection lines by repeated backcrossing. In previous experiments on our body weight lines, growth of *lit*/*lit* homozygotes on a high line background was more depressed than growth of the low selected lines in absolute terms, but was of similar proportion (Hastings *et al*., 1993). In a later study (Bünger *et al*., 1998*b*), significant differences were also found on the log scale, but they were relatively small. Further, exogenous GH administration to wild-type and to *lit*/*lit* mice gave similar proportionate responses in weight gain in the high and low lines but much larger absolute responses in the *lit*/*lit* animals (Hastings *et al*., 1993). A significant effect of the *lit* gene on fatness and a higher increase of fatness in the high than in the low body weight line were found. However, numbers were small, did not utilize our extreme fat and lean selected lines, and data comprised male body composition at an early age (49 d). Whilst these results suggest that the response to body weight selection is not greatly associated with either production of or receptors to GH, the earlier studies did not address the contribution of GH to the divergence between the fat and lean lines, nor its interaction with age and sex.

The objective of the present study was to test for the contribution of genetic variation in the GH pathway to the divergent selection response for body weight and fat content. After introgression of a null allele at a major locus in the GH pathway by repeated backcrosses into both pairs of selection lines, a check was made as to whether the 'wildtype vs knock-out differences' in body weight and fatness between the high and low line are the same by testing for interaction between GH deprivation and genetic background. The choice of scale for such a comparison is important but not easy; as effects on body mass are typically multiplicative, however, a logarithmic scale seems more appropriate than an arithmetic scale.

2. Materials and methods

(i) *Mouse lines*

Selection lines were initiated in this laboratory from a three-way cross base (two inbred and one outbred line) (Sharp *et al*., 1984). One set of lines (P, or protein lines) were divergently selected for high (PH) and low (PL) lean mass, estimated from an index of body weight and gonadal fat pad weight in males, and in subsequent generations for body weight in both sexes at 70 d of age. From the same base population divergent selection for fat content resulted in fat (F) and lean (L) lines. Selection for the first 20 generations was based on the ratio of gonadal fat pad weight to body weight at 70 d and subsequently on dry matter content of males at 98 d, both criteria strongly correlated with fat content (Hastings & Hill, 1989).

(ii) *Experimental animals*

By repeated backcrossing with progeny testing for each selection line (PH, PL, F and L), lines homozygous for the *lit* gene were produced (*l*PH, *l*PL, *l*F and *l*L). No major problems were encountered in introducing this gene into the PH, PL and F lines (Bootland *et al*., 1991; Hastings *et al*., 1993). Several attempts were necessary before the *lit* gene could be introgressed into the L line, but finally a cross *l*PH males × L females was successful. The last backcross to the PH, PL, F and L selection lines used mice from generations 57, 57, 58 and 62, with 6, 6, 4 and 2 generations of backcrossing, respectively. The resulting animals have an expected proportion of 98%, 98%, 94% and 75%, respectively, of the genotype from the selected line, with most coming from the last few generations of selection. Accidentally one 'runt' animal in line *l*PL was used for reproduction instead of a *lit*/*lit* animal, so that the *lit* gene was still segregating, but only *lit*/*lit* animals were used.

General management. Mice were fed a standard expanded breeding diet (Rat and Mouse No. 3, Special Diet Services, Witham, Essex, UK), containing: digestible crude (dg c) oil, 3·9%; dg c protein, 20·9%; starches, 27·3%; sugars, 11·2%; dg energy, 12·1 MJ/kg) from weaning onwards, and maintained with controlled lighting (12 h light) at a temperature of 21 ± 1 °C. Animals were usually housed after weaning at 21 d in groups of three–eight full sibs, except that, when litters were small, offspring of the

