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Energy expenditure and locomotor activity in mice selected for food intake adjusted for body weight

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Summary. The aim of this study was to examine the differences in physical activity and their contribution to differences in energy utilization in mice, selected either high or low for food intake, adjusted for body weight, which show correlated responses in lean content and metabolic rate. Simultaneous measurements of fasting metabolic rate and activity were made in lines of mice selected at either: a young age, 4- to 6-week food intake corrected for 4-week body weight; or an older age, 8- to 10-week food intake corrected for mean weight at 8 and 10 weeks of age. Correlated response in metabolic rate was found to have been accompanied by changes in locomotor activity near the ages at selection in both sets of lines. Activity, however, accounted for only a small proportion of variation in fasting heat production, generally less than 5%, although a highly positive correlation ($r = 0.63$) between the two traits was found. It was concluded that selection for food intake adjusted for body weight has led to correlated response in physical activity. In consequence, mice selected in the upward direction expend some of the excess energy intake rather than assimilating it as body mass and are, therefore, slightly leaner than their counterparts selected in the downward direction.

Key words: Selection response – Metabolic rate – Inheritance of activity – Mice

Introduction

Responses to selection usually produce correlated changes in several traits. For example, selection for food intake is associated with correlated responses in body weight (Sutherland et al. 1970; Pym and Nicholls 1979; Sharp et al. 1984) and metabolic rate (Pym and Farrell 1977; Bishop and Hill 1985). However, correlated responses in body composition differed between these studies. Whereas selection for absolute amount of food intake produced fatter animals (Biondini et al. 1968; Pym and Solvyns 1979) in mice and poultry, respectively, mice selected for food intake relative to body weight became leaner than either controls or divergently selected low intake lines (Hastings and Hill 1989). The sources of variation in metabolic rate associated with increased food intake, therefore, need to be understood. Several attempts have been made to explain the higher metabolic rate in the high compared to the low mouse lines of Sharp et al. (1984). Bishop (1985) found that scaling metabolic rate by lean mass could not account for the differences, nor could they be explained by changes in either mass of brown adipose tissue or temperature adaptation effects, and Parker (1988) found that the low lines had rather higher protein turnover than the high lines. Results from a separate study (S. M. Moruppa, unpublished data) also indicate no significant difference between lines in the ability to metabolize food energy. The sources of variation in energy utilization, therefore, remain unknown.

Other sources of energy expenditure include the heat increment of feeding and that due to activity. The former has no effect under basal conditions since this source of variation is removed in fasted animals. Activity, however, has been found to play a significant role in energy expenditure in pigs (Halter et al. 1980; Verstegen et al. 1982), poultry (Macleod et al. 1982; Boshouwers and Nicaise 1987) and mice (Dauncey 1986). Macleod et al. (1982) report that activity accounted for about 9% and 15% of heat production in fasting and fed chickens, respectively. Verstegen et al. (1982) found that activity accounted for about 15%–30% of total maintenance requirements in pigs. Differences in metabolic rate in lean and obese (*ob*/*ob*) mouse genotypes were associated with differences in activity (Dauncey 1986). The lean genotype had a higher metabolic rate accompanied by higher levels of activity (60% more active) than the obese genotype. There is also evidence suggesting that other behavioural traits which may affect maintenance requirement change as correlated responses to selection. Lynch and Roberts (1984) reported that lines of mice selected for high body weight build better nests than those selected divergently for low body weight, thus, the "large" mice conserve more energy for growth which would have been otherwise expended to maintain body temperature.

A simultaneous measurement of fasting heat production and activity allows for activity-related heat production to be derived. Measurement of activity in mice is, however, more often confounded with other effects, particularly reactivity, defined as the increase in levels of activity following transfer to an alien environment (Simmel and Bagwell 1983). The aims of the present study were to minimize this effect and to examine the contribution made by locomotor activity to the differences in energy utilization in mice, selected high or low for food intake corrected for body weight.

