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Variation in Plasma Concentration of Insulin-like Growth Factor-1 and its Covariation with Liveweight in Mice

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Abstract

Three experiments were undertaken to examine the degree and causes of variation in plasma concentrations of insulin-like growth factor-1 (IGF-1) in mice. The relationship between IGF-1 concentrations and liveweight was also examined. In all three experiments, a number of non-genetic factors were found to contribute significantly to the variation in IGF-1 concentrations, the most important of these being sex and litter size. In one experiment, where pups from 16 litters were cross-fostered to avoid the confounding of maternal and direct genetic effects, a heritability of $0 \cdot 40 \pm 0 \cdot 27$ was estimated for plasma IGF-1 concentration at 35 days of age. To examine further the existence of genetic variation in plasma concentrations of IGF-1 and the genetic covariation between plasma IGF-1 levels and other body traits, a selection experiment with mice has been initiated. Moderate to strong phenotypic correlations between IGF-1 concentrations and weight at an early age have been found in all three experiments.

Introduction

The existence of a strong phenotypic relationship between the plasma concentration of insulin-like growth factor-1 (IGF-1) and growth rate or mature size has been reported by several workers (Falconer 1981*b*; Holder *et al.* 1981; Eigenmann *et al.* 1984). However, few reports have attempted to establish a genetic link between plasma IGF-1 and growth potential. If this link were demonstrated, plasma concentrations of IGF-1 could provide a valuable selection criterion in animal industries where rapid liveweight gains are economically beneficial.

If IGF-1 concentration were to be of use as a selection criterion, it would be necessary to identify and adjust for any non-genetic factors which contributed to variability in plasma IGF-1. Examples of factors which might obscure the true genetic basis of the trait include sex of the individual, number of siblings born and/or reared in the litter and age of dam. Adjustment for non-genetic factors increases the accuracy with which replacement breeding stock can be ranked for selection purposes, thereby allowing more rapid rates of genetic improvement to be achieved.

This paper reports on the magnitude of non-genetic effects which must be adjusted for when selecting for IGF-1 levels in mice and presents further evidence of a phenotypic relationship between plasma concentrations of IGF-1 and liveweight.

Materials and Methods

Mice were generated by crossing and interbreeding four inbred strains (NOS, CHI, TSA and C3H). The four strains of mice had been maintained as distinct inbred lines for more than 50 generations at Massey University prior to cross-breeding.

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Mice were kept in cages with 1 litter per cage. The light to dark ratio was maintained at 14 h : 10 h, and the temperature at 20°C, throughout all experiments. A pelleted, complete diet was fed on an *ad libitum* basis.

Two blood-sampling procedures were used to obtain sufficient plasma for the IGF-1 assay. If the animals were required for further work (e.g. as breeding stock or for weight gain trials), blood was obtained by tail-snipping. Blood samples were then volumetrically bulked on a within-litter basis. Depending on the experiment, four, five or six individuals contributed to the bulked sample. Where mice were not required for further experimentation, individual blood samples were obtained by decapitation. At all blood-samplings, except in the preliminary experiment, mice were anaesthetized using 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 interassay coefficients of variation were 5·0% and 9·8%, respectively.

Analyses of (co)variance were performed using the ordinary least-squares section of the computer package REG (Gilmour 1985). At the outset of each analysis all known fixed effects, and first-order interactions between them, were included in the model to find those which significantly contributed to the variation in the dependent variable. Where further calculations were required (variance components or residuals), non-significant effects were first removed from the analysis. Both unadjusted and adjusted pairs of variables were used in the calculation of correlations. To enable the calculation of adjusted correlations, residual values were retrieved after fitting significant fixed effects. Standard errors for the heritabilities based on full-sib data were calculated using the method shown by Becker (1975).

Details of the three experiments were as follows:

Preliminary Experiment

This experiment was conducted to establish the magnitude of the relationship between plasma IGF-1 and body weight in mice and to obtain preliminary information on non-genetic factors which should be examined further. Seventy mice (five from each of 14 full-sib litters born over a 5-day period) were blood-sampled and weighed at about 30 and 80 days of age. Weaning occurred when pups averaged 24 days of age. The number of pups weaned per litter (NPW) varied between 7 and 10. The five individuals from each litter comprised either three males and two females or two males and three females. Blood collection at 30 days was undertaken without the use of ether anaesthetic, animals being warmed in a flow of hot air to increase blood flow to the tail.