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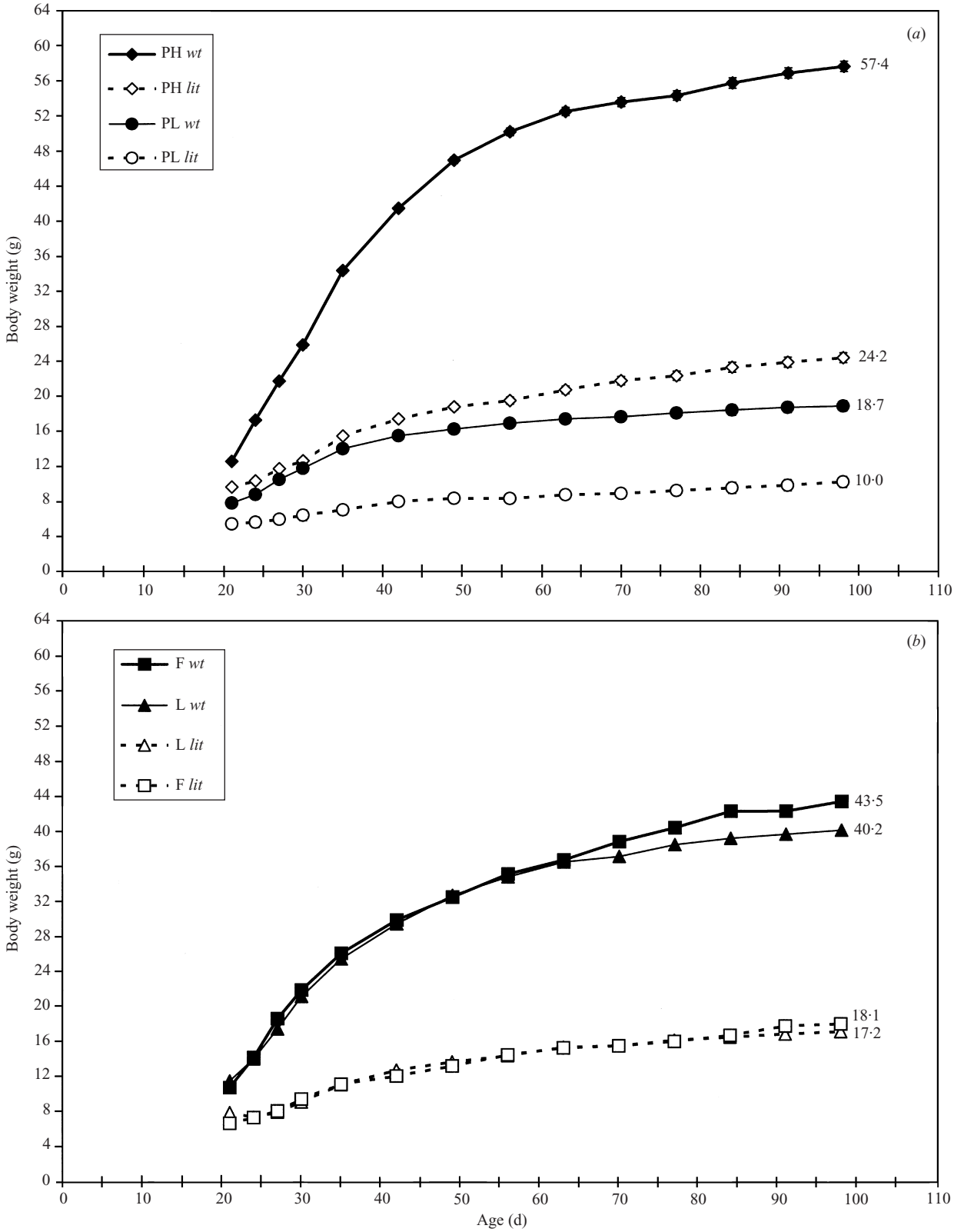

Fig. 1. Body weights for wild-type (*wt*) and homozygous (*lit*) males at each age. (*a*) PH and PL, selected for high and low body weight. (*b*) F and L selected for fatness and leanness.

same sex of two dams were weaned into one cage. After weaning, mice were housed in plastic cages (MB1, Kents Plastics Ltd).

Body weights. In each of the eight lines (four each wild-type (*wt*) and *lit*/*lit*) 16–27 litters were reared. Body weights were taken routinely at 42 d and 70 d in