Materials and methods

Animals

The mice used in this study were sampled from generation 34 of selection for appetite (A) lines, selected for 4- to 6-week food intake, adjusted by phenotypic regression on 4-week body weight to minimize its change; and generation 12 of the maintenance (M) lines, selected for 8- to 10-week food intake, adjusted for mean weight at 8 and 10 weeks of age. The history of these selection lines is reported elsewhere (Moruppa et al. 1989). Replicates "6" of A and 1, 2 and 3 of M lines were used. From each line, eight (for A lines) and four (for M lines) full-sib families were chosen at random, and from within each of these families, two male and two female mice were sampled at weaning (3 weeks of age) when available; otherwise, mice from other families of the same line and sex were used to make up numbers. Mice were housed in groups of six per sex-line in stainless steel cages, and were allowed free access to the stock diet, Beta diets Rat and Mouse no. 1 modified expanded maintenance diet (14.8% crude protein, 10.636 kJ/g ME), before metabolic and activity trials.

Laboratory procedures

Measurements of body weight, fasting heat production and locomotor activity were made both at a young age (3–4 weeks of age) and at an old age (10–11 weeks of age) in the A lines. The M lines were observed at 10–11 weeks of age alone. These age intervals were chosen because they represent ages at or about selection as well as age approaching maturity. Fasting heat production and locomotor activity were measured using a closed-circuit respiration calorimeter and a video tape recorder, respectively. The design of the calorimeter was outlined by Bishop (1985). Basically, it consists of three metabolic chambers (desiccator jars), equipped with independent gas collection apparatus. Two of the chambers were identical in size, with radii of 8.5 cm and volumes of 4,540 cm^3. The third had the same radius but was slightly larger (6,356 cm^3). Simmell and Bagwell (1983) cautioned that the size of the "testing field" may have some effect on the analysis of activity data. The metabolic chambers were enclosed in a wood/perspex box fitted with a fluorescent tube light. Observations were made at temperatures of 29 ± 1 °C, within the thermoneutral zone. Measurements were made on pairs of mice of the same sex and line. Pairs of mice rather than singles were used in an attempt to reduce stress, which can be exaggerated by isolation in the calorimeter chambers.

Fasting heat production and activity were measured at the same time during the initial 3-h period, and fasting heat production alone was measured for the remaining 3 h. Mice were placed in the calorimeter at about 09.00 h, using the first hour as an habituation period; measurements started at about 10.00 h every day. Total heat production was evaluated from standardized volumes (in litres) of oxygen consumed and carbon dioxide output using the formula of Miller et al. (1981):

$$\text{Heat production (kJ)} = 16.17\ V_{O_2} + 5.02\ V_{CO_2}$$

where V is volume of respiratory gases in litres at standard temperature and pressure. A constant (4) was used to multiply estimated heat production over the 6-h period to a 24-h basis (commencing at about 16.00 h the previous day), over which the mice were starved.

Activity score was obtained by observing both mice of a pair over the entire 3-h period, and allocating the different forms of locomotion into two activity indices: either minor activity (1) for a mouse that walks, sitting with little movements, or rearing, i.e. standing on the hind legs; or major activity (2) for running across or around the chamber, or jumping. No score was allotted to a mouse that lay quietly. A unit of activity was defined as the activity performed until a mouse stops or changes the pattern of activity from one form to another. Different types of movements were almost discrete. There were no indications of persistent movements, e.g. continuous running across or around the chambers, in any of the lines. Perhaps it is because these forms of activity demand more energy. To enable observations on both members of a pair during fast movements, e.g. when both were running or jumping concurrently, one mouse was observed at a time. Rapid movements were noticed only during the first hour of observation. The data presented are sums of the observations on both animals of a pair over the entire 3-h period.

Energy expendidure related to body mass can be expressed by scaling it with body mass raised to an exponent (*b*), the "*b*" value being the regression coefficient of log energy expenditure on log body mass. The coefficient is less than unity in most mammals but differs between species and, to some extent, within species (for a review, see McNab 1988). A regression analysis of logarithms of heat production on logarithms of body weight in the present data revealed that the *b* values were 0.64 ± 0.12, 0.63 ± 0.09 and 0.65 ± 0.11 in 3- to 4-week and 10- to 11-week A lines, and in M lines, respectively. The average value, 0.64, was chosen as the mass (kg) exponent to scale total heat production in an attempt to remove differences due to body size. Because of heterogeneity of variances and skewness in the raw data for activity score, logarithmic transformations were applied to bring the data closer to normality. However, raw data were used to evaluate and account for metabolic heat production associated with activity score.

