Genet. Res., Camb. (1991), **58**, *pp.* 123–127 *Printed in Great Britain*

Analysis of lines of mice selected for fat content 3. Flux through the *de novo* lipid synthesis pathway

EMMANUEL A. ASANTE[1], WILLIAM G. HILL[2] AND GRAHAME BULFIELD[1]

[1] *Cellular and Molecular Biology Department, AFRC Institute of Animal Physiology and Genetics, Edinburgh Research Station, Roslin, Midlothian EH25 9PS*

[2] *Institute of Cell, Animal and Population Biology, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT**

(*Received 26 September 1990 and in revised form 24 April 1991*)

Summary

The flux through the *de novo* fatty acid synthesis pathway was estimated in lines of mice which differed substantially in fat content following 26 generations of selection at 10 weeks of age. Previous estimates of lipogenic enzyme activities had indicated an increase in the capacity for lipogenesis in the Fat compared to the Lean line. Therefore the *in vivo* flux in lipogenesis was measured in both liver and gonadal fat pad (GFP) tissues of males at 5 and 10 weeks of age, using the rate of incorporation of ^{3}H from $^{3}H_2O$ and ^{14}C from acetate and citrate into total lipids. At both ages and in both tissues the Fat line had a higher flux, about 20% increase in the liver and up to three-fold increase (range 1·2- to 3·4-fold) in the GFP. We conclude that direct selection for fatness in mice has resulted in metabolic changes in the rate of *de novo* fatty acid synthesis, and that the changes are largely detectable before 10 weeks, the age of selection.

1. Introduction

This study utilizes lines of mice divergently selected for body fat content. These were part of a larger selection programme to create a resource for physiological and biochemical analyses of the basis of genetic changes in traits of growth and composition (Sharp, Hill & Robertson, 1984). Depending on age, the Fat and Lean F lines differed two-fold or more in fat content at generation 26, when this study was conducted (Hastings & Hill, 1989, 1990; I. M. Hastings, personal communication). Previous studies on these lines have shown that the specific activities of enzymes generating NADPH, an essential cofactor for lipogenesis, are about two-fold higher in the Fat than the Lean lines (Asante, Hill & Bulfield, 1989), and that the specific activities of enzymes in the *de novo* fatty acid synthesis pathway are 2- to 3·5-fold higher in the Fat line, particularly in the gonadal fat pad of young mice (Hastings & Hill, 1990). Unselected controls were generally intermediate between the Fat and Lean lines.

These previous results indicate that lipogenic capacity in the Fat line has increased, particularly in the fat pad, before 10 weeks, the age at selection in these lines. It is not known, however, whether this increase in potential for lipogenic activity actually results in an increase in lipogenic flux *in vivo* (as might be expected). This study was therefore undertaken to determine the flux by measuring the rate of incorporation of ^{3}H from $^{3}H_2O$ and of ^{14}C from acetate and citrate. This was necessary because significant differences in degradation rates alone could account for the observed differences in fat content.

2. Materials and methods

(i) *Animals*

Details of the origins of the lines (the F lines) and the selection procedure are given elsewhere (Sharp *et al.* 1984; Hastings & Hill, 1989, 1990). Briefly, three replicates of Fat and Lean lines were selected for 20 generations from the same base population using the ratio of gonadal fat pad weight (GFPW) to body weight of males at 10 weeks of age as the criterion of fatness. The 3 Fat replicates were then intercrossed to form a new Fat line (F6 Fat), and similarly for the Leans (F6 Lean), without replicates. Selection was continued in F6, now using the ratio of dry weight to

* Formerly, Department of Genetics, University of Edinburgh.