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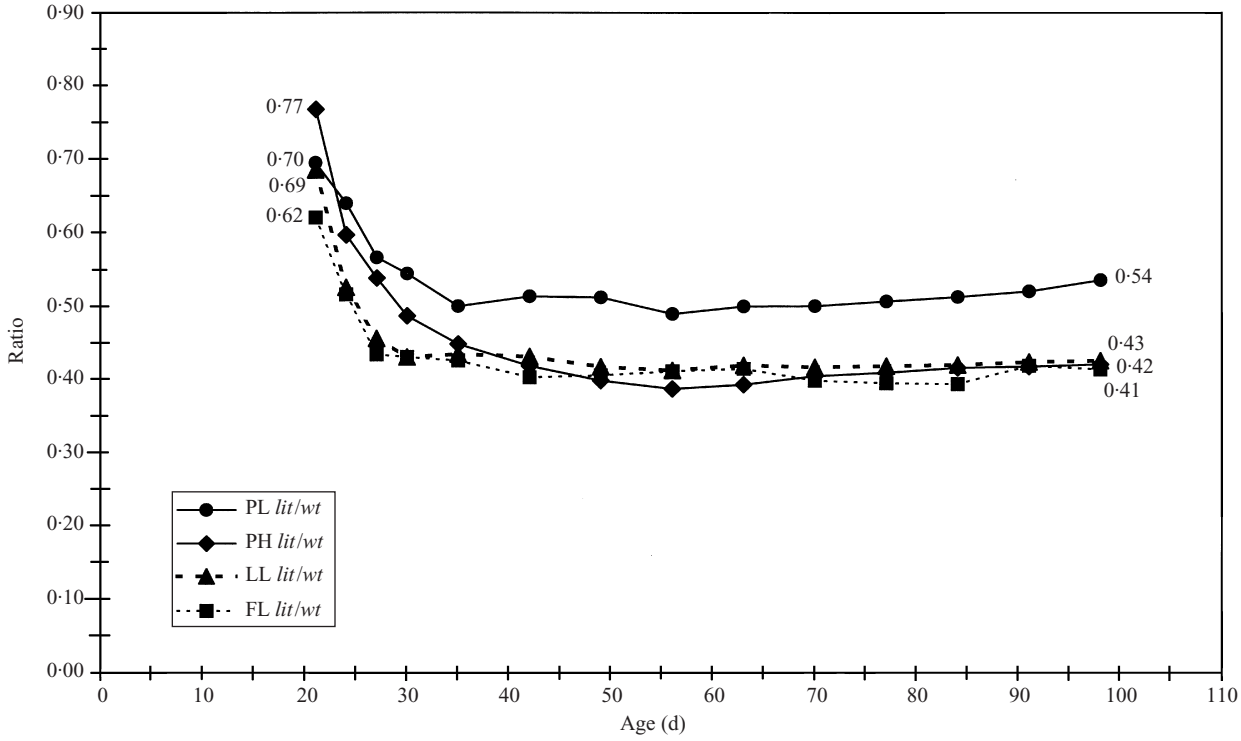

Fig. 2. Body weight of homozygous animals expressed as a ratio of wild-type animals for all selection lines at each age. Data were averaged over sexes.

all lines on all animals. In addition 20 males per line (usually not more than two males from one litter, up to four in *l*PL) were chosen at random, marked and weighed at 21 d, 24 d, 27 d, 30 d, 35 d and later weekly until 98 d. The animals used in this longitudinal study will be referred to as 'recorded' (r), whereas their other full sibs will be denoted 'non-recorded' animals (nr). The recorded animals remained in their full-sib groups and any difference between them is due to handling frequency. Four r males died during the experimental period and their data were excluded.

Body composition. Two hundred and thirty-six nr animals were killed at 71 d and 455 at 99 d (299 nr and all 156 r) by cervical dislocation after fasting for about 18 h. Body weight (BW) was then recorded. Gonadal fat pads of males were removed, weighed and returned to the carcass. The dry matter weight (DM) of the whole body carcass was determined by freeze-drying and used to predict fat percentage (fat %), based on regression of fat % on dry matter content. The regression equation (fat % = 110 × DM/BW − 28·8) was derived from chemical fat analysis of 147 bulk freeze-dried samples (each of 2–5 mice of the same line, sex and age; on average 4·1 mice per sample were ground together) which underwent standard chemical fat extraction. This regression prediction is very similar to one derived previously on wild-type males at 70 d (fat % = 113 × DM/BW − 30·2; Hastings & Hill, 1989). A linear regression (intercept and slope) was also fitted separately to both the *lit*/*lit* and *wt* animals. Although these fitted significantly better than a single regression equation, the correlation coefficient of the common regression was 0·968; and at the ends of the range of DM/BW (0·30 and 0·53), the predicted fat % differed by only 1·4 % and 1·6 % respectively. Therefore only a single regression line was used.

(iii) *Data analysis*

Data on body weights at 42 d, 70 d and 98 d for all (r plus nr) males and females were analysed using the following model:

$$Y = M + G + L + S + R + LG + LS + GS + GSL + F(L,G) + e,$$

where M is an overall mean, G (1–2) is the effect of genotype (*lit*/*lit* vs *wt*), L is a selection line effect (1–4), S (1–2) is the sex effect, R (1–2) is the effect of recording an animal, LG, LS, GS and GSL are interactions, $F(L,G)$ is a family within-line and genotype effect, and e is the residual error. All effects were fitted as fixed except $F(L,G)$ and e, fitted as random. Effects L, G and LG were tested against $F(L,G)$, and the rest were tested against the error term. Body weight data on r males at each age were

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analysed with the same model, but with *S*, *R* and the corresponding interactions excluded. Body composition data were also analysed with basically the same model, except that data at both ages were included and corresponding fixed effects (70 d, 98 d) fitted. ANOVA was undertaken using the GLM procedure of the SAS System for Windows release 6·08 (SAS Institute, Cary, NC 27513, USA).