Statistical analysis

Data from the two selection criteria (A and M) were analyzed separately using least-squares analysis of variance (LSMLMW, Harvey 1985). For the A lines, the sample size was reduced to 59 because of some occasional problems with the calorimetry equipment and the death of some animals during the course of the experiment. The model used for analysis was:

$$Y_{ijklm} = \mu + D_i + A_j + S_k + C_l + \text{(all two-way interactions)} + P_{ikm} + e_{ijklm}$$

where:

Y_{ijklm} is the record on the m^{th} pair, in the l^{th} chamber, of the k^{th} sex, at the j^{th} age, in the i^{th} direction of selection,
μ is the overall mean,
D_i is the effect of the i^{th} direction of selection (high or low),
A_j is the effect of the j^{th} age (young or old),
S_k is the effect of the k^{th} sex (male or female),
C_l is the effect of the l^{th} metabolic chamber (1, 2, 3),
P_{ikm} is the random effect of the m^{th} pair of the k^{th} sex in the i^{th} direction of selection, with σ_p^2 being its component of variance.
e_{ijklm} is the error term, with σ_e^2 being its variance component.

Repeatability was defined as $\sigma_p^2/(\sigma_e^2 + \sigma_p^2)$.

Terms for the partial regression on either activity score or body weight were added to the model for metabolic heat production and activity, respectively, to account for differences in these traits. Effects were tested against pairs, while pairs were tested against the residual. For the M lines, the same model was used, but with an effect for replicate ($R_j = 1, 2, 3$). Replicates were maintained under the same management conditions: differences between replicates, if found, would therefore include those due to random genetic drift variance, so the appropriate main effects were tested against their interaction with the replicates.

Table 1. Least-squares means, divergence[a] and coefficient of variation (C.V.) in body weight and energy expenditure traits for the appetite high (AH) and appetite low (AL) lines measured at (a) 3–4 and (b) 10–11 weeks of age

Line	Body weight (g)	Total heat production (kJ/pair/day)	Metabolic heat production (kJ/kg$^{0.64}$/day)	Activity score (per pair/3 h)
(a) 3–4 weeks of age				
AH	20.6	51.9	306	252
AL	15.4	43.6	309	204
Divergence (%)	29	17	−0.9	21
C.V. (%)	14.1	14.9	13.6	21.5
Males	19.0	45.5	256	256
Females	17.0	50.0	312	200
(b) 10–11 weeks of age				
AH	34.9	60.0	261	88
AL	28.9	50.6	241	94
Divergence (%)	18	18	4	−7
C.V. (%)	9.9	8.3	6.4	30.4
Males	35.7	55.5	244	101
Females	28.2	55.1	260	81

[a] Divergence = 2(H − L)/(H + L) × 100

Results

A beween-line difference in levels of activity was observed in the A lines at 3–4 weeks of age. The high line (AH) mice were more active than the low line (AL) mice (Table 1), although not significantly so (Table 2). There were, however, corresponding differences in body weight at this age, although the intention of the selection index was to minimize differences in this trait. This observation was partly due to slight and unintentional differences in age at calorimetry, the AH line mice being older (with an average of 3.7 weeks of age), while the AL line mice were at an average of 3 weeks of age. This was because mice had to be taken when available from the main selection experiment.

Total heat production was also higher in the AH line, but there was no significant difference between lines when scaled by metabolic weight (kg$^{0.64}$). At an older age (10–11 weeks old), the level of activity has declined sharply in both lines. The AH line mice were still heavier and had a higher metabolic rate, but the AL line became slightly more active. The differences in activity at the older age were associated with the differences in body weight. Adding a term for within-age partial regressions on body weight showed that activity was negatively correlated ($r = -0.58$, $n = 59$; $P < 0.01$) with body weight, overall. Levels of activity had little effect on either metabolic rate itself or high-low differences, although there was a positive association ($r = 0.63$, $n = 59$; $P < 0.01$) between levels of activity and metabolic rate. There were sex differences for all traits, but these differences were not consistent. Whereas the males were heavier and more active at both ages, the females had a higher metabolic rate at 10–11 weeks.