Fostering Experiment

It is well established that the weight of young animals is affected by a maternal component (particularly milk yield and litter size), and that this effect can carry over beyond weaning. The main objective of this trial was to provide an estimate of IGF-1 heritability, free of the maternal effect. Eight pups from each of 16 full-sib litters were cross-fostered such that each dam reared eight pups, no dam reared any of her own pups and each pup in a foster litter was from a different natural litter. The age at fostering varied between 1 and 5 days. Nine fostered litters consisted of five females and three males, six had four of each sex and one had five males and three females. Pups were separated from their foster-dams at about 44 days of age, this being somewhat later than normal because of the slower than expected growth. Nine pre-weaning deaths occurred. Individuals were weighed at approximately weekly intervals from 27 to 110 days of age. Two males and two females from each natural litter were blood-sampled by decapitation at 35 days of age. The remaining 54 mice (one pregnant female was excluded) were blood-sampled by decapitation at 110 days of age.

Selection Experiment

This experiment involved three lines of mice from a selection study. Two lines were selected for either high or low plasma IGF-1, whilst the third line was maintained as a randomly bred control. Each generation of the high and low lines consisted of 20 females and 10 males selected on the basis of litter mean plasma IGF-1. The control group comprised 10 pairs in each generation. Weaning and blood sampling occurred at 35–45 days of age. The parents of generation 0 were randomly allocated to the three lines from an unselected base population which was generated as previously described. The first measurements of IGF-1 and weight were made on the offspring of these unselected parents. In generation 0, six individuals contributed to the estimate of the litter mean IGF-1. Where possible, equal numbers of males and females were sampled to provide the estimate of the litter mean IGF-1 value.

Results

Preliminary Experiment

Non-genetic factors

Estimates of plasma IGF-1 concentration at 30 days were based on 14 litter means. Hence, correlations between this trait and other traits were also generated using litter means. Sex ratio (three males/two females or two males/three females) significantly affected 80-day plasma IGF-1 ($P = 0 \cdot 05$) and 80-day weight ($P < 0 \cdot 01$), but had no effect on 30-day plasma IGF-1 or 30-day weight. Time of blood-sampling (0900–1230 h), age (range of 5 days) and litter size (7, 8, 9 or 10) were non-significant for all traits. Correlations between litter mean plasma IGF-1 concentrations at 30 days and other traits (before and after adjusting for sex ratio) are shown in Table 1.

Table 1. Mean values for, and correlations among, 30-day and 80-day plasma IGF-1 concentrations and body weights in the preliminary experiment

Variable	Mean ± s.d.	Correlation (unadjusted/adjusted) with: 30-day weight	80-day IGF-1	80-day weight
		Litter means[A] ($n = 14$)		
30-day IGF-1 (ng/ml)	69·5 ± 18·8	0·77***/0·77***	−0·11/0·31	0·18/0·52*
		Individual observations[B] ($n = 70$)		
30-day weight (g)	13·6 ± 1·8	–		
80-day IGF-1 (ng/ml)	119·3 ± 14·8	0·01/−0·15	–	
80-day weight (g)	24·7 ± 3·2	0·57***/0·29*	0·34**/0·23†	–

[A] Correlations based on 14 litter means and adjusted for sex ratios: ***$P<0 \cdot 001$; *$P<0 \cdot 05$.
[B] Correlations based on 70 individual observations and adjusted for sex (male *v.* female): ***$P<0 \cdot 001$; **$P<0 \cdot 01$; *$P<0 \cdot 05$; †$P<0 \cdot 10$.

Since individual observations were available for all traits except 30-day IGF-1, correlations amongst the remaining traits were based on 70 individual records. The effects of litter size, sex and age on each of the traits were investigated. The only significant factor was sex, the difference between males and females being 1·5 g for 30-day weight ($P < 0 \cdot 001$), and 5·5 g for 80-day weight ($P < 0 \cdot 001$). The effect of sex on 80-day IGF-1 was non-significant ($P = 0 \cdot 11$). Correlations between these traits (before and after adjusting for sex) are shown in Table 1.

Heritability of plasma IGF-1 concentration at 80 days

The variance remaining in 80-day IGF-1 after adjusting for the effect of sex was apportioned into two components, that due to full-sib litters and that due to error. The resulting variance components were 20 $(\text{ng/ml})^2$ and 196 $(\text{ng/ml})^2$ for full-sib litters and error, respectively, implying a heritability of $0 \cdot 19 \pm 0 \cdot 21$ for 80-day IGF-1.

Fostering Experiment

When the pups reached 35 days of age (range 33–37 days), individual blood samples were obtained by decapitation from four mice from each of the 16 natural full-sib litters (total = 64 observations). The mean ± s.d. for 35 day IGF-1 was $97 \cdot 6 \pm 30 \cdot 9$ ng/ml.

Non-genetic factors

An analysis of the variance in plasma IGF-1 at 35 days revealed that sex, age at fostering and their interaction all had highly significant effects ($P < 0 \cdot 002$). Interactions between

foster dam, natural litter and age at fostering could not be examined due to insufficient data. Effect of the foster dam was non-significant ($P = 0 \cdot 06$). The difference between males and females in plasma IGF-1 at 35 days was 25·3 ng/ml.