Data were analysed using untransformed data and also, where there was an indication or expectation of a multiplicative scale effect, using a natural log transformation.

3. Results

(i) *Body weight*

In all lines GH deprivation had a dramatic effect on growth (Fig. 1). Whereas *wt* PH and *wt* PL differ at weaning by a factor of 1·6, this ratio increases to about 3 from approximately 60 d. The GH-deprived groups still differ to a large extent, but the ratio (PH/PL) of weights is smaller, reaching 2·5 at most, reflecting the stronger growth depression of GH deprivation in the high body weight line. Homozygous *lit*/*lit* PL animals reach only 10 g on average at 98 d and were still growing very slowly when the experiment was terminated. Although *wt* F are clearly heavier than *wt* L, especially after about 65 d when there is continuing fat deposition in the F line, there are only small differences when the lines are GH deficient.

Homozygous *lit*/*lit* animals at weaning at 21 d weighed 62–77% as much as *wt* animals, but this percentage decreased to 41–54% at 98 d (Fig. 2). The growth depression at weaning was lowest in the PH line and highest in the F line; soon afterwards, the only striking line difference was between PL and the other three lines. These results were confirmed by the analysis of all males at 42 d, 70 d and 98 d. A similar picture emerged from the analysis of body weights of all females at these ages, where PL females showed the lowest growth depression (by 40%) followed by the L females (55%) (Table 1).

Analysis of variance at each age point using the log-transformed data of all r males found large effects of line and genotype. The interaction between line and genotype does not reach significance before 42 d but persists thereafter (not shown). Using log-transformed data of all animals ($n = 987$) this interaction was significant from 42 d ($P < 0{\cdot}001$), as was the sex by genotype interaction ($P < 0{\cdot}001$) (Table 2). Analysis of variance of untransformed data (not shown) gave the same significance for the main effects and interactions as for log-transformed data, but F ratios were generally larger and they were significant for genotype × sex at all ages.

Table 1. *Least square means and their average standard error (SE) for body weights (BW, g) of all animals at 42, 70 and 98 days (d)*

	Males								Females							
	42 d			70 d			98 d		42 d			70 d			98 d	
	n	BW	log BW	*n*	BW	log BW	*n*	BW	log BW	*n*	BW	log BW	*n*	BW	log BW	*n*	BW	log BW
PH *wt*	81	41·1	3·71	70	54·0	3·98	31	56·7	4·04	79	33·7	3·50	66	46·0	3·82	25	52·1	3·94
PH *lit*	69	17·4	2·85	65	21·4	3·06	39	24·6	3·20	58	16·0	2·76	56	18·1	2·89	35	20·7	3·01
PL *wt*	103	15·0	2·71	98	17·2	2·84	34	18·8	2·93	78	12·2	2·50	73	13·6	2·61	25	15·3	2·72
PL *lit*	22	8·2	2·10	22	8·7	2·14	21	10·0	2·29	21	7·2	1·97	15	8·1	2·09	15	9·2	2·21
F *wt*	93	29·7	3·38	84	38·9	3·65	24	43·6	3·76	78	25·6	3·23	62	33·8	3·50	21	37·5	3·59
F *lit*	39	12·3	2·50	42	15·5	2·73	31	18·5	2·90	45	11·6	2·45	42	13·7	2·61	26	15·3	2·71
L *wt*	52	29·8	3·39	24	37·3	3·62	32	40·5	3·70	41	24·8	3·21	4	28·2	3·35	27	35·3	3·55
L *lit*	64	13·4	2·58	60	15·8	2·76	36	17·2	2·84	64	12·1	2·49	64	14·1	2·64	44	15·8	2·75
Sums/*SE*	**523**	*0·32*	*0·013*	**465**	*0·67*	*0·022*	**248**	*0·73*	0·025	**464**	*0·29*	*0·012*	**382**	*0·44*	*0·015*	**218**	*0·59*	*0·021*

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Table 2. *ANOVA results on log-transformed body weight data at 42, 70 and 98 days (d)*

	d.f.	42 d MS	70 d MS	98 d MS
L	3	19·4123***	22·0512***	12·4535***
G	1	85·4396***	43·4719***	46·2544***
LG	3	0·4236***	0·5746***	0·3658***
F(*L*, *G*)	151[a]	0·0387***	0·0398***	0·0364***
S	1	2·2026***	1·4672***	0·9658***
LS	3	0·0264**	0·0141	0·0139
GS	1	0·3470***	0·1335***	0·0083
LGS	3	0·0021	0·0435***	0·0460**
R	1	0·0348*	0·0154	0·0416*
Error	815[a]	0·00619	0·00822	0·00958

Effects *L*, *G* and *LG* were tested against *F*(*L*, *G*), and the rest were tested against the error term.
***$P < 0{\cdot}001$; **$P < 0{\cdot}01$; *$P < 0{\cdot}05$.
[a] d.f. given for 42 d; d.f. for F (L, G) at 70 d and 98 d were 138 and 102, respectively; and for Error were 692 and 347, respectively.

