THE ROLE OF TESTOSTERONE IN LINES OF MICE DIVERGENTLY SELECTED ON FAT CONTENT

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SUMMARY

Lines of mice have been divergently selected on, and now differ five fold in, estimated fat content at 14 weeks of age. Individuals from each line were castrated or sham operated and subsequently given either exogenous testosterone or the appropriate control. Analysis of body weight, fat content and lean mass failed to detect any interaction between these treatments and genetic background. It is concluded that testosterone metabolism has not contributed disproportionately to the response to artificial selection.

INTRODUCTION

There is considerable interest in identifying the type of genes underlying quantitative traits, and two main methods are employed. The first, "bottom up" approach, is to search directly for such loci, for example by mapping crosses between divergent strains, and then attempting to identify candidate trait loci in that region. The second, "top-down" approach is to identify the physiological basis of the altered phenotype: this gives an idea as to the type of genes likely to affect quantitative traits and may ultimately allow the phenotype to be directly manipulated by transgenic technology. A common strategy employed in this second approach is measure the levels of, for example, enzyme activities, circulating hormone levels, sensitivity or hormone receptors. This can give counter-intuitive results (for example, the level of circulating growth hormone is typically lower in high growth lines of mice, e.g. Medrano *et al.* (1991) and the interpretation of such results is unclear. An alternative to this observational approach is the direct experimental approach employed here.

Testosterone is known to be lipogenic, and mice from the line of mice selected for increased carcass fat content (the Fat line) have smaller testes than those selected for decreased fat content (the Lean line) (Hastings *et al.*, 1991). Briefly, the experimental rationale is to castrate animals from both lines and/or administer exogenous testosterone (with appropriate controls in each case). If one line responds disproportionately to the removal of testosterone by castration, it indicates that response has been disproportionately large in that part of metabolism. Similarly, if response to exogenous testosterone is greater in one line, we may conclude that response has been achieved, at least in part, by an increased sensitivity to testosterone. The logic is identical to that employed previously when investigating the role of growth hormone in lines of mice divergently selected on body weight (Hastings *et al.*, 1992).

MATERIALS AND METHODS.

Mouse lines. Individuals were taken from lines of mice divergently selected for 50 generations on estimated carcass fat content in males at 14 weeks of age; at the time of this experiment they diverged five fold in estimated fat content at this age (4% vs 20%). Twelve litters were taken from the Fat line, 18 from the Lean, and male mice were randomly assigned to each of the four treatments with group sizes as shown in Table 1. The litters were born over an eight day period and were split at weaning into cages within lines (to avoid differences in, for example, aggression which may occur between mice from different lines housed together); each cage contained only mice injected with the same substance (testosterone or oil) to minimise the chance of errors. There were 19 cages in total (of the metal type described by Hastings and Hill, 1993) and each contained between 4 and 6 mice. They were maintained in a 14:10 hour light dark cycle at 23 ± 1^{o}C. Mice were killed at 70 ± 2 days and were denied access to food ("fasted") during the 6 hours prior to

killing to minimise variation in gut content. The presence or absence of testes was confirmed by dissection, and the carcass freeze dried (which required two separate batches). Freeze drying allows the carcass fat content to be accurately predicted by regression on the ratio of dry weight to body weight (Hastings and Hill, 1989). Lean weight is correlated with the weight of water in the carcass and was also estimated as fat-free body weight.

Experimental Protocol. This protocol is based on that of Siddiqui *et al.* (1989). Male mice were anaesthetised at 10 days of age with halothane, castrated or sham operated (to act as controls), and the wound closed with acrylate glue. They were subsequently given tetracycline in their drinking water between days 10 and 14 (following surgery) and between days 18 and 21 (as pre-weaning mortality is typically high in the Fat lines at this age). Injections were at 14 day intervals commencing at 14 days of age with an additional injection at 63 days of age to ensure high levels of testosterone immediately prior to termination of the experiment at age 70 days. Injections were made between 6.5 and 7.5 hours into the light period and mice were weighed weekly at the same time. All castrations and 14 day injections were done on the correct day. Thereafter they were split into two "injection groups" so that each could be injected on the appropriate day ± 2 days. Injections were made s/c with testosterone enanthate (Schering AG) diluted in peanut oil at a concentration of $1gl^{-1}$ at 14 days of age, $2gl^{-1}$ at 28 days of age, and $5gl^{-1}$ thereafter. Dosage was 0.5ug per gram body weight per day, based on the weight at injection (i.e. a 10g mouse would receive 0.5x10x14=70ug); controls received the appropriate volume of oil.

