*Genet. Res., Camb.* (1989), **53**, *pp.* 187–191 *With 2 text-figures Printed in Great Britain* 

# Responses to divergent selection for plasma concentrations of insulin-like growth factor-1 in mice

H. T. BLAIR,† S. N. McCUTCHEON,[1] D. D. S. MACKENZIE,[1] P. D. GLUCKMAN,[2] J. E. ORMSBY[3] AND B. H. BRIER[2]

[1] *Department of Animal Science, Massey University, Palmerston North, New Zealand*
[2] *Department of Paediatrics, University of Auckland Medical School, Auckland, New Zealand*
[3] *Small Animal Production Unit, Massey University, Palmerston North, New Zealand*

(*Received 6 January 1988 and in revised form 29 November 1988*)

## Summary

A divergent selection experiment with mice, using plasma concentrations of insulin-like growth factor-1 (IGF-1) at 42 days of age as the selection criterion, was undertaken for 7 generations. Lines were not replicated. To obtain sufficient plasma for the IGF-1 assay, blood from four individuals was volumetrically bulked to obtain a litter mean IGF-1 concentration. This necessitated the use of between family selection. Although inbreeding accumulated in a linear fashion in each of the high, control and low lines, the rates were different for each line (3·6, 1·6 and 5·3% per generation for the high, control and low lines, respectively). As a consequence, the effects of selection and inbreeding are confounded in this experiment. Divergence between the high and low lines in plasma concentrations of IGF-1 continued steadily until generation 5. In generations 6 and 7, there was a reduced degree of divergence and this contributed towards the low realized heritability value of $0.15 \pm 0.12$. Six-week liveweight showed a steady positive correlated response to selection for or against plasma concentrations of IGF-1 until generation 4 (high-low difference = 1·7 g = 12%). In generation 5, a substantial drop in 6-week liveweight in the low line relative to both the high and control lines occurred (high-low difference, 3·9; g, 25%). This difference was maintained until generation 7.

This experiment suggests that genetic variation exists at 6 weeks of age in plasma concentrations of IGF-1 in mice. Furthermore, genetic covariation between plasma IGF-1 concentrations and liveweight at 6 weeks of age is likely to be positive. Further experiments have been initiated to examine these theories.

## 1. Introduction

Intensive research during the 1980s has investigated the role of insulin-like growth factor-1 (IGF-1) in the somatotrophic axis (Hall & Sara, 1983; Froesch *et al.* 1985; Breier *et al.* 1986) and other physiological functions such as reproduction (Adashi *et al.* 1985) and lactation (Gluckman *et al.* 1987). The possible involvement of IGF-1 in several important production traits, including liveweight gain, reproductive efficiency and milk production, has led to interest in its potential as a selection criterion.

Blair *et al.* (1987) reported a number of non-genetic factors affecting plasma IGF-1 concentrations which, in order to maximize the rate of genetic gain, would need to be corrected for prior to ranking animals for selection. They also reported a moderate heritability estimate (0·40) for plasma concentrations of IGF-1 at about 6 weeks of age in mice, suggesting that genetic variation exists for this trait. Phenotypic correlations between plasma concentrations of IGF-1 and liveweight at 6 weeks of age were positive and generally high in the above trial.

In an effort to provide more information about the level of genetic variation in plasma concentrations of IGF-1, and the degree of genetic covariation with other traits, a mouse selection experiment was initiated in 1985. This paper reports on direct responses to selection for plasma concentrations of IGF-1 at 6 weeks of age and correlated responses in 6-week liveweight.

## 2. Materials and methods

Four inbred strains of mice maintained at the Massey University Small Animal Production Unit (SAPU)

† Corresponding author.

were crossed and interbred to provide genetic variation in the base population. These strains, and the period for which they have been inbred at the SAPU, are 'NOS' or 'White mice' (Flux, 1957), CHI (Flux & Munford, 1957) and TSA (Cockrem, 1959), 30 years; and C3H/He (Anon, 1987), 5 years. All mice were housed in one room with a constant light to dark ratio of 14 h:10 h and a temperature of about 20 °C. A complete pelleted diet was fed *ad libitum*. After blood sampling and weighing of pups in generation 4, 3% tallow was added to soften the pellets. Analysis of the diets showed that addition of the tallow increased digestible energy content by only 4·5% (which is within the normal range of between-batch variation).