(ii) *Body composition*

Fasted body weight and fat-free body weight. In the longitudinal study on non-fasted males, the lowest depression in weight was found in *l*PL males, compared with their *wt* controls (Fig. 1*a*). A very similar picture emerged for the fasted body weights of males and females at 99 d. Males and females of *l*PL showed the lowest growth depression, by 44% and 40%, respectively, whilst the reduction in the other lines was between 55% and 60%. As numbers of *l*PL males were low, none were dissected at 71 d, making a line comparison difficult, but it is of note that at 71 d the L males and females showed a low growth depression as well. Changes in fat-free body weight (ffBW) are not confounded with changes in fat and a similar situation was found for ffBW as for the weight at 99 d, with a lower depression for *l*PL males and females than for the other lines (Table 3). Genotype, line and sex effects were significant ($P < 0{\cdot}001$), as were the interactions $G \times L$ and $G \times S$ in transformed and untransformed data (Table 4).

Fatness. In general *lit* mice had a higher fat percentage (fat%) than *wt* mice ($P < 0{\cdot}001$), and males responded more than females in fatness ($G \times S$, $P < 0{\cdot}001$). GH-deficient *lit* males were on average about 4·5% fatter at both ages than *wt* males, whereas the differences for females were about 1·8% and 1·3% at 70 d and 98 d (Table 3). There were also significant line × genotype interactions in the log-transformed data (*LG*, $P < 0{\cdot}01$), males from both P lines reacting more strongly than those of the other lines. Males at both ages were fatter than females (*S*, $P < 0{\cdot}001$), more so in the GH-deficient groups (*GS*,

Table 3. *Least square means (LSM) and their averaged standard errors (SE) for predicted body composition traits at 71 and 99 days*

	Males (least square means)							Females (least square means)					
	n	BW (g)	Fat (g)	ffBW (g)	DM/BW	Fat[a] (%)	GFPW/BW (mg/g)	*n*	BW (g)	Fat (g)	ffBW (g)	DM/BW	Fat[a] (%)
At 71 days													
PH *wt*	20	43·4	2·27	41·2	0·307	5·0	0·78	17	35·1	1·37	33·7	0·296	3·8
PH *lit*	16	19·0	2·73	16·2	0·387	13·8	1·31	19	16·0	1·26	14·8	0·330	7·5
PL *wt*	19	17·1	0·74	16·3	0·305	4·7	0·30	13	13·9	0·52	13·4	0·301	4·3
PL *lit*	0							6	7·0	0·25	6·8	0·313	5·6
F *wt*	17	36·2	7·20	29·0	0·431	18·6	3·62	18	29·9	5·72	24·2	0·423	17·7
F *lit*	10	14·2	2·74	11·5	0·442	19·8	2·81	20	11·8	1·92	9·8	0·422	17·7
L *wt*	12	30·1	0·91	29·2	0·289	3·0	−0·01[b]	15	26·0	0·66	25·3	0·286	2·7
L *lit*	18	14·4	0·97	13·4	0·324	6·8	0·50	16	12·9	0·61	12·3	0·306	4·8
Sums/SE	**112**	*1·2*	*0·56*	*0·86*	*0·015*	*1·6*	*0·26*	**124**	*1·3*	*0·59*	*0·91*	*0·015*	*1·7*
At 99 days													
PH *wt*	34	53·0	3·69	49·3	0·330	7·5	1·31	25	48·5	4·36	44·2	0·342	8·8
PH *lit*	40	22·3	3·23	19·1	0·388	13·9	1·81	27	19·4	2·12	17·2	0·356	10·3
PL *wt*	34	16·2	1·16	15·0	0·323	6·7	0·57	25	13·2	0·63	12·5	0·307	5·0
PL *lit*	21	9·0	1·37	7·6	0·378	12·8	1·25	15	7·9	0·55	7·3	0·326	7·1
F *wt*	28	39·2	8·63	30·6	0·462	22·0	4·40	25	34·4	7·55	26·9	0·450	20·7
F *lit*	30	17·3	4·70	12·6	0·502	26·4	3·57	24	14·4	3·12	11·3	0·446	20·2
L *wt*	33	37·1	1·69	35·5	0·306	4·8	0·49	27	32·2	1·00	31·2	0·289	3·0
L *lit*	38	15·2	1·12	14·0	0·325	6·9	0·59	29	13·6	0·69	12·9	0·309	5·2
Sums/SE	**258**	*0·68*	*0·31*	*0·49*	*0·008*	*0·91*	*0·14*	**197**	*0·79*	*0·37*	*0·57*	*0·010*	*1·1*

