# The effect of testosterone in mice divergently selected on fat content or body weight

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**Summary**

Lines of mice have been divergently selected on one of two traits: (i) estimated fat content at 14 weeks of age, which has resulted in a 5-fold divergence, and (ii) body weight at 10 weeks of age, which has resulted in a 3-fold divergence. Individuals from each line were castrated or sham operated at 10 days of age and subsequently given either exogenous testosterone or the appropriate control from 14 days of age. Castration increased fat content and decreased lean weight in all lines, an effect which was not reversed by administration of testosterone. Body weight was reduced by around 10% as a result of castration and this effect was at least partially reversed by exogenous testosterone. Analysis of body weight, fat content and lean mass at 10 weeks of age failed to detect any interaction between these treatments and genetic background. It is therefore concluded that testosterone metabolism has not contributed disproportionately to the response to artificial selection in spite of its known effects on growth and body composition.

## 1. 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 phenotypically divergent strains, and 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 of the actions 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, for example, to measure the activities of enzymes, concentrations of circulating hormone or the levels of hormone receptors. However, this can sometimes give counter-intuitive results and the interpretation of such results is unclear: for example, the level of circulating growth hormone is typically lower in high growth lines of chickens (Goddard *et al*., 1988) irrespective of the genetic basis of the growth difference (poly- or mono-genetic). Medrano *et al*. (1991) found no difference between mouse lines differing in growth as a consequence of divergent artificial selection but reported reduced levels of circulating growth hormone in the same mouse lines containing a single mutation increasing growth. An alternative to this observational approach of investigating the relationship between physiology and genetic control of growth is the direct experimental approach employed here.

Testosterone is one of the main regulators of murine growth, acting both directly and indirectly via the growth hormone/insulin-like growth factors (GH/IGF-1) system (for access to the literature on its general effects see, for example, Buttery *et al*. (1986) and for its specific role in rodent models see, for example, Donahue *et al*. (1993)). In particular, testosterone is lipolytic, and mice from lines selected for increased carcass fat content have smaller testes than those selected for decreased fat content (Hastings *et al*., 1991). Similarly, mouse lines divergently selected on testis weight show (negatively) correlated response in fat content (Hill *et al*., 1990). Thus testosterone metabolism seems a likely target on which artificial selection may operate, and the obvious question is whether putative genetic variation associated with this hormone system has been utilized in the response to artificial selection on body weight or composition.

Briefly, the experimental protocol for testing this hypothesis is to castrate animals and/or administer

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exogenous testosterone (with appropriate controls in each case) to animals from divergently selected lines. If one of the lines 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 (Pidduck & Falconer, 1978; Hastings *et al*., 1993). Physiological regulation of growth is a delicate and highly complex system and we do not argue that administration of exogenous testosterone should completely reverse the effects of castration (nor, in our previous paper, that exogenous growth hormone would completely remove the effects of a mutation disrupting growth hormone metabolism). Rather we argue that this type of experiment is a way of directly manipulating specific aspects of physiology affecting growth and that an interaction of treatment with selection criterion would provide evidence that selection has altered the degree to which the hormonal axis under question affects growth.

## 2. Materials and methods

### (i) *Mouse lines*

Two pairs of mouse lines were used. The *F* line has been divergently selected on fat content in adult male mice and the *P* line has been divergently selected on body weight in adults of both sexes; for further details see Hastings *et al*. (1991) and Beniwal *et al*. (1992) respectively. Mice were maintained in a 14:10 h light–dark cycle at $23 \pm 1$ °C and fed *ad libitum* on Rat and Mouse Diet No. 1 (Special Diet Services, England CM8 3AB). Experimental details specific to the *F* and *P* lines are detailed below.

### (a) *F 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 5-fold in estimated fat content at this age (4% *v*. 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 8 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, see later) to minimize the chance of errors. There were 19 cages in total (of the metal type described by Hastings & Hill, 1993), each containing between four and six mice.