Three lines were established in August 1985 by random allocation of males and females from a common base population. Two lines were subjected to divergent selection for plasma concentrations of IGF-1 and the third was maintained as a randomly bred control. Animals were used for breeding only once, resulting in discrete generations. Each of the selection lines was maintained at 20 females and 10 males while the control line consisted of 10 pairs. Mating of full-sibs was avoided. In order to maximize the effective population size in the control line, one male and one female were randomly chosen from each litter to become the replacement breeding stock for the following generation. Furthermore, a cyclical pattern of mating was employed to reduce the rate of accumulation of inbreeding. The lines were not replicated.

Selection was based on IGF-1 levels estimated from blood samples which were pooled within litters. Six individuals per litter were sampled in generation 0 and four in subsequent generations. Pooled, rather than individual, samples were used to minimize the effects of blood sampling on health and growth of the mice (low-line mice with weaning weights as low as 10 g have been observed and the 200 $\mu$l blood sample required for the assay would represent about 30 of their total blood volume). Because IGF-1 levels in mice are influenced by sex (Blair *et al.* 1987), equal numbers of males and females were sampled where possible. Blood sampling was carried out between 09.00 and 13.00 h. Blood was obtained by tail-snipping after anaesthetizing the mice with ether. Plasma was prepared from heparinized blood and stored at −20 °C. IGF-1 concentrations were measured by radioimmunoassay following acid-ethanol extraction using the procedure of Gluckman and Butler (1983). Intra- and inter-assay coefficients of variation were 5·0 and 9·8%, respectively.

Selection of breeding stock for the high (low) line was based on those litters having the highest (lowest) adjusted means for IGF-1 levels. IGF-1 concentrations were adjusted for the number of pups weaned (NPW) and age at sampling.

Analysis of litter mean IGF-1 concentrations was undertaken by applying a linear model with line as a fixed effect and a number of pups weaned (NPW) and age at sampling as covariates. Liveweight was analysed using a similar linear model, with sex included as an additional fixed effect. Due to the unbalanced nature of the data, line was always fitted last, to enable comparisons between lines to be free from sex, NPW or age biases. All analyses were undertaken using the statistical package REG (Gilmour, 1985).

Selection differentials for IGF-1 concentrations were calculated by deviating the weighted average of the litters providing parents of the next generation from the simple generation mean IGF-1 concentration. The weighting of litter mean IGF-1 concentration by the number of parents provided to the next generation accounted for (*a*) unequal litter sizes, and (*b*) partial selection of the lowest ranked litter providing parents.

Conversion of the components of the formula presented by Hill (1972) to those relevant for family selection allowed the approximation of a standard error for the realized heritability which included a random genetic drift component.

## 3. Results

### (i) *Inbreeding*

The regression of average inbreeding on generation yielded rates of increase in breeding of 3·6, 1·6 and 5·3% per generation for the high, control and low lines, respectively, assuming zero inbreeding at generation 0. The different rates at which inbreeding has accumulated in the 3 lines could potentially have interfered with the rates at which changes due to selection accrued. For example, Falconer (1981) noted that, in mice, 6-week liveweight could be depressed by 0.58 g per 10% increase in inbreeding. Since the effects of inbreeding were completely confounded with the effects of selection in this experiment, it was not possible to separate changes due to selection from those due to inbreeding.

### (ii) *Direct response*

The mean IGF-1 concentrations for each line within generation are presented in Table 1. There was considerable between-generation fluctuation in the IGF-1 concentrations and this could not be accounted for by assay drift. Of particular concern is that values for the control line did not appear to vary in a similar fashion to those of the high and low lines. Therefore, direct response to selection in IGF-1 concentrations was calculated as the difference between high and low line means (Fig. 1). Under these circumstances, it was not possible to determine whether response was symmetrical or asymmetrical. However, it is clear that response to selection continued until generation 5. The reduced response in generations 6 and 7 could reflect reduced levels of genetic variation in either or