Table 1. *Mean body weight (BW), liver weight (LW) and gonadal fat pad weight (GFPW) of mice injected with ^3H_2O, [1,5-^{14}C]citrate and [U-^{14}C]acetate at 5 and 10 weeks of age, and ratios of means (F/L ratio) averaged over treatments*[a]

Experiment (tracer used)		^3H_2O	[1,5-^{14}C]citrate	[U-^{14}C]acetate	F/L ratio
			5 weeks		
BW (g)	Fat	18·6	25·7	25·8	
	Lean	24·9	25·6	24·7	0·93
LW (g)	Fat	1·04	1·37	1·60	
	Lean	1·43	1·34	1·36	0·97
GFPW (g)	Fat	0·143	0·317	0·310	
	Lean	0·115	0·139	0·119	2·06
			10 weeks		
BW (g)	Fat	35·9	43·1	39·7	
	Lean	30·0	34·4	32·7	1·22
LW (g)	Fat	1·94	1·79	1·91	
	Lean	1·59	1·46	1·63	1·21
GFPW (g)	Fat	1·00	1·55	1·35	
	Lean	0·241	0·279	0·234	5·17

[a] $n = 8$ mice per cell, except for [1,5-^{14}C]citrate in Lean line at 5 and 10 weeks where $n = 7$.

wet body weight of males at 14 weeks as a criterion of overall fatness, GFP in the Lean lines having become very small. Contemporaneous unselected control populations were not maintained for use in the present study. Mice for the present study were sampled from generation 26 (i.e. six generations after lines were crossed).

(ii) *Experimental*

Three groups of eight mice were sampled from each of the lines at both 5 and 10 weeks of age and injected intraperitoneally with 1·5 mCi of ^3H_2O or 5 μCi [U-^{14}C]acetate or 5 μCi [1,5-^{14}C]citrate, each in 0·1 ml saline (0·9% w/v). The mice were left in a fume cupboard for 60 min after ^3H_2O injection and 30 min after acetate or citrate injections, the linearity of radiolabel incorporation from each substrate into liver and GFP tissues having been checked in preliminary experiments. At these times after injection, the mice were anaesthetized with diethyl-ether and the abdominal cavity quickly opened up.

The heart was punctured and blood samples were collected with syringes rinsed with 5% heparin and aliquoted into heparinized Eppendorf tubes. The liver and GFP were quickly removed, weighed, frozen in liquid nitrogen and stored at −80 °C. Total lipids were extracted according to the method of Folch, Lee & Sloane-Stanley (1957) as follows: the Chloroform phase (5 ml) containing the total lipids was carefully pipetted into mini-scintillation vials and dried under air. Radioactivity in the dried total lipids was counted in a scintillation counter after addition of 0·5 ml methanol and 4 ml Optiphase MP. The specific radioactivity of ^{3}H in plasma of each mouse was determined by counting 10 μl of 100-fold diluted plasma. The weight of water in 1·0 ml of pooled plasma was determined for the group receiving ^3H_2O and this was used to convert the counts in dpm for each mouse to μg ^{3}H per gram tissue (Rath & Thenen, 1980). This conversion accounts for isotope dilution and errors in the amounts of radioactivity administered per mouse. Protein contents (mg/g) of the liver and GFP tissue were determined as previously described (Asante *et al.* 1989). Means for each age-label subclass were used to convert counts per gram tissue to counts per mg protein.

(iii) *Statistical*

Data on counts were transformed to logarithms to reduce heterogeneity of variance and non-normality. Results given in the tables are of back transformed means, with standard errors also obtained by back transformation as a linear approximation. The ratios of means for Fat and Lean lines were tested for departure from unity, using t tests of the difference of log means. Results were also pooled over ages, tissues and substrates, using the log means weighted by the inverse of their variance.

3. Results

The mean body, liver and gonadal fat pad weights are shown in Table 1; the group at 5 weeks from the Fat line injected with ^3H_2O had a lower mean body weight than usual for these lines, but there was no indication that other results were unusual for these mice (Hastings & Hill, 1989). By 10 weeks of age the GFPW of the Fat line was over six-fold higher than that of the Lean and as a ratio of body weight was five-fold higher. In the liver the rate of incorporation of ^{3}H and ^{14}C into

https://doi.org/10.1017/S0016672300029773 Published online by Cambridge University Press

Table 2. *Mean*[a] (±S.E.)[b] *rate of incorporation of radioactivity* (^3H *or* ^{14}C) *into liver and gonadal fat pad (GFP) total lipids of three groups of Fat and Lean mice injected with either* ^3H_2O (*μg* ^3H *per mg protein*), [1,5-^{14}C]*citrate* (*dpm* × 10^3/*mg protein*) *or* [*U*-^{14}C]*acetate* (*dpm* × 10^3/*mg protein*) *at* 5 *and* 10 *weeks of age*