### (b) *P lines*

At the time of this experiment the *P* lines had undergone 51 generations of divergent selection resulting in a nearly 3-fold difference in male body weight at age 10 weeks (18·3 g *v*. 52·8 g; mean of generations 49, 50 and 51). Sixteen families were used from the High line and 17 families from the Low; litters were split across cage and treatment. There were 17 cages in total and the number of mice per cage was between two and six. The experiment was performed in standard plastic cages (Hastings & Hill, 1993) in contrast to the experiment in the *F* lines. This type of cage is known to result in lower fat content but was deemed satisfactory, firstly because the High and Low lines differ only slightly in fat content, and secondly because the *P* lines were routinely maintained and selected in this type of cage.

### (ii) *Experimental protocol*

The experimental protocol is based on that of Siddiqui *et al*. (1989). Male mice were anaesthetized at 10 days of age with halothane, castrated or sham operated (to act as controls), and the surgical site 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 6·5–7·5 h into the light period and mice were weighed weekly at this time. All castrations and injections at 14 days of age were performed at the exact age.

Thereafter the mice were split into two 'injection groups' so that each could be injected on the appropriate day $\pm 2$ days. Injections were of subcutaneous testosterone enanthate (Schering AG) diluted in peanut oil at a concentration of 1 g $l^{-1}$ at 14 days of age, 2 g $l^{-1}$ at 28 days of age, and 5 g $l^{-1}$ thereafter. Dosage was 0·5 $\mu$g per gram body weight per day, based on the weight at injection (i.e. a 10 g mouse would receive $0{\cdot}5 \times 10 \times 14 = 70$ $\mu$g); controls received the appropriate volume of oil. Mice were killed at $70 \pm 2$ days and were denied access to food ('fasted') during the 6 h prior to killing to minimize variation in gut content. The presence or absence of testes was confirmed by dissection, and the carcasses freeze-dried (which required two separate drying batches of equal size in each of the lines) enabling carcass fat content to be predicted for each individual mouse by regression of dry matter content on body weight

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Table 1. *F lines: means and group sizes of weights between 2 and 10 weeks of age, fasted 10 week weight, weight of water in the carcass (water wt), and percentage and weights of fat, lean/protein and ash in the carcass; the latter measures were either estimated on an individual basis from dry matter content or obtained chemically from analysis of pooled samples (in which case sample size was 2 for each category)*

| | Fat | | | | Lean | | | | Mean SE | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Castrated | | Sham operated | | Castrated | | Sham operated | | | |
| | Testosterone ($n = 11$) | Oil ($n = 8$) | Testosterone ($n = 8$) | Oil ($n = 8$) | Testosterone ($n = 14$) | Oil ($n = 15$) | Testosterone ($n = 13$) | Oil ($n = 11$) | Fat | Lean |
| *Body weights (g)* | | | | | | | | | | |
| 2 weeks of age | 5·3 | 5·4 | 5·2 | 5·3 | 6·0 | 6·2 | 6·2 | 6·0 | 0·30 | 0·44 |
| 4 weeks of age | 13·4 | 12·2 | 11·9 | 10·8 | 13·0 | 11·9 | 14·1 | 13·5 | 1·18 | 1·06 |
| 6 weeks of age | 22·7 | 22·3 | 22·7 | 23·7 | 21·7 | 21·3 | 23·8 | 23·7 | 1·51 | 0·94 |
| 8 weeks of age | 30·6 | 28·4 | 29·6 | 31·0 | 27·1 | 25·9 | 28·9 | 29·1 | 1·46 | 0·83 |
| 10 weeks of age | 36·3 | 33·3 | 34·1 | 35·4 | 30·8 | 28·9 | 32·1 | 31·9 | 1·82 | 0·85 |
| 10 weeks of age (fasted) | 34·6 | 31·7 | 32·4 | 34·0 | 29·4 | 27·3 | 30·4 | 29·9 | 1·73 | 0·82 |
| *Estimates from dry matter content* | | | | | | | | | | |
| Water wt (g) | 18·7 | 17·3 | 18·3 | 18·5 | 20·0 | 18·7 | 21·0 | 20·8 | 0·69 | 0·57 |
| Fat (% wet wt)[a] | 21·1 | 20·5 | 18·8 | 21·1 | 6·0 | 5·2 | 4·6 | 4·2 | 1·29 | 0·36 |
| Fat wt (g) | 7·5 | 6·7 | 6·2 | 7·3 | 1·8 | 1·4 | 1·4 | 1·2 | 0·74 | 0·12 |
| Lean wt (g) | 27·0 | 25·0 | 26·2 | 26·7 | 27·7 | 25·8 | 29·0 | 28·7 | 1·07 | 0·79 |
| *Chemical carcass composition* | | | | | | | | | | |
| Fat (% dry wt)[a] | 52·8 | 50·6 | 47·1 | 48·6 | 20·6 | 19·0 | 19·4 | 18·5 | 0·87 | 1·33 |
| Protein (% dry wt) | 36·1 | 37·1 | 41·0 | 38·8 | 62·4 | 64·0 | 63·1 | 64·7 | 0·78 | 1·23 |
| Ash (% dry wt) | 6·2 | 6·6 | 7·2 | 7·7 | 11·5 | 11·4 | 11·2 | 11·4 | 0·39 | 0·21 |
| Fat (g) | 9·00 | 7·51 | 6·59 | 7·28 | 1·93 | 1·63 | 1·90 | 1·68 | 0·52 | 0·15 |
| Protein (g) | 6·15 | 5·49 | 5·71 | 5·82 | 5·84 | 5·51 | 6·17 | 5·88 | 0·31 | 0·19 |
| Ash (g) | 1·06 | 0·98 | 1·01 | 1·15 | 1·08 | 0·98 | 1·09 | 1·03 | 0·06 | 0·04 |