BW, body weight (after overnight fasting); DM, dry matter; GFPW, gonadal fat pad weight; ffBW, fat-free body weight.
[a] Fat percentage is predicted from fat% = 110 × DM/BW − 28·8 and used to calculate fat (g) and ffBW.
[b] Note LSM with very unbalanced family sizes.

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Table 4. *ANOVA results on log transformed body composition traits and untransformed fat percentage, ages (A) combined*

	d.f.	Fat % MS	Log 'fat (%)' MS	Log 'fat (g)' MS	Log 'ffBW (g)' MS
L	3	5577·3***	48·973***	59·800***	5·4230***
G	1	589·9***	9·552***	9·198***	40·8421***
LG	3	53·1	1·362**	1·977**	0·1685***
F(*L*, *G*)	118	32·4***	0·285***	0·433***	0·0263***
S	1	440·5***	4·652***	12·981***	1·4242***
A	1	73·3*	0·508	1·491**	0·1592***
R	1	117·5**	0·934*	1·461**	0·0140
LS	3	12·1	0·117	0·097	0·0059
GS	1	245·7***	1·532**	1·233*	0·1061***
LA	3	29·5	0·124	0·183	0·0223*
GA	1	0·4	0·104	0·145	0·0039
SA	1	2·3	0·086	0·163	0·0141
LGS	3	27·6	0·174	0·202	0·0116
LGA	3	4·7	0·054	0·127	0·0287**
LGS	3	27·6	0·174	0·202	0·0116
LSA	3	38·2*	0·771**	0·868**	0·0005
GSA	1	2·0	0·001	0·002	0·0045
LGSA	2	14·5	0·703*	0·851*	0·0043
Error	541	12·13	0·1779	0·2150	0·00638

Effects *L*, *G* and *LG* were tested against *F*(*L*, *G*), and the rest against error.
***$P < 0{\cdot}001$; **$P < 0{\cdot}01$; *$P < 0{\cdot}05$.

$P < 0{\cdot}001$). It is striking that there is no obvious change in fat percentage in F line females at either age when GH deprived. The ratio of gonadal fat pad weight (GFPW) to BW of males indicates an increase in fatness in GH-deprived animals. Whereas this ratio increased in *lit*/*lit* at 70 d and 98 d in *l*PH, *l*PL and in *l*L, it decreased significantly in *l*F males at both ages (Table 3), in contrast to fat estimated from dry matter content which also increased in that line. A high correlation between GFPW/BW and fat % at 99 d was found in both *lit*/*lit* (0.95) and *wt* (0·92) males, however, with a higher slope in the *lit* animals and a very similar picture at 70 d.

In general the total amount of fat (fat g) in the body was lower in the *lit* than the *wt* animals (*G*, $P < 0{\cdot}001$), but there were large line and sex differences. Substantial reductions in fat occurred in the F line, *l*F animals aggregating only 34–54 % of the fat amount of *wt* animals. In all other lines the reduction was very much smaller (*LG*, $P < 0{\cdot}01$) and in some cases GH-deficient mice aggregated slightly more fat than *wt* animals (Table 3).

4. Discussion

Genetic variation for such traits as body weight and fatness is ubiquitous, and provides the primary source for response to artificial selection and for evolutionary adaptation. However, the nature of the genes underlying quantitative trait variation is still poorly understood. A complete description of the genetic differentiation between two lines divergently selected on a quantitative trait would comprise a complete list of the genes involved, their effects and interactions – at present unattainable. 'Candidate genes', identified through their major effects on phenotypes, are known to contribute to variation, e.g. in bristle number in *Drosophila* (Mackay, 1996). However, the extent to which candidate genes that may have large effects contribute to variation for complex traits such as growth and fatness remains unknown (Keightley *et al*., 1998). The method employed for the first time by Pidduck & Falconer (1978) of knocking out candidate genes and thereby related metabolic pathways by an introgression of known mutations or of specially constructed transgenes by repeated backcrosses seems a powerful way to elucidate their role in selection responses.

