Genet. Res., Camb. (1989), **54**, *pp.* 155–160 *Printed in Great Britain*

Analysis of lines of mice selected for fat content. 1. Correlated responses in the activities of NADPH-generating enzymes.

EMMANUEL A. ASANTE[1], WILLIAM G. HILL[2] AND GRAHAME BULFIELD[1]*
[1] *Gene Expression Group, AFRC Institute of Animal Physiology and Genetics Research, Edinburgh Research Station, Roslin, Midlothian EH25 9PS*
[2] *Institute of Animal Genetics, University of Edinburgh, West Mains Road, Edinburgh EH9 3JN*

(*Received 7 November 1988 and in revised form 2 March 1989*)

Summary

Estimates of the activities (V_{max}) of four enzymes that generate the coenzyme NADPH, an absolute requirement for tissue fatty-acid synthesis, and of the concentration of NADP plus NADPH were made in lines of mice differing in fat content. These lines had been selected from the same base population for 20 generations, and 3 high, 3 low replicates and 1 unselected control were used. Analyses were performed on liver and gonadal fat pad (GFP) of males at 5 and 10 weeks of age. In both the liver and the GFP, measurable activities of the four enzymes: glucose-6-phosphate dehydrogenase (G6PDH), 6-phosphogluconate dehydrogenase (6PGDH), isocitrate dehydrogenase (IDH) and malic enzyme (ME) expressed per mg soluble protein were, with minor exceptions, higher in the Fat (F) than in the Lean (L) lines at both ages; the highest ratio being 2·2 for ME in the GFP. The relationships between these measurable activities (V_{max}) and *in vivo* lipogenesis are not however known. When expressed per gram tissue, the ratios for F to L in the GFP were less than 1 in most cases, presumably because of the very different adipocyte numbers and/or sizes between the lines. There were no significant differences between the lines in the concentration of NADP plus NADPH per gram tissue in liver or GFP, suggesting that F lines converted NADP to NADPH faster than L lines. It is predicted that selection on the enzyme activities would be less efficient than direct selection at changing fat content.

1. Introduction

Carcass fat content of meat animals is of great concern to both the consumer and the producer. It shows substantial genetic variation; for example it is possible to select lines to differ greatly in body fat content (e.g. LeClercq, Blum & Boyer, 1980; Sharp, Hill & Robertson, 1984). Carcass fat deposition is a component of growth presumably controlled by many genes, whose identities and functions are not known. An understanding of the genetic basis of fatness will be improved by an increased knowledge of its underlying biochemical and physiological mechanisms.

One approach that has been adopted in pigs is to investigate the relationship between NADPH-generating enzyme activities and backfat thickness by selecting high and low lines of pigs for eight generations on the sum of the specific activities of four NADPH-generating enzymes (Muller, 1986). This experiment was based on the premise that NADPH is required in the *de novo* biosynthesis of fatty acids (Langdon, 1957) and, therefore, the activities of the enzymes producing this coenzyme could be expected to have a direct effect on rates of lipogenesis. The enzymes measured were glucose 6-phosphate dehydrogenase (G6PDH), 6-phosphogluconate dehydrogenase (6PGDH), malic enzyme (ME), and isocitrate dehydrogenase (IDH). There was a direct response of 2·6 phenotypic standard deviations (high–low) in the sum of enzyme activities and a correlated response of 3·6 phenotypic standard deviations in backfat thickness, the line with higher enzyme activities being fatter. The corollary of this selection experiment is that if animals were selected directly for fatness the activities of NADPH-generating enzymes would be altered.

Lines of mice have been selected for high and low fatness (Sharp *et al.* 1984) and these can now be used to investigate the genetic control of fatness at the

* Corresponding author.

https://doi.org/10.1017/S0016672300028536 Published online by Cambridge University Press

physiological level. We report on the findings of an experiment conducted in mice selected for fat content and extend it to the total tissue concentration of NADP and NADPH.

2. Materials and methods

(i) *Animals*

Fat (F) and Lean (L) lines of mice were established (Sharp *et al.* 1984) through divergent selection for 20 generations on the ratio of gonadal fat pad (GFP) weight to body weight in 10-week-old males (Hastings & Hill, 1989). There were three replicates and within each there were three contemporary lines: F, L and Control (C). Selection was relaxed after generation 20 and the lines were then maintained without selection for biochemical/physiological analyses. These relaxed lines were analysed again at generation 29 and found to have shown little regression towards C. The percentage body fat estimated from the ratio of dry weight to wet body weight at 10 weeks of age had decreased from 16·0 to 15·3% in F line and increased from 6·42 to 7·55 in L line (I. M. Hastings, personal communication).