[a] Note that percentage fat content is, by convention, expressed on a wet weight (% wet wt) basis when estimated from dry matter content, but expressed on a dry weight basis (% dry wt) when obtained chemically.

Table 2. *F lines: REML estimates* (±*SE*) *of the effects of genetic background* (*backgr*), *castration* (*castr*), *injection* (*inj*), *and interactions thereof, on selected growth and composition traits as estimated from dry matter content*

| | 10 week wt (g) (fasted) | Fat (% wet wt) | Fat (g) | Lean wt (g) |
|---|---|---|---|---|
| (i) Untransformed | | | | |
| Main effects | | | | |
| backgr (Fat − Lean) | 3·02 ± 1·31* | 14·86 ± 0·90*** | 5·17 ± 0·50*** | −2·14 ± 0·93* |
| castr (full − sham) | −0·58 ± 0·65 | 1·68 ± 0·43*** | 0·62 ± 0·23** | −1·29 ± 0·53* |
| inj (testosterone − control) | 0·45 ± 0·64 | −0·49 ± 0·42 | −0·19 ± 0·23 | 0·64 ± 0·52 |
| Interactions | | | | |
| backgr∗castr | 1·18 ± 1·28 | 0·48 ± 0·86 | 0·32 ± 0·47 | 0·86 ± 0·96 |
| backgr∗inj | −0·91 ± 1·27 | 1·38 ± 0·85 | −0·53 ± 0·47 | −0·40 ± 0·95 |
| castr∗inj | 2·15 ± 0·92* | 1·06 ± 0·60 | 0·68 ± 0·32* | 1·46 ± 0·74 |
| backgr∗cast∗inj | 0·70 ± 1·54 | 0·45 ± 1·02 | 0·84 ± 0·56 | 0·38 ± 1·19 |
| (ii) Natural log transformed | | | | |
| Main effects | | | | |
| backgr (Fat − Lean) | 0·09 ± 0·04* | 1·39 ± 0·08*** | 1·47 ± 0·10*** | −0·08 ± 0·04* |
| castr (full − sham) | −0·02 ± 0·02 | 0·17 ± 0·04*** | 0·15 ± 0·04** | −0·05 ± 0·02* |
| inj (testosterone − control) | 0·02 ± 0·02 | 0·02 ± 0·03 | 0·03 ± 0·04 | 0·02 ± 0·02 |
| Interactions | | | | |
| backgr∗castr | 0·04 ± 0·04 | −0·07 ± 0·07 | −0·03 ± 0·10 | 0·03 ± 0·04 |
| backgr∗inj | −0·03 ± 0·04 | −0·11 ± 0·07 | −0·14 ± 0·10 | −0·01 ± 0·04 |
| castr∗inj | 0·07 ± 0·03* | 0·08 ± 0·05 | 0·16 ± 0·06* | 0·06 ± 0·03 |
| backgr∗castr∗inj | 0·02 ± 0·05 | −0·01 ± 0·09 | 0·004 ± 0·11 | 0·01 ± 0·04 |