Data on the M lines are presented in Table 3, and their corresponding analyses of variance in Table 4. An important feature in these lines was the use of replication. There were consistent differences between replicates, replicate 2 being heavier and less active than the other replicates. As in the A lines, the difference between directions of selection in activity score was not significant in the raw data, which was again attributed to heterogeneity of variance. The coefficient of variation of activity score was higher in the MH, about twice that in the ML lines.

A log transformation revealed that the high lines were significantly ($P < 0.05$) more active than the low lines. These differences were accompanied by similar differences in metabolic heat production. However, no significant difference in body weight was found. There were sex differences for all traits, males having higher values. In general, however, the proportion of fasting metabolic rate accounted for by activity, i.e. metabolic heat production less that corrected for activity, was found to be generally small, mostly less than 5%. The corrected means were 272 and 259 kJ/kg$^{0.64}$/day for MH and ML lines. Corresponding values for AH and AL lines at 3–4 weeks of age

Table 2. Mean squares for body weight and energy expenditure traits: total heat production (THP), metabolic heat production (MHP) and after regression on activity score, activity score (AS), and $\log_{10}$ AS for the A lines

Source	*d.f.*	Body weight (g)	Total heat production (kJ/pair/day)	Metabolic heat production (kJ/kg$^{0.64}$/day)	MHP after regression on activity (kJ/$^{0.64}$/day)	AS (per pair/3 h)	AS after regression on body weight (per pair/3 h)	Log_{10} AS (per pair/3 h)
Selection direction	1	395.7**	987.8**	336	273	5,695	2,735	0.0290
Age	1	2,400.1**	702.2**	46,547**	4,184	230,139**	6,160	1.8147**
Sex	1	23.0	4.4	2,883	2,697	1,476	2,317	0.0159
Chamber	2	20.9	0.8	885	884	404	478	0.0127
Dir × age	1	1.7	3.5	458	325	8,583	2,819	0.0181
Dir × sex	1	34.8	51.4	19	295	1,673	2,676	0.0082
Dir × chm	2	0.7	3.1	297	293	594	516	0.0037
Age × sex	1	98.2*	72.5	28	25	3 737	535	0.0097
Age × chm	2	1.4	21.5	489	486	4,469	4,651	0.0283
Sex × chm	2	23.3	57.0	591	591	239	469	0.0053
Pairs	28	18.1*	71.3	2,209	1,948	3,816*	3,677*	0.0278**
Regression		–	–	–	0.12[a]	–	699[c]	–
Residual	16	7.1	49.7	1,379	1,471[b]	1,405	1,527[d]	0.0081
Repeatability[e]		0.50	0.22	0.28	0.19	0.41	0.48	0.61

* $P<0.05$, ** $P<0.01$; pairs tested against residual, all other effects against pairs

[a, b, c] and [d] *d.f.* = 1 (pooled regression), 15, 2 (within-age regressions) and 14, respectively

[e] Repeatability was defined as the ratio of the variance between pairs to the sum of variance between pairs and error variance

Table 3. Least-squares means and coefficient of variation of body weight and energy expenditure traits for the high food intake (MH) and low food intake (ML) M lines at 10–11 weeks of age

Line	Rep.	Body weight (g)	Total heat production (kJ/pair/day)	Metabolic heat production (kJ/kg$^{0.64}$/day)	Activity score (per pair/3 h)
MH	1	26.8	54.5	272	170
	2	32.0	57.2	257	96
	3	27.3	58.1	291	123
	Mean	28.7	56.6	274	130
	C.V. (%)	4.5	6.4	4.9	42.6
ML	1	27.4	52.3	256	111
	2	31.3	54.2	247	80
	3	28.9	57.3	275	94
	Mean	29.2	54.6	260	95
	C.V. (%)	6.1	6.1	4.8	24.8
	Males	31.2	58.4	268	127
	Females	26.8	52.8	266	98

were almost the same as that of uncorrected means, and slightly lower at 10–11 weeks of age, 259 and 241 kJ/kg$^{0.64}$/day for AH and AL, respectively.