The remaining 54 mice were individually blood sampled at 110 days of age. One record was discarded as it was the only representative for one of the original full-sib litters. This left 15 full-sib litters represented by between 2 and 4 offspring. The mean ± s.d. for 110-day plasma IGF-1 was 103·4 ± 22·1 ng/ml. The only factor to contribute significantly to variation in 110-day plasma IGF-1 was sex, the difference between males and females being 27·1 ng/ml ($P < 0 \cdot 001$).

Age at fostering, sex and foster dam all had significant effects on liveweight. The first-order interaction between age at fostering and sex was not significant. The effects of foster dam and age at fostering diminished as the animals aged such that by 49 days foster-dam became non-significant and by 84 days age at fostering became non-significant.

Heritability of plasma concentration of IGF-1

The variation remaining in plasma IGF-1 at 35 days after adjusting for non-genetic effects was divided into a full-sib litter variance component of 82 $(ng/ml)^2$ and an error variance component of 326 $(ng/ml)^2$. These components combine to give a heritability estimate of 0·40 ± 0·27 for plasma IGF-1 at 35 days.

The full-sib litter component of variance for 110-day plasma IGF-1 was 30 $(ng/ml)^2$ and the error variance component was 314 $(ng/ml)^2$. These values provide a heritability estimate of 0·17 ± 0·28 for 110-day plasma IGF-1.

Relationship of plasma IGF-1 to weight

The unadjusted and adjusted correlations between 35-day plasma IGF-1 and 35-day weight were 0·79 ($P < 0 \cdot 001$) and 0·31 ($P < 0 \cdot 05$), while the corresponding correlations between 110-day plasma IGF-1 and 110-day weight were 0·53 ($P < 0 \cdot 05$) and 0·20 ($P > 0 \cdot 10$). Correlations between 35-day plasma IGF-1 and later weights were based on 15 pairs of litter means. All unadjusted and adjusted correlations were non-significant.

Selection Experiment

In generation 0 of the selection experiment, six individuals from each litter contributed to the pooled blood sample from which the litter mean plasma IGF-1 was determined. Measurements were undertaken at about 35 days of age and a total of 47 litters were sampled (19 from each selection line and nine from the control).

Because of the random assignment of parents to lines, there were no significant differences between the lines for IGF-1. The NPW (range of 5–12) significantly affected both plasma IGF-1 (−6·0 ng/ml per pup weaned) and weight (−0·9 g per pup weaned) at 35 days of age ($P < 0 \cdot 001$). Because the intention of this experiment was to investigate the potential for changing IGF-1 by genetic means, selection is being based on litter means after adjustment for NPW. The correlation between plasma IGF-1 (adjusted for NPW) and weight at 35 days for the 47 litter means was 0·58 (unadjusted value = 0·72).

Blood sampling took place between 0930 and 1145 h and 1315 and 1430 h (on the same day). The means ± s.e. for IGF-1 were 130·3 ± 3·7 ng/ml and 124·9 ± 3·8 ng/ml for the morning and afternoon periods, respectively ($P > 0 \cdot 10$).

Discussion

Non-genetic Factors Affecting Plasma IGF-1

Sex

Eighty-day IGF-1 in the preliminary experiment and 35- and 110-day IGF-1 in the fostering experiment were all significantly higher in males. Males were also significantly

heavier than females. In contrast, no differences in IGF-1 concentrations were observed between female and castrated male pigs (Ringberg Lund-Larsen and Bakke 1975) or male and female children (Luna *et al.* 1983), while Copeland *et al.* (1985) showed that female chimpanzees had higher levels of IGF-1 than males up to 8 years of age. Although not consistent with previous reports, the results of this study clearly indicate a sex difference in plasma concentrations of IGF-1 in mice.

Litter size

In the selection experiment there was a significant effect of litter size on litter mean IGF-1. The effect of rearing an extra pup was to decrease litter mean IGF-1 by 6 ng/ml. An increase in litter size also decreased the average weight of pups, presumably because of competition between litter mates for the limited supply of milk. There have been no previous reports on the effect of litter size on IGF-1.

Age at fostering

In an attempt to separate the maternal effect from the direct effect on IGF-1, all pups in the fostering experiment were cross-fostered at ages ranging from 1 to 5 days. This inadvertently contributed a further effect which systematically altered both IGF-1 and liveweight at an early age such that the pups which were older when fostered had higher IGF-1 levels at 35 days and heavier weights until about 80 days of age. The effect of age at fostering on IGF-1 at 110 days was non-significant. An explanation for this induced effect is that the older pups had a competitive advantage in obtaining milk from their foster dam. However, it was not possible from the data presented here to establish whether there was any cause and effect involved between weight and plasma IGF-1 concentrations.