	^3H_2O	[1,5-^{14}C]citrate	[U-^{14}C]acetate
		Liver	
5 weeks			
Fat	1·272 ± 0·087	0·257 ± 0·17	3·70 ± 0·63
Lean	1·119 ± 0·065	0·213 ± 0·15	2·91 ± 0·95
F/L ratio	1·14 ± 0·10	1·22 ± 0·11	1·27 ± 0·46
10 weeks			
Fat	1·048 ± 0·098	0·311 ± 0·038	3·55 ± 0·79
Lean	0·872 ± 0·057	0·242 ± 0·029	2·84 ± 0·54
F/L ratio	1·20 ± 0·14	1·29 ± 0·22	1·25 ± 0·37
		GFP	
5 weeks			
Fat	2·936 ± 0·941	0·576 ± 0·104	51·7 ± 17·3
Lean	1·933 ± 0·294	0·469 ± 0·054	19·9 ± 3·6
F/L ratio	1·52 ± 0·54	1·23 ± 0·26	2·60 ± 0·99*
10 weeks			
Fat	0·717 ± 0·101	0·565 ± 0·076	30·3 ± 15·8
Lean	0·515 ± 0·047	0·281 ± 0·038	8·86 ± 2·91
F/L ratio	1·39 ± 0·23	2·01 ± 0·38**	3·42 ± 2·06
		Mean[c]	
F/L ratio	1·23 ± 0·09*	1·32 ± 0·09**	1·65 ± 0·31**

* $P < 0·05$; ** $P < 0·01$; test for F/L ratio > 1.
[a] $n = 8$ observations per cell, except for [1,5-^{14}C]citrate in lean at 5 and 10 weeks where $n = 7$.
[b] Log transformed data were analysed. Back transformed means and standard errors shown.
[c] Weighted means using log transformed data, with approximate standard errors.

total lipids was consistently about 20% higher in the Fat than the Lean line at both ages in all three groups of mice, although this was not significant (Table 2). In the gonadal fat pad the differences between lines were more variable than in the liver depending on the type of radiolabelled substrate used (Table 2). The ratio of Fat to Lean animals in rate of incorporation ranged from 1·2 to 2·6 at 5 weeks and from 1·4 to 3·4 at 10 weeks. Mean rates generally dropped from 5 to 10 weeks in the GFP, but not in the liver, both when expressed per mg protein (Table 2) and per gram tissue (data not shown). Mean ratios of Fat to Lean (computed from the log transformed data) significantly exceeded unity for each substrate.

The rate of incorporation of ^{14}C into hepatic total lipids in both selected lines from acetate was much higher than from citrate at both ages in all lines (Table 2). In the liver there was an approximately 12-fold difference at both ages in both lines whereas in the GFP, the ratio ranged from 30 to 90, being higher in the Fat lines and at the younger age.

4. Discussion

Two relevant biochemical investigations have previously been undertaken on these F lines. First, the specific activities were determined (per mg protein) at 5 and 10 weeks of age in the liver and GFP of four NADPH-generating enzymes: glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, malic enzyme and isocitrate dehydrogenase (Asante *et al.* 1989). Their activities were in general higher in the Fat lines and the largest differences were in the GFP at 5 weeks of age where the ratio of Fat to Lean lines were 1·31, 1·11, 2·25 and 1·27, respectively (Table 3). Second, specific activities were estimated at the same ages for six enzymes involved in *de novo* lipogenesis: ATP-citrate lyase (ACL), acetyl coenzymeA carboxylase (ACC), fatty acid synthetase (FAS), cytoplasmic malate dehydrogenase (MDH), malic enzyme (ME) and pyruvate kinase (PK) (Hastings & Hill, 1990) (Table 3). Activities were generally higher in the Fat line and again the largest differences occurred in the GFP at 5 weeks, where for the F6 lines the ratios of Fat to Lean for ACL, ACC, FAS (the three main enzymes directly involved in the *de novo* synthesis pathway) and ME were 3·53, 2·37, 2·52 and 3·51, respectively. Two other enzymes not directly or solely involved in lipogenesis, MDH and PK, showed little difference in activity between Fat and Lean lines.