For further details see heading and notes to Table 1.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$; approximate significance obtained using a *t*-test with 50 d.f.

(Hastings & Hill, 1989). 'Lean weight' was estimated as fat-free body weight. To confirm the accuracy of these estimates, samples of mice were analysed chemically to determine fat, crude protein and ash contents. Two samples were taken from each of the 16 experimental groups (i.e. two selection criteria × two directions of selection × two treatments × two injection protocols), each sample consisting of three mice sampled from different cages and families.

(iii) *Statistical analysis*

Body weight, estimated fat content, and estimated lean weight at age 70 days were analysed for each line separately using the restricted maximum likelihood (REML) option of the Genstat statistical package (Genstat, 1988). The model fitted the (fixed) effects of genetic background (high- or low-selected), castration (full or sham) and injection (testosterone or oil) with the additional random effects of family (incorporating differences in date of birth, litter size, etc.) and cage, and the fixed effect of injection batch and, where appropriate, the fixed effect of drying batch. Significance of effects was tested using a *t*-test with 50 degrees of freedom (d.f.) for data obtained from individual animals, and 7 d.f. for data obtained from pooled chemical analysis. The data were also transformed onto a natural logarithmic scale and re-analysed to check that the large differences between the lines did not generate interactions which could be attributed to scale effects.

## 3. Results

The group means for the *F* lines are shown in Table 1, and the results of their statistical analysis in Table 2. Similarly the group means and statistical analyses of the *P* lines are shown in Tables 3 and 4. The chemical analysis of body composition is presented, as is normal, as a percentage of dry weight and the corresponding weights of each component are also presented. The components are estimated independently and, as is usual in this situation, do not sum to 100%. The results are in good agreement with the estimates made on water content (and expressed on a 'wet' body weight basis). There is known to be a close relationship between fat content estimated from water content and fat weight obtained chemically (e.g. Hastings & Hill, 1989); in the present study the correlation coefficient is 0·98 (analysis of data from Tables 1 and 3). Thus only the analyses of estimated body composition are given in Tables 2 and 4 as there are more degrees of freedom, and random effects such as cage and family can be removed from the analysis. The analyses of chemical composition gave the same pattern of results and are omitted for brevity. The fixed factors of drying batch and injection group were significant for some traits. This means that the

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Table 3. *P lines: means of various growth and composition traits*