(ii) *Design of experiment*

Ten male mice were sampled from each of the three replicates of F and L lines and one replicate of C of generations 24 and 25 at 5 and 10 weeks of age. These two ages were chosen because 10 weeks was the age of selection and 5 weeks was at the maximum growth phase and 2 weeks after weaning.

A separate experiment was carried out to determine the metabolite pool size of NADP+NADPH. Six male mice per line were sampled from the same lines at both 5 and 10 weeks of age.

(iii) *Sample preparation*

(*a*) *For enzyme assay*. To eliminate the possible effects of diurnal variation on lipogenesis, the mice were killed by cervical dislocation after stunning between 09·00 and 10·00 h. The abdominal cavity was opened up and the liver and gonadal fat pads (GFP) quickly excised, weighed and placed on ice-cooled aluminium foil. The GFPs, like the liver, are a discrete depot that can be dissected out quickly and accurately. At 5 weeks of age the GFPs were pooled from pairs of mice because of their small sizes.

The ice-cooled liver and GFP were homogenized five times with three times v/w of ice-cold buffer (0·25 M Aristar sucrose, 1 mM dithiothreitol, 1 mM-EDTA di-sodium salt, 0·05 M Tris pH 7·4) using a Tri-R (Camlab) homogenizer. The homogenization lasted about 10 s per sample after which the tube was quickly returned to an ice bucket. The homogenate was transferred into 10 ml centrifuge tubes and spun at 60000 ***g*** for 60 min at 5 °C. The mitochondria and other subcellular particles were sedimented at this speed, their integrity being preserved by the isotonic sucrose in the buffer.

The cell-free supernatant was aspirated with fine-tip Pasteur pipettes, put into disposable Eppendorf tubes pre-cooled on ice, and used as the tissue extract for all enzyme assays. All operations were carried out at 0–5 °C before incubation to 30 °C in an LKB Reaction Rate Analyser. Any dilutions necessary for liver extracts were done with the extraction buffer. No dilution was necessary for the GFP extracts. The enzyme assays were carried out immediately and the remaining supernatants were stored at −80 °C for protein determination later.

(*b*) *For coenzyme assay*. The mice were killed under similar conditions except that the liver and GFPs were quickly frozen in liquid nitrogen and kept at −80 °C until analysed. No pooling of GFP tissue was done at 5 weeks of age.

The tissues were partially thawed and homogenized as for the enzymes. For the liver a 10-fold dilution and for the GFPs 5-fold dilution was made with buffer. The diluted homogenates in test tubes were immersed in a boiling water bath for 5 min. The tubes were cooled on an ice bath and then centrifuged for 10 min at 4 °C, at 1000 ***g***. The supernatant solution was aspirated and kept on ice and used for the assay of NADP plus NADPH.

(iv) *Assays*

(*a*) *Enzymes*. The activities of all the four enzymes (G6PDH, EC 1·1·1·49; 6PGDH, EC 1·1·1·44; ME, EC 1·1·1·40; IDH, EC 1·1·1·42) were measured by connecting them to NADP-linked reactions according to Bulfield & Moore (1974). Spontaneous reaction

Table 1. *Means pooled over replicates of body weight (BW), liver weight (LW) and gonadal fat pad weight (GFPW) of mice used for enzyme activity determinations, and ratios of values for the fat and lean lines (F/L ratio). Ten mice were taken at each age in each replicate (3 fat, 1 control, 3 lean)*

	BW (g)	LW (g)	GFPW (g)	GFPW/BW (mg/g)
5 weeks				
Fat	23·6	1·45	0·381	16·1
Control	25·8	1·64	0·377	14·1
Lean	23·1	1·42	0·221	9·6
F/L ratio	1·02	1·02	1·72	1·68
10 weeks				
Fat	37·9	2·22	0·845	22·3
Control	41·0	2·27	0·529	12·9
Lean	35·9	2·13	0·284	7·9
F/L ratio	1·06	1·05	3·04	2·82

https://doi.org/10.1017/S0016672300028536 Published online by Cambridge University Press