Scale. The conclusions drawn from such an approach will largely depend from the scale on which the traits are measured as the most important interaction in this study, genotype by line, is much smaller on the log scale than on the arithmetic scale relative to the main effects. As growth is a geometrical rather than an arithmetical process, a geometrical scale appears to be the most 'natural' for body weights (Falconer & Mackay, 1996) and fat amount. As fat percentage is itself a ratio we focused on untransformed values but showed also the results for log-transformed values.

Body weight. GH seems to be an obvious candidate pathway contributing to genetic change in growth of mice due to selection on body weight. Its predominant

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role in postnatal growth is evident in studies on GH-deficient mutant mice (e.g. Cheng *et al*., 1983). Among these mutants, those such as *lit* which primarily affect a single hormone are of particular value.

In this experiment we found indication that genetic changes in the GH axis are involved in selection response for body weight, but, although significant, only a small part of the total variance was attributable to line × *lit* gene interaction. The ratio of PH to PL for *wt* and *lit* for body weight at 98 d was 3·1 and 2·4, respectively, from which it can be concluded that about 23% of the total response is due to the GH system. This is in agreement with earlier findings for these lines using *lit* (Bünger *et al*., 1998*b*) and in other lines using *dw* (Pidduck & Falconer, 1978). Hastings *et al*. (1993) found a stronger weight reduction with the *lit*/*lit* on the high (PH) than low (PL) selected background for body weight at 28 d and 49 d but the interaction between genetic status and line was small and non-significant for log-transformed body weights. Because of some differences in the methodology it is difficult to explain this small discrepancy, but in the present study the *F* value for the interaction term increased from 21 d to about 50 d of age, when the earlier study ended.

Eisen *et al*. (1993) made a single cross between males, hemizygous for a dwarf mutant bovine growth hormone transgene (acting as a GH antagonist) and females of a high-growth selected and a control line. The mutant gene had a slightly but significantly, greater effect in the selection line.

Previously we investigated the implications of genetical thyroid ablation on the same set of lines as in this study, which caused a deficiency of both thyroxine and GH (Bünger *et al*., 1998*b*). The growth depression in the L line was exceptionally low, a result not confirmed by the present study. The reasons remain unclear and may lay in methodological differences (e.g. only two backcross generations were made for the *l*L line and it was GH but not thyroxine deprived). In general, however, thyroid ablation on body weights led to the same conclusion.

In summary, differences in the GH axis are obviously not the sole cause of the observed divergence in body weight produced by selection, because lines differ also when GH-deficient. The presence of a relatively small although significant interaction between gene action and genetic background on log transformed data implies some GH involvement, but not in the major way that might be expected from its significance for postnatal growth.

Body composition. GH has profound effects on body composition in mammals, higher GH values promoting leaner animals and vice versa, so some of the selection response in fatness could be associated with the GH axis. In earlier backcross generations of *lit* into the P-lines, different increases in fat proportion on 49-day-old males were found (Hastings *et al*., 1993). In the present experiment an increased fat percentage in mice with GH deficiency was found at the later ages (70 d and 98 d) investigated. Mice with a combined thyroxine and GH deficiency (Bünger *et al*., 1998*b*), however, showed a similar growth depression at the end of the experiment (at nearly 100 d) but were leaner than wild-type.

Significant line × genotype and genotype × sex interactions for fat percentage (log-transformed) were found in the present study, but both interactions were small compared with the corresponding main effects. However, the exceptional behaviour of the F line is of note. The fat percentage in GH-deprived males of all other lines increased by a factor of 1·4–2·8 but only by 1·1–1·2 in F males; and GFPW/BW ratio increased by a factor of 1·2–2·2 in the other lines but decreased by a factor of 0·8 in the F line. Fat percentage in females showed a similar but less pronounced picture, with no increase in fat percentage in GH deprived F females. The high overall fatness in F animals therefore seems relatively independent of the GH axis. Long-term selection on fatness might therefore have resulted in a GH decrease in the F lines selected for fatness (as found in fat-selected sheep: Francis *et al*., 1998) and/or loss of sensitivity to its action. Proof of this hypothesis would need data on levels of GH and GH receptor or insulin-like growth factor (IGF) and its binding protein (IGFBP). In an earlier study at generation 20, where the line divergence was very much smaller, there were no significant differences in IGF-1 levels between the F and L lines (McKnight & Goddard, 1989).

There are some puzzles about GH in our context. From what is known about the predominant role of GH in postnatal growth and in fatness, one could expect: (i) higher GH levels and/or GH sensitivity in lines with higher growth, and (ii), because higher GH level oppose fatness, heavier lines would be leaner.