The differences in lipogenic and NADPH-generating enzyme activities indicated that the Fat lines have an elevated capacity for lipogenesis and the results of the present study confirm that lipogenesis *in*

https://doi.org/10.1017/S0016672300029773 Published online by Cambridge University Press

Table 3. *Summary of differences (F/L ratios) in enzyme activities and lipogenic fluxes between Fat and Lean F line or F6 mice*

	5 weeks		10 weeks	
	Liver	GFP	Liver	GFP
Enzyme activities [a]				
G6PDH	1·56	1·31	0·99	1·05
6PGDH	1·43	1·11	1·16	1·48
ME	1·26	2·25	1·17	2·26
IDH	1·15	1·27	1·06	1·47
ACL[b]	1·52	3·53	1·05	1·53
ACC	1·12	2·37	0·97	1·52
FAS	1·21	2·52	1·04	1·46
MDH	0·99	1·09	1·02	0·81
ME	1·29	3·51	1·13	1·57
PK	1·11	1·38	1·03	0·85
Flux[c]				
^3H_2O	1·14	2·15	1·22	1·45
[U-^{14}C]acetate	1·11	3·14	1·30	4·21
[1,5-^{14}C]citrate	1·23	1·31	1·30	1·99

Table was derived from Asante *et al.* (1989)[a], Hastings & Hill (1990)[b] and the present paper[c].

vivo has indeed been altered by divergent selection for fatness. There are three characteristic features of the present study that are common to the studies of Asante *et al.* (1989) and Hastings & Hill (1990). First, the magnitude of the differences between lines (ratio of Fat/Lean) in lipogenic rates or enzyme activities were less in liver than in the GFP, perhaps because the GFP is a relatively more active site for lipogenesis or perhaps because selection up to generation 20 was practised directly on this organ (although other fat depots also responded to selection).

Second, whereas absolute activities in the liver remained largely unchanged across ages, in the GFP there was a consistent decline in absolute activities from 5 to 10 weeks of age. Third, in all three studies, between-line differences (ratio of Fat/Lean) were generally as high or higher at 5 weeks as at 10 weeks of age (the age when selection was practised), the only exception being that the difference in incorporation of ^{14}C substrates into GFP was lower at 5 weeks (Table 2). Although the F6 lines used in the present study are not replicated, the previous analyses of enzyme activities generally showed good agreement among the replicated F lines (from which the F6 were derived).

Radiolabels provide a powerful tool for estimating flux through the lipid synthesis pathway, but there are problems inherent in the techniques involved. There can be a high variance between estimates (see for example, standard errors for acetate in GFP in Table 2), and the choice of radiolabelled substrates appears to greatly influence experimental results. It has been argued that whereas ^{3}H from ^3H_2O is incorporated into newly formed lipids regardless of source of substrate or carbon, ^{14}C precursors are subject to isotope dilution from endogenous unlabelled acetyl-CoA, especially in the liver (Hems, Rath & Terence, 1975; Baker, Learn & Bruckdorfer, 1978). The use of ^{14}C precursors will therefore underestimate hepatic contribution and overestimate extra hepatic contribution to total fatty acid synthesis.

Both [^{14}C]citrate and [^{14}C]acetate were used in the present study because citrate appears to be a more physiological substrate derived from glucose metabolism than acetate but is rarely used in lipid labelling experiments. In all our comparisons ^3H_2O, [^{14}C]acetate and [^{14}C]citrate have been treated as three different means of looking for differences between the Fat and Lean lines. The emphasis is on between-line differences and no attempt has been made to compare directly absolute values of ^3H_2O (μg/g) and [^{14}C]citrate or [^{14}C]acetate (dpm/g). The big difference between acetate and citrate may be a function of the relative efficiencies of labelling intracellular acetate and citrate, the relative pool sizes, the number of pathways each substrate is subjected to as well as the kinetics of the system. Though the differences between acetate and citrate are themselves interesting, the aim of the present study was not to investigate these biochemical differences, but rather the differences between the selected lines.