| | High | | | | Low | | | | Mean SE | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Castrated | | Sham operated | | Castrated | | Sham operated | | | |
| | Testosterone ($n = 9$) | Oil ($n = 6$) | Testosterone ($n = 8$) | Oil ($n = 11$ | Testosterone ($n = 11$) | Oil ($n = 13$) | Testosterone ($n = 10$) | Oil ($n = 11$) | High | Low |
| Body weights (g) | | | | | | | | | | |
| 2 weeks of age | 8·1 | 7·7 | 8·6 | 8·9 | 5·1 | 5·6 | 5·3 | 5·4 | 0·39 | 0·24 |
| 4 weeks of age | 19·8 | 18·9 | 21·7 | 22·3 | 8·6 | 9·5 | 9·0 | 8·9 | 1·38 | 0·50 |
| 6 weeks of age | 34·8 | 30·2 | 37·7 | 38·0 | 13·6 | 13·6 | 14·9 | 14·0 | 1·54 | 0·49 |
| 8 weeks of age | 45·4 | 39·4 | 47·6 | 46·8 | 16·1 | 15·3 | 16·4 | 15·7 | 1·50 | 0·62 |
| 10 weeks of age | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a |
| 10 weeks of age (fasted) | 51·7 | 42·9 | 49·9 | 49·4 | 16·8 | 15·9 | 16·5 | 15·6 | 1·57 | 0·59 |
| Estimates from dry matter content | | | | | | | | | | |
| Water wt (g) | 34·2 | 27·8 | 33·9 | 34·2 | 11·3 | 10·5 | 11·4 | 10·5 | 1·09 | 0·38 |
| Fat (% ww) | 8·1 | 9·4 | 6·1 | 4·6 | 6·9 | 7·9 | 4·7 | 6·4 | 1·02 | 0·68 |
| Fat wt (g) | 4·2 | 4·1 | 3·0 | 2·2 | 1·2 | 1·3 | 0·79 | 0·98 | 0·51 | 0·13 |
| Lean wt (g) | 47·5 | 38·7 | 46·8 | 47·2 | 15·6 | 14·6 | 15·8 | 14·6 | 1·49 | 0·53 |
| Chemical carcass composition | | | | | | | | | | |
| Fat (% dry wt) | 30·3 | 34·1 | 24·9 | 24·0 | 21·7 | 24·1 | 17·1 | 24·0 | 1·93 | 2·17 |
| Protein (%) | 54·2 | 51·1 | 59·2 | 59·6 | 62·4 | 60·0 | 65·5 | 60·0 | 1·50 | 1·79 |
| Ash (%) | 9·0 | 9·0 | 9·9 | 10·3 | 10·5 | 11·0 | 11·9 | 11·2 | 0·35 | 0·44 |
| Fat wt (g) | 5·28 | 5·39 | 3·95 | 3·78 | 1·31 | 1·28 | 0·92 | 1·19 | 0·51 | 0·13 |
| Protein wt (g) | 9·40 | 8·02 | 9·40 | 9·36 | 3·73 | 3·20 | 3·52 | 3·02 | 0·19 | 0·14 |
| Ash wt (g) | 1·57 | 1·41 | 1·57 | 1·62 | 0·63 | 0·59 | 0·64 | 0·56 | 0·04 | 0·02 |

For further details see heading and notes to Table 1.

Table 4. *P lines: REML estimates (±SE) of the effects of genetic background (backgr), castration (castr), injection (inj), and interactions thereof, on selected growth and composition traits as estimated from dry matter content*

| | 10 week wt (g) (fasted) | Fat (% wet wt) | Fat (g) | Lean (g) |
|---|---|---|---|---|
| (i) Untransformed | | | | |
| Main effects | | | | |
| backgr (High − Low) | 31·57 ± 1·06*** | 0·74 ± 0·59 | 2·36 ± 0·25*** | 29·37 ± 0·98*** |
| castr (full − sham) | −0·98 ± 0·61 | 3·09 ± 0·46*** | 1·06 ± 0·19*** | −2·14 ± 0·60** |
| inj (testosterone − control) | 3·33 ± 0·60** | −0·46 ± 0·44 | 0·21 ± 0·19 | 3·14 ± 0·62** |
| Interactions | | | | |
| backgr∗castr | −1·62 ± 1·10 | 0·73 ± 0·71 | 0·60 ± 0·31 | −2·47 ± 1·06* |
| backgr∗inj | 2·06 ± 1·09 | 0·61 ± 0·70 | 0·28 ± 0·30 | 1·89 ± 1·07 |
| castr∗inj | 2·32 ± 0·86* | 0·04 ± 0·64 | 0·03 ± 0·27 | 2·18 ± 0·86* |
| backgr∗castr∗inj | 2·19 ± 1·37 | 0·86 ± 0·94 | 0·16 ± 0·40 | 2·45 ± 1·34 |
| (ii) Natural log transformed | | | | |
| Main effects | | | | |
| backgr (High − Low) | 1·07 ± 0·03*** | 0·06 ± 0·09 | 1·13 ± 0·10*** | 1·07 ± 0·03*** |
| castr (full − sham) | −0·01 ± 0·02 | 0·52 ± 0·07*** | 0·50 ± 0·08*** | −0·05 ± 0·02* |
| inj (testosterone − control) | 0·09 ± 0·02** | −0·02 ± 0·07 | 0·07 ± 0·07 | 0·10 ± 0·02** |
| Interactions | | | | |
| backgr∗castr | −0·04 ± 0·04 | 0·12 ± 0·11 | 0·07 ± 0·12 | −0·06 ± 0·04 |
| backgr∗inj | 0·02 ± 0·04 | 0·14 ± 0·11 | 0·17 ± 0·12 | 0·02 ± 0·04 |
| castr∗inj | 0·05 ± 0·03 | −0·04 ± 0·10 | 0·02 ± 0·11 | 0·05 ± 0·03 |
| backgr∗castr∗inj | 0·04 ± 0·05 | 0·16 ± 0·15 | 0·11 ± 0·16 | 0·06 ± 0·05 |