Table 1. *Adjusted[a] means (±S.E.) by line and generation for 6-week plasma IGF-1 concentrations and 6-week liveweights*

| | IGF-1 concentration (ng/ml) | | | Liveweight (g) | | |
|---|---|---|---|---|---|---|
| Generation | High | Control | Low | High | Control | Low |
| 0 | 132±4 | 125±5 | 131±4 | 21·6±0·2 | 21·2±0·2 | 21·9±0·2 |
| 1 | 102±5 | na | 94±5 | 20·8±0·2 | 20·2±0·2 | 19·5±0·2 |
| 2 | 105±3 | 76±5 | 83±3 | 19·6±0·2 | 18·5±0·2 | 17·3±0·2 |
| 3 | 137±5 | 133±7 | 120±5 | 18·1±0·2 | 16·8±0·2 | 15·5±0·2 |
| 4 | 88±9 | 87±10 | 65±9 | 15·8±0·4 | 15·1±0·4 | 14·1±0·4 |
| 5 | 146±4 | 114±5 | 113±4 | 19·5±0·2 | 19·1±0·3 | 15·6±0·3 |
| 6 | 102±3 | 100±4 | 80±4 | 21·9±0·2 | 21·4±0·2 | 17·2±0·2 |
| 7 | 85±2 | 72±3 | 58±2 | 22·5±0·2 | 21·3±0·2 | 18·5±0·2 |

[a] Adjusted for age at weighing/sampling and litter size; liveweights also adjusted for sex.

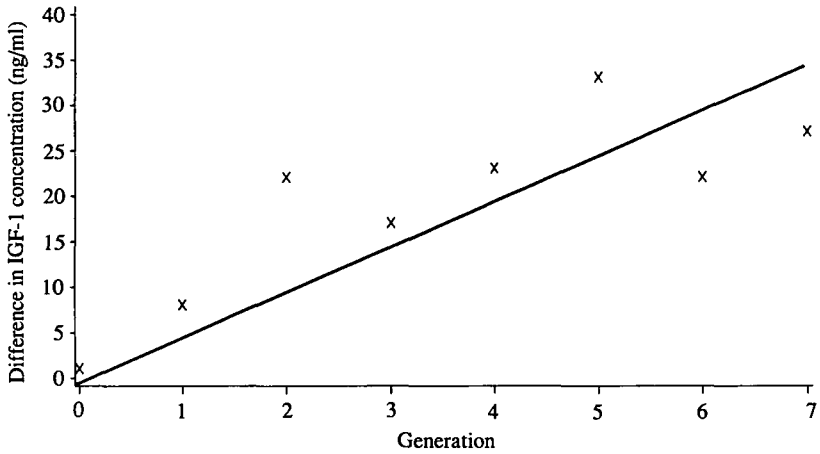


Fig. 1. Differences between high and low line IGF-1 concentrations by generation and the regression fitted through the origin ($b = 4{\cdot}86$).

both lines due to selection or inbreeding. Alternatively, the reduced response could be caused by the inefficiency of family selection. It is unlikely that the change in feed pellet composition after generation 4 contributed to the reduced response, since further divergence occurred in generation 5. The average rates of accumulation of selection differential per generation were 16·0 ng/ml in the high line and −15·7 ng/ml in the low line. There was no indication of a decline in the selection differentials in later generations. Since generations were discrete in this trial, cumulative selection differentials were obtained by adding successive selection differentials together. A realized heritability of 0·15±0·12 based on response to family selection was generated by regressing the divergence between the high and low lines on the cumulative selection differential. However, this value would be significantly affected by the poor responses found in the last two generations. Since the reason for the decline in response is unknown, this realized heritability estimate must be treated with caution. Furthermore, the use of family selection contributed to a high standard error via random genetic drift.

(iii) *Correlated response in 6-week liveweight*

Mean adjusted 6-week liveweights for the various generations are presented in Table 1. Perusal of the generation means reveals a downward trend in liveweights until generation 4. From generation 5 onwards, liveweights steadily improved until the high and control lines returned to weights similar to those obtained at the outset of the experiment. The reason for these changes is unknown; the consistency of change does not suggest a nutritional effect (although a diet modification occurred after generation 4), and the steady increase in mean liveweight in generations 5–7 is the opposite of what would be expected due to inbreeding.