The present data indicate, however, that the Fat and Lean F6 mice differ in lipogenic rates regardless of the radiolabelled substrate used, so selection for fat content has indeed altered the rate of flux through the *de novo* lipid synthesis pathway. This may have been achieved through changes in the activities of the main enzymes involved in the specific *de novo* lipid synthesis pathway (Hastings & Hill, 1990) and ancillary enzymes that generate NADPH essential for the reduction of

https://doi.org/10.1017/S0016672300029773 Published online by Cambridge University Press

malonyl CoA to fatty acids (Asante *et al.* 1989). The general occurrence of similar or larger differences between lines at 5 weeks than 10 weeks of age (the age of selection) suggests that the metabolic events relevant to lipid deposition are being established by 5 weeks of age when the lines differ little in GFPW or total fat.

Although the changes in enzyme activities and fluxes in the Fat and Lean lines are consistently in the same direction (Table 3), the magnitude of the changes are unequal. This raises the question of whether or not the changes in flux can explain the differences in fat deposition between the fat and lean mice. There is evidence that fat accretion in the Fat line increases linearly with age beyond 10 weeks, but tails off in the Lean line by 10 weeks of age (Hastings, 1989). Yet fat synthesis rate as determined in the present study, though much reduced in the Lean line by 10 weeks of age, is not negligible. As there is no net increase in fat deposition some amount of degradation must be taking place.

For the Fat line, as fat deposition continues above 14 weeks of age and fat is a terminal product, a small increase in synthetic flux would result in a large increase in fat deposition, provided degradation rates are negligible. The extent of degradation and whether it differs between the Fat and Lean lines is not known.

This work was supported by a Linked Research Group grant from the Agricultural and Food Research Council and a Commonwealth Scholarship to E. A. Asante. We are grateful to Dr Ian Hastings for unpublished information and helpful comments on the manuscript.

References

Asante, E. A., Hill, W. G. & Bulfield, G. (1989). Analysis of lines of mice selected for fat content. 1. Correlated responses in the activities of NADPH-generating enzymes. *Genetical Research* **54**, 155–160.

Baker, N., Learn, B. D. & Bruckdorfer, K. R. (1978). Re-evaluation of lipogenesis from dietary glucose carbon in liver and carcass of mice. *Journal of Lipid Research* **19**, 879–893.

Bulfield, G., Issacson, J. H. & Middleton, R. J. (1988). Biochemical correlates of selection for weight-for-age in chickens: twenty-fold higher muscle ornithine decarboxylase levels in modern broilers. *Theoretical and Applied Genetics* **75**, 432–437.

Folch, J., Lee, M. & Sloane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry* **226**, 497–509.

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 Biochemistry and Physiology* **82B**, 581–586.

Goodridge, A. G. (1968). Metabolism of glucose U-^{14}C *in vitro* in adipose tissue from embryonic and growing chicks. *American Journal of Physiology* **214**, 897–901.

Hastings, I. M. (1989). Genetical and biochemical analyses of growth. PhD. Thesis, University of Edinburgh.

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.

Hastings, I. M. & Hill, W. G. (1990). Analysis of lines of mice selected for fat content. 2. Correlated responses in the activities of enzymes involved in lipogenesis. *Genetical Research* **55**, 55–61.

Hems, D. A., Rath, E. A. & Terence, R. V. (1975). Fatty acid synthesis in liver and adipose tissue of normal and genetically obese (*ob/ob*) mice during the 24-hour cycle. *Biochemical Journal* **150**, 167–173.

Jansen, G. R., Hutchison, C. F. & Zanetti, M. E. (1966). Studies on lipogenesis *in vivo*: Effect of dietary fat or starvation on conversion of [^{14}C]glucose into fat and turnover of newly synthesised fat. *Biochemical Journal* **99**, 323–332.

Leveille, G. A., Romsos, D. R., Yeh, Y. Y. & O'Hea, E. K. (1975). Lipid biosynthesis in the chick. A consideration of site of synthesis, influence of diet and possible regulatory mechanisms. *Poultry Science* **54**, 1075–1093.

Rath, E. A. & Thenen, S. W. (1980). Influence of age and genetic background on *in vivo* fatty acid synthesis in obese (*ob/ob*) mice. *Biochimica et Biophysica Acta* **618**, 18–27.

Rogers, P. & Webb, G. P. (1980). Estimation of body fat in normal and *obese* mice. *British Journal of Nutrition* **43**, 83–86.

Sharp, G. L., Hill, W. G. & Robertson, A. (1984). Effects of selection on growth, body composition and food intake in mice 1. Responses in selected traits. *Genetical Research* **43**, 75–92.