Table 2. *Mean activities, pooled over replicates, of the four enzymes with residual standard deviations* (S.D.), *and standard error* [S.E.(F–L)] *and significance of the difference between fat and lean lines estimated from between replicate line mean square*[a]

	Liver				Gonadal fat pad			
	G6PDH	6PGDH	ME	IDH	G6PDH	6PGDH	ME	IDH
5 weeks	Activity as μmol/min per g tissue							
Fat	2·18	3·73*	7·71	30·7	4·06	2·00	2·89*	2·66
Control	1·50	3·30	4·42	28·9	5·20	2·05	3·33	2·81
Lean	1·41	2·80	6·71	28·1	4·88	2·10	1·84	2·61
F/L ratio	1·55	1·35	1·14	1·10	0·83	0·95	1·57	1·02
S.D.	0·560	0·402	1·83	2·78	1·183	0·461	0·859	0·609
S.E. (F–L)	0·520	0·214	2·032	2·59	0·576	0·254	0·339	0·275
	Activity as nmol/min per mg soluble protein							
Fat	29·5	49·4	94·0	404	427	207	311	256
Control	8·3	41·8	92·2	356	344	179	234	212
Lean	18·9	34·5	74·5	352	327	186	138	201
F/L ratio	1·56	1·43	1·26	1·15	1·31	1·11	2·25	1·27
S.D.	8·14	5·77	24·5	37·2	133	97·0	74·3	63·2
S.E. (F–L)	10·05	6·38	45·7	76·0	111	89·8	97·4	75·7
10 weeks	Activity as μmol/min per g tissue							
Fat	1·02	2·79*	7·75	32·9	1·88**	0·84**	0·82	1·20**
Control	0·82	2·37	6·32	29·1	3·72	1·41	1·07	1·86
Lean	1·01	2·35	6·51	30·0	4·47	1·48	0·89	2·06
F/L. ratio	1·01	1·19	1·19	1·10	0·42	0·57	0·92	0·58
S.D.	0·239	0·276	2·59	2·74	0·878	0·330	0·274	0·284
S.E. (F–L)	0·131	0·142	0·703	1·57	0·333	0·134	0·105	0·140
	Activity as nmol/min per mg soluble protein							
Fat	11·4	31·2**	86·8	369*	284	131**	122**	185
Control	8·9	25·7	68·2	319	233	82	64	96
Lean	11·5	26·9	74·4	348	270	89	54	126
F/L ratio	0·99	1·16	1·17	1·06	1·05	1·48	2·26	1·47
S.D.	2·84	3·25	18·15	38·6	82·0	41·5	36·9	43·1
S.E. (F–L)	1·30	0·73	6·26	7·2	12·5	5·3	9·8	25·7

* $P < 0·05$; ** $P < 0·01$.

[a] Degrees of freedom: 63 for residual and 4 for replicate line, except at 5 weeks for: liver per mg soluble protein, 45 and 2, GFP per mg soluble protein, 20 and 2, and GFP per g tissue, 28 and 4, respectively, due to pooling of samples and lack of a replicate.

was observed for only G6PDH, but this was largely reduced by leaving the reaction mixture plus enzyme extract on the bench for 15 min prior to addition of the substrate.

(*b*) *Protein.* Protein concentration in the stored (−80 °C) supernatants was determined with the BCA Protein Assay Reagent supplied by Pierce Chemical Company (UK Ltd).

(*c*) *Coenzyme.* The microassay of the concentration of NADP and NADPH was done according to Nisselbaum & Green (1969), with the following modifications: G6PDH was used at a concentration of 200 U/ml and reliable absorbance was achieved after 5 min of incubation.

(v) *Statistical analysis*

The specific activities of enzymes were expressed on both a per gram tissue and a per mg soluble protein basis. The analysis of variance was done within tissues, and ages using the following model

$$Y_{ijk} = u + D_i + L_{ij} + e_{ijk},$$

where Y_{ijk} is the observation on the kth sample of the jth replicate of the ith direction of selection; u is the overall mean; D_i is the effect of the ith direction of selection (F, L, C; $i = 1, 2, 3$); L_{ij} is the effect of genetic drift or sampling of replicate lines ($j = 1, 2, 3$); and e_{ijk} is the residual error. Differences between F

https://doi.org/10.1017/S0016672300028536 Published online by Cambridge University Press

and L mice were compared using the L_{ij} mean square as the valid error to include drift effects. These tests lack power, however, as there were only 4 D.F. for the replicate line mean square. Also, activity per mg soluble protein was not determined for replicate 1 at 5 weeks; so there are then 2 D.F.