The divergence of the high and low lines from the control line in 6-week weight is shown in Fig. 2. The high-low difference was significant from generation 1 onwards, while the high-control and control-low differences were significant only on an irregular basis. The rate of divergence appeared relatively steady up to generation 4. However, a major difference between the high and control lines and the low line developed



https://doi.org/10.1017/S0016672300028159 Published online by Cambridge University Press

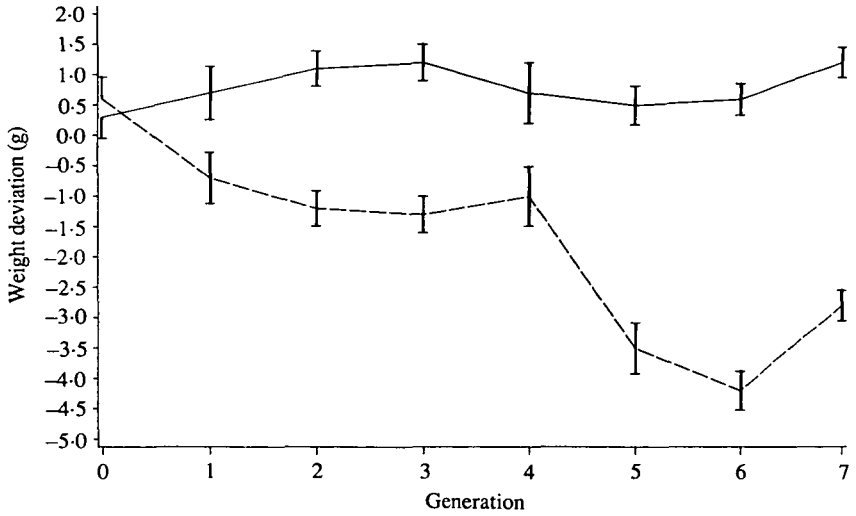


Fig. 2. Deviation of the high and low lines from the control line for liveweight (g). (—, high line; ----, low line).

in generation 5 and persisted in the following 2 generations. Examination of the mean low-line weights in Table 1 shows that liveweights actually increased after generation 4, but at a lesser rate compared with the high and control lines. there was no major change in the levels of inbreeding in any of the lines at generation 5. Rather, inbreeding accumulated in a relatively linear fashion.

## 4. Discussion

It is clear from the results that any discussion of responses to selection in IGF-1 concentrations and liveweight must be undertaken with care, given the different rates of accumulation in inbreeding.

Fig. 1 clearly shows that response to selection for IGF-1 concentrations has occurred, but it is not possible to conclude whether change has been in one or both directions. Since there was a much reduced response in generations 5 and 6 the realized heritability value of 0·15 is of limited value in making comparisons with other information in the literature. Blair *et al.* (1987) reported a heritability of 0·40 for IGF-1 concentrations at 35 days of age in mice. This estimate was based on a small number of full-sib litters from one unselected generation. The only other evidence for genetic variation in IGF-1 levels comes from two studies where selection has been for body-size or liveweight [Lund-Larsen & Bakke, 1975, (pigs); Eigenmann *et al.* 1984, (dogs)]. A further study by Buonomo *et al.* (1987) with pigs is of limited value in supporting the current argument since the 3 body-sizes investigated did not come from common genetic stock. A substantial body of evidence is accumulating to show that IGF-1 concentrations are often involved in major growth disturbances (particularly dwarfism), but these tend to involve major genes rather than the quantitative aspects being addressed here (Willeberg *et al.* 1975; Holder & Willis, 1977; Huybrechts *et al.* 1985; Merimee *et al.* 1987).

Although substantial changes have occurred in 6-week liveweight, it would be unwise to attribute all of the difference to a correlated response to selection for IGF-1 levels. The substantial change in generation 5 is of particular concern and would be consistent with the appearance of a major gene for dwarfism. However, in 4 randomly bred generations observed since selection was suspended, no evidence to support this theory has emerged.

There is no direct evidence in the literature regarding the possible existence of a genetic correlation between IGF-1 concentrations and liveweight. The studies of Lund-Larsen & Bakke (1975) and Eigenmann *et al.* (1984) provide some indirect support.

In conclusion, the use of family selection caused unequal rates of inbreeding accumulation in the three lines. As a consequence, it was not possible to separate the effects of selection and inbreeding. However, results were sufficiently favourable to suggest that further work is warranted to investigate the genetic nature of the IGF-1 concentration/liveweight relationship. To this end, a selection experiment involving Romney sheep has been established in which plasma levels of IGF-1 in individuals at about 5 months of age are being used as the selection criterion.

Financial assistance from the organizing committee of the First World Congress on Sheep and Beef Cattle Breeding, the C. Alma Baker Trust Fund and the Medical Research Council of New Zealand is gratefully acknowledged. The contribution of staff of the Small Animal Production Unit, Masey University, in maintaining the mice is also greatly appreciated.