Statistical analysis. Body weight, water weight, estimated fat content, and estimated lean weight at age 70 days were analysed using the restricted maximum likelihood (REML) option of the Genstat statistical package (Genstat, 1988). The model fitted the (fixed) effects shown in Table 2 with the additional random effect of family (incorporating differences in date of birth, litter size, etc.), the random effect of cage, the fixed effect of injection batch and, where appropriate, the fixed effect of drying batch.

RESULTS

The group means are shown in Table 1, and the results of the statistical analysis in Table 2. Mice with the Fat genetic background are slightly heavier, due to a greatly increased amount of fat and despite a slightly lower lean mass, a result found in previous analyses of these lines (Hastings *et al.*, 1992). Castration decreased 10 week body weight by about 8-9% in each line (comparison of sham/oil with castration/oil in Table 1). Administration of exogenous testosterone restored normal growth (castration/testosterone vs. sham/oil in Table 1). This reduced the fixed (or independent) effect of castration which is non-significant on Table 2, whereas the interaction between treatment and injection is positive and significant. A similar result was noted for fat weight, in which the effect of castration in increasing fat weight was reversed by administration of exogenous testosterone (comparison of castration/testosterone with sham/oil); the data in this case were not entirely consistent as castration in the Fat line in the absence of testosterone seemed to reduce fat content slightly. Castration significantly altered the proportional body composition in both lines, increasing fat percentage and decreasing estimated lean weight. Exogenous testosterone did not reverse these effects. Apart from the interaction between treatment and injection in the analysis of body weight and fat weight, there were no significant interactions. In particular those between genetic background and both experimental treatments were non-significant. Analysis of the data after transformation onto a log scale gave essentially the same results, suggesting that these conclusions are not artefacts of the scale employed (data not shown).

DISCUSSION

The results of this study are similar to those obtained by Siddiqui *et al.* (1989) for lines divergently selected on IGF1 levels (which also exhibited a correlated divergence in 10 week weight) in three important respects. Firstly, castration resulted in an 8-9% reduction in 10 week body weight. Secondly, the administration of

Table 1. Means and group sizes of weights between 2 and 10 weeks of age, fasted 10 week weight (10 wk wt -f) weight of water in the carcass, estimated percentage fat, estimated weight of fat and estimated lean weight.

	FAT				LEAN				
	castrated		sham operated		castrated		sham operated		
	testost.	oil	testost.	oil	testost.	oil	testost.	oil	mean.
N:	11	8	8	8	14	15	13	11	s.e.
2 wk wt. (g)	5.3	5.4	5.2	5.3	6.0	6.2	6.2	6.0	0.4
3 wk wt. (g)	9.8	9.6	9.1	8.8	9.0	9.4	9.8	9.9	0.6
4 wk wt. (g)	13.4	12.2	11.9	10.8	13.0	11.9	14.1	13.5	1.1
5 wk wt. (g)	18.6	16.8	17.3	16.6	18.0	17.4	19.6	19.5	1.2
6 wk wt. (g)	22.7	22.3	22.7	23.7	21.7	21.3	23.8	23.7	1.2
7 wk wt. (g)	27.4	25.5	27.1	28.1	24.8	23.5	26.9	26.9	1.2
8 wk wt. (g)	30.6	28.4	29.6	31.0	27.1	25.9	28.9	29.1	1.0
9 wk wt. (g)	34.0	31.0	31.9	33.0	29.5	27.5	30.3	30.0	1.3
10 wk wt. (g)	36.3	33.3	34.1	35.4	30.8	28.9	32.1	31.9	1.3
10wk wt.-f (g)	34.6	31.7	32.4	34.0	29.4	27.3	30.4	29.9	1.3
water wt. (g)	18.7	17.3	18.3	18.5	20.0	18.7	21.0	20.8	0.6
est. fat (%)	21.1	20.5	18.8	21.1	6.0	5.2	4.6	4.2	0.8
est. fat wt. (g)	7.5	6.7	6.2	7.3	1.8	1.4	1.4	1.2	0.4
est. lean wt. (g)	27.0	25.0	26.2	26.7	27.7	25.8	29.0	28.7	0.9

Table 2. REML estimates (± s.e.) of the effects of genetic background (backgr), treatment (treat), injection (inj), and interactions thereof, on 10 wk wt (fasted), weight of water in the carcass, estimated percentage fat, estimated weight of fat and estimated lean weight.