3. Results

The lines had similar body and liver weights at both ages (Table 1). F lines averaged 1·7-fold higher GFP weight than L line at 5 weeks and 3-fold higher at 10 weeks of age (Table 1).

Regardless of whether the data were expressed on a per gram wet weight or on a per mg protein basis, F lines showed higher hepatic enzyme activities than L lines, the only exception being for G6PDH at 10 weeks where there was no difference between them. (Table 2). Depending on how data were expressed F lines averaged 10–55 and 1–19% higher enzyme activities than L at 5 and 10 weeks of age respectively. Of the four NADPH-generating enzymes, IDH had the highest measurable activity (V_{max}) followed by ME, 6PGDH and G6PDH. The two hexose monophosphate shunt enzymes, G6PDH and 6PGDH, exhibited lower hepatic measurable activity than ME and IDH, but a higher F/L ratio.

In the GFP the activities of G6PDH and 6PGDH were higher in L than F lines at both 5 and 10 weeks of age when specific activities were expressed on a per gram GFP basis (Table 2). IDH and ME showed higher activities per gram GFP in F than L lines at 5 weeks. When activities were expressed on a per mg soluble protein basis, activities of all four enzymes were higher in F than L lines, but ME was the only enzyme which showed a 2-fold difference on a per mg protein basis at both 5 and 10 weeks of age. The sum of activities of the four enzymes followed closely the pattern of changes in the activities of G6PDH and 6PGDH. Though the control was sometimes higher than the selected lines, as for example in G6PDH activity on a per gram basis at 5 weeks of age, no significant asymmetry [i.e. $(F+L)/2-C$, data not shown] was found for any enzyme at either age.

In the GFP the ranking in a descending order on enzyme activities on a per mg protein basis was as follows: G6PDH > 6PGDH = ME = IDH (Table 2), this order being completely different from that found in the liver.

The concentration of the coenzymes NADP+ NADPH in each tissue were similar in F and L lines (Table 3), but C line had higher levels and also higher body and organ weights than the selected lines (data not shown). Whereas there was no significant difference between high and low lines in concentration, there was significant asymmetry and substantial differences between replicates (data not shown).

Table 3. *Mean concentrations, pooled over replicates of NADP+NADPH. Other symbols as Table 2. Six mice were taken at each age, in each replicate (3 fat, 1 control, 3 lean)*

	NADP+NADPH concentration (μg/g)			
	5 weeks		10 weeks	
	Liver	GFP	Liver	GFP
Fat	43·0	17·7	43·4	17·3
Control	59·8	24·7	50·8	18·3
Lean	49·0	16·1	43·8	17·9
F/L ratio	0·88	1·10	0·99	0·97
S.D.	5·87	3·05	6·65	5·88
S.E. (F–L)	1·70	1·51	0·80	0·60

Table 4. *Sum of activities of the four enzymes, pooled over replicates*

	Activity (nmol/min per mg soluble protein)			
	5 weeks		10 weeks	
	Liver	GFP	Liver	GFP
Fat	577	1200	498	722
Control	498	969	421	475
Lean	480	853	460	538
F/L ratio	1·20	1·41	1·08	1·34

4. Discussion

Muller (1986) altered backfat thickness by direct selection of pigs on the sum of NADPH-generating enzyme activities. We have found that the specific activities (V_{max}) of G6PDH, 6PGDH, ME and IDH are higher in the liver and GFP of our F-line mice than the L mice. Although the capacity to synthesize NADPH is higher in F than L lines, the actual rate of synthesis of NADPH *in vivo* is not known. The association between fatness and the activity of NADPH-generating enzymes is likely to be a direct response to selection because replicated lines were used, minimising the possibility that it is due to genetic drift (Falconer, 1973). It is therefore likely that the 10–55% increase in the activity of the four enzymes in the liver at 5 weeks may be a biologically significant metabolic change.

In spite of the relatively lower hepatic activities of G6PDH and 6PGDH, the between-line differences (F/L) at 5 weeks of age suggest that selection may have had a greater effect on these two enzymes (35–55%) than on ME and IDH (10–14%).