## References

Adashi, E. Y., Resnick, C. E., D'Ercole, A. J., Svoboda, M. E. & van Wyk, J. J. (1985). Insulin-like growth factors as intraovarian regulators of granulosa cell growth and function. *Endocrine Reviews* **6**, 400–420.

Anon. (1987) *Mouse News Letter* **79**, 25.

Blair, H. T., McCutcheon, S. N., Mackenzie, D. D. S., Gluckman, P. D. & Ormsby, J. E. (1987). Variation in plasma concentration of insulin-like growth factor-1 and its covariation with liveweight in mice. *Australian Journal of Biological Sciences* **40**, 287–293.

Breier, B. H., Bass, H. J., Butler, J. H. & Gluckman, P. D. (1986). The somatomedin axis in young steers: influence of nutritional status on pulsatile release of GH and circulating concentrations of IGF-1. *Journal of Endocrinology* **111**, 209–215.

Buonomo, F. C., Lauterio, T. J., Baile, C. A. & Campion, D. R. (1987). Determination of insulin-like growth factor-1 (IGF-1) and IGF binding protein protein levels in swine. *Domestic Animal Endocrinology* **4**, 23–31.

Cockrem, F. (1959). Selection for relationships opposite to those predicted by the genetic correlation between two traits in the house mouse (*Mus musculus*). *Nature* **183**, 342–343.

Eigenmann, J. E., Patterson, D. F. & Froesch, E. R. (1984). Body-size parallels insulin-like growth factor-1 levels but not growth hormone secretory capacity. *Acta Endocrinologica* **106**, 448–453.

Falconer, D. S. (1981). *Introduction to Quantitative Genetics*. Longman Group Ltd, U.K.

Flux, D. S. (1957). The growth-stimulating effect of growth hormone and L-thyroxine on the mammary glands and uterus of the mouse. *Journal of Endocrinology* **15**, 266–272.

Flux, D. S. & Munford, R. E. (1957). The effect of adrenocorticotrophin on the mammary glands of intact mature female mice of the CHI strain. *Journal of Endocrinology* **14**, 343–347.

Froesch, E. R., Schmid, C., Schwander, J. & Zapf, J. (1985). Actions of insulin-like growth factors. *Annual Reviews of Physiology* **47**, 443–467.

Gilmour, A. R. (1985). *REG – A Generalized Linear Models Program*. New South Wales Department of Agriculture, Australia.

Gluckman, P. D., Breier, B. H. & Davis, S. R. (1987). Physiology of the somatotropic axis with particular reference to the ruminant. *Journal of Dairy Science* **70**, 442–466.

Gluckman, P. D. & Butler, J. H. (1983). Parturition related changes in the insulin-like growth factors 1 and 2 in the perinatal lamb. *Journal of Endocrinology* **99**, 223–232.

Hall, K. & Sara, V. R. (1983). Growth and somatomedins. *Vitamins and Hormones* **40**, 175–233.

Hill, W. G. (1972). Estimation of realised heritabilities from selection experiments I. Divergent selection. *Biometrics* **28**, 747–765.

Holder, A. T. & Wallis, M. (1977). Actions of growth hormone, prolactin and thyroxine on serum somatomedin-like activity and growth in hypopituitary dwarf mice. *Journal of Endocrinology* **74**, 223–229.

Huybrechts, L. M., King, D. B., Lauterio, T. J., Marsh, J. and Scanes, C. B. (1985). Plasma concentrations of somatomedin-C in hypophysectomized, dwarf and intact growing domestic fowl as determined by heterologous radioimmunoassay. *Journal of Endocrinology* **104**, 233–239.

Lund-Larsen, T. R. & Bakke, H. (1975). Growth hormone and somatomedin activities in lines of pigs selected for rate of gain and thickness of backfat. *Acta Agriculture Scandinavica* **25**, 231–234.

Merimee, T. J., Zapf, J., Hewlett, B. & Cavalii-Sforza, L. L. (1987). Insulin-like growth factors in pygmies. *The New England Journal of Medicine* **316**, 906–911.

Nilsson, A., Isgaard, J., Lindahl, A., Dahlstrom, A., Skottner, A. & Isaksson, O. G. P. (1986). Regulation by growth hormone of number of chondrocytes containing IGF-1 in rat growth plate. *Science* **233**, 571–574.

Willeberg, P., Kastrup, K. W. & Andresen, E. (1975). Pituitary dwarfism in German Shepherd dogs: studies on somatomedin activity. *Nordisk Veterinaer Medicin* **27**, 448–454.