For further details see heading and notes to Table 1.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$; approximate significance obtained using a $t$-test with 50 df.

magnitude of effects estimated by fitting a full REML model may differ from those obtained by a crude comparison of group means, the most notable example being the effect of castration on fat percentage in the *F* line, which was 1·68 % ± 0·43 % by REML (Table 2) but only 1·02 % by comparison of means (Table 1).

Castration decreased the body weight at 10 weeks by about 8–9 % in the Fat and Lean *F* lines (comparison of sham/oil with castration/oil in Table 1). Administration of exogenous testosterone restored normal growth (castration/testosterone *v*. sham/oil in Table 1). This reduced the fixed (or independent) effect of castration, which is non-significant (Table 2), whereas the interaction between treatment and injection is positive and significant. The results were less clear in the *P* lines: castration seemed to reduce body weight in the High but not the Low lines (sham/oil *v*. castration/oil in Table 3). The injection of exogenous testosterone increased body weight by an average of 10 %, apparently as a result of increased lean mass. There was a significant interaction between treatment and injection in the *P* lines on analysis of untransformed data, but this effect was removed by logarithmic transformation. The effects on body composition were the same in the *F* and *P* lines: 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 treatment were non-significant.

## 4. Discussion

The results of this study are similar to those obtained previously by Siddiqui *et al.* (1989) for lines divergently selected on IGF-1 levels (which also exhibited a correlated divergence in weight at 10 weeks of age) in three important respects. Firstly, castration resulted in an 8–9 % reduction in the body weight at 10 weeks. Secondly, the administration of exogenous growth hormone restored normal growth rate. Thirdly, interactions with genetic background were absent in both studies. This last result is at variance with that reported by Hooper *et al.* (1986), who noted a significant interaction with genetic background: castration reduced body weight by approximately 16 % in a line selected for increased body weight compared with 8 % in an unselected control line (testosterone levels had altered in these lines: O'Kean *et al.*, 1986). There are two possible reasons for these differing results. Firstly, there may have been more genetic variance associated with testosterone metabolism in the base population of the lines studied by Hooper *et al.* (1986) and this variance was utilized in the response to selection. Secondly, testosterone metabolism may differ between their lines purely by random genetic

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drift rather than as a consequence of selection. Since their lines were not replicated, it is impossible to distinguish between the two hypotheses. The same lack of replication applies in our lines, but it seems unlikely that the effects of random genetic drift and selection would be both opposite and equal such that significant interactions were obscured.

Testosterone is known to increase aggression in most mammals, including mice (e.g. deRuiter *et al*., 1992; Sluyter *et al*., 1996), and there is currently some concern that intense selection in commercial animals may cause correlated changes in undesirable behavioural traits (although a previous behavioural study suggested this was not the case in these lines: Holmes & Hastings, 1995). 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.

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 (5-fold) response to selection in the *F* lines, despite the fact that castration altered composition in both lines, and that mice from the Fat line have smaller testes. Similarly, it had little or no differential effect in the *P* lines, which differ 3-fold in body weight. 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 & Falconer, 1978; Hastings *et al*., 1993) and between leptin sensitivity (at least in mean if not variance of response) and genetic background in lines selected on fat content (Bunger & Hill, 1997). Taken together, these results support a model of selection response in which genetic differences acting through many physiological systems contribute to the response, rather than the differences acting through one main system.