In the GFP a comparison between the two modes of expression of results (per gram tissue *vs.* per mg protein) for G6PDH and 6PGDH at 5 weeks, and for all four enzymes at 10 weeks leads to opposite conclusions from what are essentially the same results.

https://doi.org/10.1017/S0016672300028536 Published online by Cambridge University Press

On a per gram basis, L line exhibited a higher activity of G6PDH and 6PGDH than F line in most cases. Such a situation would arise if F line had substantially bigger adipocytes than L resulting in a dilution effect of the enzyme activities, but the cell numbers in the gonadal fat tissues of these mouse lines have not yet been investigated. IDH appeared to have a much lower measurable activity in the GFP than in the liver. It was concluded from experiments on isolated rat adipocytes, that cytosolic NADPH-linked isocitrate dehydrogenase does not substantially contribute to the formation of cytosolic NADPH (Kather & Brand, 1975). A possible conclusion from the present study is that IDH has a lower capacity to generate NADPH in the GFP than in the liver, though the *in vivo* situation is not known. This would represent a complete reversal of its apparent role in the liver and would serve to emphasize that observations made in one tissue within a species cannot be extrapolated to another tissue, let alone to another species.

When the results for the GFP were expressed on a per mg soluble protein basis, F line consistently had higher activities of all four enzymes than L line. This highlights the problem of the most suitable way of expressing these types of results, especially in the adipose tissue where cell sizes and numbers play an important part. Total activity merely reflects changes in organ sizes and not necessarily metabolic changes. Since enzymes are proteins and protein synthesis is a function of metabolic capacity, expressing the results on a per mg soluble protein seems more appropriate.

ME was the only enzyme in the GFP which maintained more than a 2-fold difference between F and L lines at both ages on a per mg protein basis, suggesting that this enzyme could play an important role in lipid metabolism.

Muller (1986) summed the measurable activities of the four enzymes and used that as the selection criterion. Although the sum of activities may not be biologically meaningful, in the present study, in both the liver and GFP the sum was higher in F lines than L lines, the highest difference being 41% (in GFP at 5 weeks, Table 4). Providing that the rate of turnover of lipids is very low in the selected lines, however, the cumulative effect of a smaller difference in the sum of the enzyme activities may be sufficient to account for a 3-fold difference in GFP weight at 10 weeks of age.

The higher correlated response in backfat thickness ($3{\cdot}6\sigma_B$) than the direct response in the sum of NADPH-generating enzyme activities ($2{\cdot}5\sigma_E$) obtained by Muller (1986) in pigs, would require that $h_B r_A > h_E$ where h_B^2 is the heritability of backfat, h_E^2 is the heritability of the sum of NADPH-generating enzyme activities, σ_B and σ_E are the corresponding phenotypic standard deviations and r_A is the genetic correlation between backfat thickness and NADPH-generating enzyme activities. This implies a higher heritability for backfat than for the sum of enzyme activities, and a genetic correlation of 1, or nearly, between the two traits. A realized heritability of 0·88 was reported for the sum of NADPH-generating enzyme activities (Muller, 1985) implying a heritability of backfat thickness greater than 0·88, well above typical figures, e.g. Hudson & Kennedy (1985). The size of the correlated response in fatness is therefore surprisingly large.

It is possible to predict what the correlated response in fatness (CR_F), measured as the ratio of GFP weight to body weight (F, mg/g) will be, when selection is based on enzyme activity, measured for example as the sum of activities of the four NADPH-generating enzymes in the GFP (E, nmol/mg protein) from data on the corresponding correlated responses in activity (CR_E) when selection is based on fatness (Hill, 1985). Assuming the same selection intensities were practised in each case, it can be shown that $CR_E/\sigma_E = CR_F/\sigma_F$, where σ_E and σ_F are the corresponding phenotypic standard deviations (Falconer, 1984). Using the F–L divergence at 5 weeks $CR_E = 347$, and the residual standard deviation which was computed as $\sigma_E = 288$, then $CR_E/\sigma_E = 1{\cdot}20$; the corresponding values for 10 weeks of age are 184, 173 and 1·06 respectively.

The direct response, R_F, given by the F–L divergence from selection on the ratio of GFP weight to body weight at 10 weeks is 14·4 mg/g (Table 1), and $\sigma_F = 4{\cdot}09$ (from Sharp *et al.* 1984). So $R_F/\sigma_F = 3{\cdot}52$, about three times CR_E/σ_E (1·20) which, according to the relationship given above, should be equal to CR_F/σ_F. This implies that indirect selection on enzyme activities would be about one-third as effective as direct selection if the same selection intensity were practised. There are of course many riders to this argument: total carcass fat may have been changed more effectively, selection was practised in only one sex and might have been improved by selecting in both sexes, and species differences have to be taken into consideration. Nevertheless, our results do not fit well with those of Muller (1985; 1986).