Discussion

These results imply that the correlated responses in leanness and metabolic rate to selection for food intake, corrected for body weight, may be partly ascribed to correlated response in physical activity. For both selection criteria, the mice selected in the upward direction were more active than those selected in the downward direction. In the A lines, these differences were observed at the younger age (3–4 weeks of age) near to the age at selection, although these differences were not significant. At an age approaching maturity (10–11 weeks of age), the low lines tended to become more active. However, the levels of activity had declined sharply in both lines, compatible with the fact that physical activity decreases with age (Halter et al. 1980; Verstegen et al. 1982). The M lines showed differences in both activity and metabolic rate at 10–11 weeks of age, which in these lines represents an age interval immediately following the age at selection (8–10 weeks of age). These lines provide a more useful model for studying the various components of maintenance requirement, for they have responded to selection, i.e. they show consistent divergence in food intake in the direction of selection, but have changed very little in body weight. Therefore, an account of the excess energy ingested would provide some explanation of the genetic variation in maintenance requirement. The present study indicates a small, but significant increase in metabolic rate and activity in the high lines. Although there were consistent between-replicate differences in both body weight and the energy expenditure traits, line differences within replicates were consistently observed.

The present study also suggests that there is some relationship between locomotor activity and energy expenditure, as in other studies where simultaneous mea-

Table 4. Mean-squares for body weight and energy expenditure traits: total heat production, metabolic heat production (MHP) and after regression on activity score (AS), and $\log_{10}$ AS for the M lines

Source	*d.f.*	Body weight (g)	Total heat production (kJ/pair/day)	Metabolic heat production ($kJ/kg^{0.64}/day$)	MHP after regression on activity ($kJ/kg^{0.64}/day$)	AS (per pair/3 h)	Log_{10} AS (per pair/3 h)
Selection direction	1	3.12	44.89	1,928 *	1,385**	13,549	0.1541
Replicate	2	88.13**	72.62*	3,648**	3,479**	10,679	0.1227**
Sex	1	220.42**	372.95**	42	0.23	9,709	0.0772
Chamber	2	5.17	38.30	185	113	1,598	0.0155
Dir × rep	2	5.72	4.37	37	14	1,775	0.0073
Dir × sex	1	0.01	0.00	14	92	6,482	0.0494
Dir × chm	2	12.82*	33.29	304	228	1,837	0.0253
Rep × sex	2	0.81	183.74**	4,475**	4,068**	6,880	0.0528
Sex × chm	4	12.68*	10.69	243	215	1,412	0.0189
Sex × chm	2	5.12	30.79	1,070*	957*	2,290	0.0442
Regression (within direction)	2	–	–	–	198	–	–
Residual	28	2.90	14.76	202	207[a]	1,768	0.0200

* $P < 0.05$, ** $P < 0.01$; effects of direction, sex and chamber were tested against their respective interactions with replicates, all others against residual

[a] *d.f.* = 26

surements were conducted. The proportion of basal energy expenditure accounted for by activity was small, mostly less than 5%, but similar to other previous reports for mice (Trayhurn et al. 1979). The differences in activity found in the present study were observed under bright illumination and fasting conditions, suggesting that they are likely to be sustained or possibly exaggerated under normal conditions in stock cages. Mice and rats tend to have increased nocturnal activity and metabolic rate (Mount and Willmott 1967; Anderson and Smith 1987). Reports in the chicken, however, indicate that activity-related energy expenditure is positively correlated with light intensity (Boshouwers and Nicaise 1987). These differences are mainly due to the difference in circadian rhythm between these species, the rodents being nocturnal whereas chickens are diurnal.

The results of the present study, therefore, provide some evidence to suggest that selection for food intake adjusted for body weight has led to correlated changes in physical activity. Consequently, mice selected for high food intake relative to body weight expend some of the excess assimilated food energy intake on activity, rather than using it to produce body mass and are, therefore, slightly leaner than their low intake counterparts.