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## References

Beniwal, B. K., Hastings, I. M., Thompson, R. & Hill, W. G. (1992). Estimation of changes in genetic parameters in selected lines of mice using REML with an animal model. II. Body weight, body composition and litter size. *Heredity* **69**, 361–371.

Bunger, L. & Hill, W. G. (1997). Effects of leptin administration on long-term selected fat mice. *Genetical Research*, in press.

Buttery, P. J., Haynes, N. B. & Lindsay, D. P. (1986). *Control and Manipulation of Animal Growth*. London: Butterworth.

DeRuiter, A. J. H., Koolhaas, J. M., Keijser, J. N., Van-Oortmerssen, G. A. & Bohus, B. (1992). Differential testosterone secretory capacity of the testes of aggressive and nonaggressive house mice during ontogeny. *Aggressive Behaviour* **18**, 149–157.

Donahue, L. R., Watson, G. & Beamer, W. G. (1993). Regulation of metabolic water and protein compartments by insulin-like growth factor-I and testosterone in growth hormone deficient *lit/lit* mice. *Journal of Endocrinology* **139**, 431–439.

Genstat 5 Committee (1988). *Genstat 5 Reference Manual*. Oxford: Oxford University Press.

Goddard, C., Wilkie, R. S. & Dunn, I. C. (1988). The relationship between insulin-like growth factor-1, growth hormone, thyroid hormones and insulin in chickens selected for growth. *Domestic Animal Endocrinology* **5**, 165–176.

Hastings, I. M. & Hill, W. G. (1989). A note on the effects of different selection criteria on carcass composition in mice. *Animal Production* **48**, 229–233.

Hastings, I. M. & Hill, W. G. (1993). The effects of cage type on murine body composition. *Mouse Genome* **91**, 329–330.

Hastings, I. M., Yang, J. & Hill, W. G. (1991). Analysis of mice selected on body weight. 4. Correlated responses in growth and reproduction. *Genetical Research* **58**, 253–259.

Hastings, I. M., Bootland, L. H. & Hill, W. G. (1993). The role of growth hormone in lines of mice divergently selected on body weight. *Genetical Research* **61**, 101–106.

Hill, W. G., Marks, P. J., Jenkins, J. C. & Land, R. B. (1990). Selection on testis size as an indicator of maturity in growing animals. I. Direct and correlated responses in growth. *Genetics*, *Selection*, *Evolution* **22**, 231–246.

Holmes, I. & Hastings, I. M. (1995). Behavioural changes as a correlated response to selection in mice. *Genetical Research* **66**, 27–33.

Hooper, A. C. B., Brien, T. G. & Lawlor, P. G. (1986). The effects of orchidectomy and the role of testosterone in determining the growth of male mice selected for increased body weight. *Andrologia* **18**, 509–515.

Medrano, J. F., Pomp, D., Sharrow, L., Bradford, G. E., Downs, T. R. & Frohman, L. A. (1991). Growth hormone and insulin-like growth factor-1 measurement in high growth (*hg*) mice. *Genetical Research* **58**, 67–74.

O'Kean, J. C., Brien, T. G., Hooper, A. C. B. & Graham, A. (1986). Testicular activity in mice selected for increased body weight. *Andrologia* **18**, 190–195.

Pidduck, H. G. & Falconer, D. S. (1978). Growth hormone function in strains of mice selected for large and small size. *Genetical Research* **32**, 195–206.

Siddiqui, R. A., McCutcheon, S. N., MacKenzie, D. D. S., Blair, H. T., Ormsby, J. E., Gluckman, P. D. & Breier, B. H. (1989). Role of testosterone in regulating the growth of mice from lines selected for low *v*. high plasma insulin-like growth factor-1 concentrations. *Acta Endocrinologica* **121**, 686–690.

Sluyter, F., VanOortmerssen, G. A., DeRuiter, A. J. H. & Koolhaas, J. M. (1996). Aggression in wild house mice-current state of affairs. *Behaviour Genetics* **26**, 497–504.