Maternal effect

In the fostering experiment, foster dam had a significant ($P < 0 \cdot 01$) effect on 35-day weight while the effect on 35-day plasma IGF-1 approached significance ($P = 0 \cdot 06$). As is common with traits measured before or soon after weaning, the maternal environment has distorted the true genetic basis of both weight and IGF-1 levels in the fostered offspring, as predicted by their phenotype. To maximize the rates of genetic gains in such traits it would be desirable to use statistical models that separate direct genetic effects from maternal effects.

Age

Because litters were born over a relatively compact period, it was not possible to investigate the contribution of age at each blood sampling to the variation in plasma IGF-1 concentrations.

Weight as a covariate

The contributions of the above non-genetic factors to the variation in IGF-1 concentration could be removed by first adjusting for weight. This indicated that the effect of these factors was most likely mediated via weight. However, if the objective of selecting for IGF-1 was to increase weight or growth rate, correction for weight would be inappropriate since this would most likely reduce the rate at which correlated changes in body growth occurred. Under these circumstances it would be necessary to adjust for the non-genetic factors individually.

Heritability of Plasma Concentrations of IGF-1

The information presented above makes it clear that when young animals are being chosen for breeding purposes on the basis of plasma concentrations of IGF-1, adjustments

must be used to maximize the rate of genetic progress. Although the effects studied here may not directly relate to other species, it would appear reasonable to expect that any factor which affected the rate of growth could also alter plasma IGF-1 concentrations.

The fostering experiment was specifically designed to estimate the heritability of IGF-1 at about 35 days in the absence of confounding maternal effects. The heritability estimate of 0·40 has been calculated from limited data (four full-sibs from each of 16 litters) and should be interpreted more as an indication of possible genetic variation rather than as a firmly established value. The existence of genetic variation in plasma IGF-1 at between 35 and 45 days of age is now being further examined via a selection experiment.

There have been no previous estimates of the heritability of plasma IGF-1 concentrations, although indirect evidence would support the hypothesis that genetic variation exists (Ringberg Lund-Larsen and Bakke 1975; Eigenmann *et al.* 1984; Huybrechts *et al.* 1985).

Heritability estimates were also calculated for plasma IGF-1 at 80 days (preliminary experiment) and 110 days (fostering experiment). Both estimates of the heritability were less than 0·2, implying that only a small proportion of the total variation is genetic in origin at these older ages. While it is unlikely that attempts would be made to directly alter IGF-1 at older ages, it is important to know that genetic variation exists as this indicates that mature IGF-1 concentrations have the potential to change. Such changes could have important repercussions, depending on the role of IGF-1 at older ages.

Phenotypic correlations between plasma IGF-1 at 30 or 35 days and 80 or 110 days (based on litter means) were small (−0·18 to 0·32). If it is assumed that the genetic correlation is of a similar sign and magnitude to the phenotypic correlation (Falconer 1981*a*), then it is unlikely that selection for IGF-1 levels at an early age would cause any major changes in plasma levels of IGF-1 at older ages.

Relationship of Plasma IGF-1 to Liveweight

A moderate to strong relationship between IGF-1 and liveweight at a young age was found in each of the three experiments. The adjusted correlation of 0·31 obtained in the fostering experiment was somewhat smaller than those from the preliminary and selection experiments (0·77 and 0·58). However, Sara *et al.* (1986) suggested that the preweaning period was critical for the development of the mature IGF system. It is possible that the stress of fostering somehow interfered with this development process. It was also demonstrated that a number of non-genetic factors had the potential to alter the magnitude of this relationship. This is consistent with the findings of Ringberg (1979) who showed that IGF-1 concentrations at 6–9 months were significantly different in two groups of bull calves chosen on the basis of high or low weight gains between 3 and 12 months of age.

The relationships between plasma IGF-1 at an early age and later weights observed in the preliminary and fostering experiments were not consistent. In the preliminary experiment the correlation with adjusted 80-day weight was moderately positive, whilst in the fostering experiment all correlations with weights subsequent to 35 days were small negative values. However, these relationships were assessed via litter means, with only a small number of observations, and may not accurately reflect the relationship between pairs of observations on individuals.

Conclusions

Several non-genetic factors were found to contribute significantly to the variation in plasma concentrations of IGF-1. Adjustment for these factors would increase the rate at which genetic progress in IGF-1 concentrations could be made. From the limited data presented here, it was not possible to establish with certainty the magnitude of genetic variation in IGF-1 levels at about 6 weeks of age. A selection experiment has been established to further explore this problem. A moderate to strong association between plasma concentrations of IGF-1 and weight at about 6 weeks of age concurred with previous reports.

The possibility that part of this association is genetic in origin is currently being examined in the above-mentioned selection experiment.

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