Considering (i), large lines of mice might have markedly lower plasma GH values, as in mice carrying the recessive *hg* mutation, but these animals were shown to have higher plasma IGF-1 levels (Medrano *et al*., 1991). Similar results were found in divergently growth selected pigs (Norton *et al*., 1989). As the physiological mode of GH secretion is highly pulsatile, with frequency and amplitude as signalling elements (Waxman *et al*., 1991), its full characterization is complicated. In addition, as many actions of GH are mediated by IGF-1, the measurement of IGF and IGFBP seems a good alternative. Using mice from earlier generations of our lines (McKnight & Goddard, 1989) it was found that PH mice had 22% higher basal IGF-1 concentrations at 70 d than PL mice (and 60% higher body weight). Similar results were found in mice from other low, control and a high body

weight selection lines, with respective mean male BW at 56 d (selection age) of 19, 31 and 54 g, where serum IGF-1 concentrations were 290, 450 and 600 ng/ml, respectively (Höflich *et al*., 1998).

It has been argued that the growth promotion of the *hg* mutation occurs through an IGF-1 mediated process, 'independently' of GH or by inhibiting directly or indirectly its expression by negative feedback (Medrano *et al*., 1991). This illustrates the complexity of growth regulation, through hypothalamic releasing and inhibiting hormones, pituitary synthesis and secretion of GH, IGF and IGFBP and their interactions. Allelic differences in any of these genes could give changes in body weight.

Now let us consider (ii), i.e. whether higher GH or IGF-1 levels oppose fatness and therefore heavier lines are leaner. Selection for body weight is usually accompanied by an increase in fatness (reviewed by McCarthy, 1982) but there are a few exceptions, however, including our P-lines (e.g. Bünger *et al*., 1998 *a*, *b*), probably due to the initial 20 generations of selection on lean mass and the relatively high age at selection (70 d). Another high body weight line with increased fatness also had increased IGF-1 levels (Höflich *et al*., 1998).

Administration of exogenous GH to wild-type PH and PL mice did not reduce fatness in either line (Hastings *et al*., 1993), but these are relatively lean lines with only 7% fat at around 100 d. GH treatment reduced the fatness in GH-deficient homozygous *lit* mice of both lines, counteracting the fatness caused by GH deficiency. Similar results have been reported in humans where fatness was reduced in adult GH-deficient patients by GH treatment (Fisker *et al*., 1997). Treatment of growing pigs with porcine GH markedly stimulated muscle growth and reduced fat deposition (reviewed by Etherton & Bauman, 1998) and neither bovine nor human GH transgenic pigs showed the enhanced growth phenotype found in mice (reviewed by Kopchick & Cioffi, 1991), but were much leaner. Transgenic mice with GH overproduction tend to be leaner, but effects seem to be age or body weight dependent (Pomp *et al*., 1992). Thus, all results indicate that GH deficiency leads to a substantial increase in fatness, but increased GH or IGF-1 levels do not always counteract fatness.

Although our results indicate that GH is involved in the selection response in the F line they do not suggest that variation in the GH axis related genes accounts for a very high proportion of the observed selection responses, and support a polygenic model of selection response. Further experiments are needed to assess the contribution of other known players in fat metabolism such as leptin (reviewed by Friedman & Halaas, 1998). Leptin administered to fat line (F) and control males reduced fatness in both lines by a similar extent (Bünger & Hill, 1997). Circulating leptin levels, however, were 60- to 300-fold higher in the F than L lines (Bünger *et al*., 1999*b*). It would therefore be informative to introgress into the F and L lines genes which knock out leptin production (e.g. Lep^{ob}) and reception (e.g. $Lepr^{db}$) and assess their contribution to observed line differences.

Candidate gene approach. Overall we have to ask whether the candidate gene approach is a very useful tool in identifying loci contributing to variation in natural or domesticated populations – an area which is very poorly understood. Although there are indications that in natural populations variation is due to alleles at loci having major effects (e.g. Mackay, 1996), this is not strongly supported by this study directed at a metabolic pathway not at a specific locus, but it has been possible to quantify the contribution of this pathway to the observed response or variation and further experiments can then be focused on its individual elements.

Without prior knowledge of changes in specific hormone or hormone receptor levels, however, a candidate gene approach resembles an attempt to find a needle in a haystack. It may therefore be necessary to consider one pathway after another, perhaps in combination, as interactions among the pathways are taken into account. In the present case, for example, we have to consider hormones such as IGF-1 when analysing the effects of GH. Granted the validity of the approach, however, selection lines are a powerful resource for finding hormonal differences as the large line differences produced may provide a strong magnet for the needle. The approach can narrow down the changes to one or a few genes, whereas coarse QTL mapping leads only to a region. Therefore, mapping studies and experiments using a candidate gene approach should be used in a synergistic way towards the goal of identifying and locating genes affecting quantitative traits.

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