There is practically no difference between F and L lines in the metabolite pool size of NADP+NADPH. These results suggest that the important metabolic alteration is not in the metabolite pool size of NADP+NADPH between the lines, nor in the activities of any individual NADPH-generating enzyme (though this aspect has been altered) but rather in the rate of interconversion between the available NADP and NADPH. The coenzyme is probably present in both F and L lines at the same level, implying that the *in vitro* measurement of rate of production of NADPH is a close reflection of *in vivo* conditions. F lines appear to turn over NADP+NADPH faster than L, suggesting a higher rate of lipogenesis.

In conclusion, NADPH-generating enzyme activities in these F and L lines of mice have been altered by divergent selection for fatness, although the concentration of NADP+NADPH remains unchanged. These results indicate that the capacity to synthesize

https://doi.org/10.1017/S0016672300028536 Published online by Cambridge University Press

NADPH and hence lipogenesis is increased in F-line mice but they do not reveal the situation *in vivo*. The next stage will be to analyse *de novo* lipogenesis in F- and L-lean mice.

This work was supported by a Linked Research Group grant from the Agricultural and Food Research Council to W. G. Hill and a Commonwealth Scholarship to E. A. Asante. Thanks are due to R. K. Koehn for spotting a howler.

References

Ballard, F. J. & Hanson, R. W. (1967). Changes in synthesis in rat liver during development. *Biochemical Journal* **102**, 952–958.

Bulfield, G. & Moore, E. A. (1974). Semi-automated assays for enzymopathies of carbohydrate metabolism in liver and erythrocytes, using a reaction rate analyser. *Clinica Chimica Acta* **53**, 265–271.

Falconer, D. S. (1983). *Introduction to Quantitative Genetics*, 2nd edn. pp. 340. Longman.

Gandemer, G., Pascal, G. & Durand, G. (1985). Comparative changes in the *in vivo* fatty acid synthesis in liver and adipose tissues during the post-weaning growth of male rats. *Comparative Biochemical Physiology* **82B**, 581–586.

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

Hill, W. G. (1985). Detection and genetic assessment of physiological criteria of merit within breeds. In *Genetics of Reproduction in Sheep* (ed. R. B. Land and D. W. Robinson), pp. 319–331. Butterworths.

Hudson, G. F. S. & Kennedy, B. W. (1985). Genetic trend of growth rate and backfat thickness of swine in Ontario. *Journal of Animal Science* **61**(1), 92–97.

Kather, H. & Brand, K. (1985). Origin of hydrogen required for fatty acid synthesis in isolated rat adipocytes. *Archives of Biochemistry and Biophysics* **170**, 417–426.

Langdon, R. G. (1957). The biosynthesis of fatty acids in rat liver. *Journal Biological Chemistry* **226**, 615–629.

Leclercq, B., Blum, J. C. & Boyer, J. P. (1980). Selecting broilers for low or high abdominal fat: initial observations. *British Poultry Science* **21**, 107–113.

Madvig, P. & Abraham, S. (1980). Relationship of Malic enzyme activity to fatty acid synthesis and the pathways of glucose catabolism in developing rat liver. *Journal of Nutrition* **110**, 90–99.

Muller, E. (1985). Genetic regulation of fat metabolism in pigs. Paper presented at the 36th Annual Meeting of the Study Commission EAAP, Kallithea, Halkidiki, Greece, 30 September–3 October, 1985.

Muller, E. (1986). Physiological and biochemical indicators of growth and composition. In *Exploiting New Technologies in Animal Breeding, Genetic Developments* (ed. C. Smith, J. W., B. King and J. C. McKay), pp. 132–139. Oxford Science Publications.

Nisselbaum, J. S. & Green, S. (1969). A simple ultramicro method for determination of pyridine nucleotides in tissues. *Analytical Biochemistry* **27**, 212–217.

Sharp, G. L., Hill, W. G. & Robertson, A. (1984). Effects of selection on growth, body composition and food intake in mice I. Responses in selected traits. *Genetical Research* **43**, 75–92.

https://doi.org/10.1017/S0016672300028536 Published online by Cambridge University Press