	10 wk wt.(g)	water wt. (g)	est. fat (%)	est. fat wt. (g)	est. lean wt. (g)
Main effects:					
backgr(Fat-Lean)	3.02±1.31*	-2.35±0.64***	14.86±0.90***	5.17±0.50***	-2.14±0.93***
treat(cast. - sham)	-0.58±0.65	-1.04±0.38**	1.68±0.43***	0.62±0.23**	-1.29±0.53*
inj(testost. - control)	0.45±0.64	0.49±0.37	-0.49±0.42	-0.19±0.23	0.64±0.52
Interactions:					
backgr*treat	1.18±1.28	0.58±0.67	0.48±0.86	0.32±0.47	0.86±0.96
backgr*inj	-0.91±1.27	-0.21±0.66	1.38±0.85	-0.53±0.47	-0.40±0.95
treat*inj	2.15±0.92*	0.97±0.52	1.06±0.60	0.68±0.32*	1.46±0.74
backgr*treat*inj	0.70±1.54	0.21±0.83	0.45±1.02	0.84±0.56	0.38±1.19

*p<0.05, **p<0.01, ***p<0.001; approximate significance obtained using a *t* test with 50 d.f.

exogenous growth hormone restored normal growth rate. Thirdly, interactions with genetic background were absent in both studies. This latter result is at variance with that reported by Hooper *et al.* (1986) who noted a significant interaction with genetic background: a reduction in body weight of approximately 16% in a line selected for increased body weight compared to 8% in an unselected control line. Testosterone levels had been altered in this selection line (O'Kean *et al.*, 1986) but it is not clear whether this interaction with genetic background was a consequence of selection directly on body weight or a chance result due to genetic drift as the line was not replicated. We are currently applying the same experimental procedure described above to (replicated) lines of mice divergently selected on body weight, and derived from the same base population as the fat-selected lines, which may clarify the situation. The inability of exogenous testosterone to reverse the effect of castration on body composition (% fat) was presumably a consequence of its mode of application, or the result of other physiological effects mediated by the testes.

The most important result is the lack of interaction between either experimental treatment and genetic background. This indicates that testosterone metabolism has not played a disproportionate part in the large (five fold) response to selection in these lines, despite the fact that castration altered composition in both lines, and that mice from the Fat line have smaller testes. A similar lack of interaction has been noted between growth hormone metabolism and genetic background in lines of mice divergently selected on body weight (Pidduck and Falconer, 1978; Hastings *et al*,. 1993) and these results support a model of the response to selection in which genetic differences acting through many physiological systems contribute to the response rather than through one main system. Testosterone is known to increase aggression in most mammals (including mice, e.g. de Ruiter *et al.*, 1992) and there is currently some concern that intense selection in commercial animals may cause correlated changes in undesirable behavioural traits. The results presented here suggest that significant changes in testosterone metabolism have not occurred as a consequence of selection on body composition (at least in mice), so reducing the likelihood of correlated changes in aggressive behaviour.

We thank S.N. McCutcheon for advice in the execution of this experiment and R.P. McInnes for technical assistance. This work was supported by the Agricultural and Food Research Council.

REFERENCES

DeRUITER, A.J.H, KOOLHAAS, J.M., KEIJSER, J.N., VanOORTMERSSEN, G.A. and BOHUS, B. (1992) Aggressive Behaviour 18:149-157.

GENSTAT 5 COMMITTEE (1988) Genstat 5 reference Manual, Oxford University Press.

HASTINGS, I.M., BOOTLAND, L.H. and HILL, W.G. (1993). Genetical Research 61:101-106.

HASTINGS, I.M. and HILL, W.G. (1989) Animal Production 48:229-233.

HASTINGS, I.M. and HILL, W.G. (1993). Mouse Genome 91:329-330.

HASTINGS, I.M., YANG, J and HILL, W.G. (1991). Genetical Research 58:253-259.

HOOPER, A.C.B., BRIEN, T.G. and LAWLOR, P.G. (1986) Andrologia 18:509-515.

O'KEAN, J.C., BRIEN, T.G., HOOPER, A.C.B., and GRAHAM, A. (1986) Andrologia 18:190-195

PIDDUCK, H.G. and FALCONER, D.S. (1978) Genetical Research 32:195-206.

MEDRANO, J.F., POMP D., SHARROW, L., BRADFORD, G.E., DOWNS, T.R. and FROHMAN, L.A. (1991). Genetical Research 58:67-74.

SIDDIQUI, R.A., MCCUTCHEON, S.N., MACKENZIE, D.D.S., BLAIR, H.T., ORMSBY, J.E, GLUCKMAN, P.D. and BREIER B.H. (1989) Acta Endocrinologica 121:686-690.