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References

Anderson VN, Smith GK (1987) Effects of feeding and light cycles on activity rhythms of maternally isolated rat pups. Physiol Behav 39:169–181

Biondini PE, Sutherland TM, Haverland LH (1968) Body composition of mice selected for rapid growth rate. J Anim Sci 27:5–12

Bishop SC (1985) Genetic and metabolic aspects of growth and its components in mice. PhD thesis, University of Edinburgh

Bishop SC, Hill WG (1985) Effect of selection on growth, body composition and food intake in mice. 3. Correlated responses; growth, body composition, food intake and efficiency and catabolism. Genet Res 46:57–74

Boshouwers FMG, Nicaise E (1987) Physical activity and energy expenditure of laying hens as affected by light intensity. Br Poult Sci 28:155–168

Dauncey MJ (1986) Activity-induced thermogenesis in lean and genetically obese (*ob/ob*) mice. Experientia 42:547–549

Halter HM, Wenk C, Schurch A (1980) Effect of feeding level and feed composition on energy utilization, physical activity and growth performance of piglets. In: Mount LE (ed) Energy metabolism. Proc 8th Symp Energy Metabolism, EAAP Publication No. 26, Butterworths, London

Harvey WR (1985) User's guide for LSMLMW, mixed model least-squares and maximum likelihood computer program. Ohio State University

Hastings IM, Hill WG (1989) A note on the effect of different selection criteria in mice. Anim Prod 48:229–233

Lynch CB, Roberts RC (1984) Aspects of temperature regulation in mice selected for large and small size. Genet Res 43:299–306

Macleod MG, Jewitt TR, White J, Verbrugge M, Mitchell MA (1982) The contribution of locomotor activity to energy expenditure in the domestic fowl. In: Ekern A, Sunstol F (eds) Energy metabolism of farm animals. Proc 9th Symp Energy Metabolism. EAAP Publication No. 29, pp 297–300

McNab BK (1988) Complications inherent in scaling the basal rate of metabolism in mammals. Q Rev Biol 63:26–53

Miller BG, Kirkwood JK, Howard K, Tuddenham A, Webster AJF (1981) A self-compensatory, closed-circuit respiration calorimeter for small mammals and birds. Lab Anim 15:313–317

Moruppa SM, Hill WG, Sinnet-Smith PA (1989) Effect of selection for growth, body composition and food intake in mice: utilization of increased energy intake by "cafeteria" feeding. Livestock Prod Sci (in press)

Mount LE, Willmott JV (1967) The relationship between spontaneous activity, metabolic rate and the 24 h cycle in mice at different environmental temperatures. J Physiol 190:371–380

Parker K (1988) Protein turnover in mice selected for appetite. PhD thesis, University of Edinburgh

Pym RAE, Farrell DJ (1977) A comparison of the energy and nitrogen metabolism of broilers selected for increased growth rate, food consumption and conversion of food to gain. Br Poult Sci 18:411–426

Pym RAE, Nicholls PJ (1979) Selection for food conversion in broilers: Direct and correlated responses to selection for body weight gain, food consumption and food conversion ratio. Br Poult Sci 20:73–86

Pym RAE, Solvyns AJ (1979) Selection for food conversion in broilers: Body composition of birds selected for increased body-weight gain, food consumption and food conversion ratio. Br Poult Sci 20:87–97

Sharp GL, Hill WG, Robertson A (1984) Effects of selection on growth body composition and food intake in mice. 1. Responses in selected traits. Genet Res 43:75–92

Simmel EC, Bagwell M (1983) Genetics of expolatory behaviour and activity. In: Fuller JL, Simmel EC (eds) Behaviour genetics; principles and application. Lawrence Erlbaum Associates, London, pp 89–113

Sutherland TM, Biondini PE, Haverland LH, Pettus D, Owen WB (1970) Selection for rate of gain, appetite and efficiency of feed utilization in mice. J Anim Sci 31:1049–1057

Trayhurn P, Thurlby PL, Woodward CJH, James WPT (1979) Thermoregulation in genetically obese rodents: the relationship to metabolic efficiency. In: Festing MFW (ed) Animal models of obesity. Macmillan, London, pp 191–203

Verstegen MWA, Van der Hel W, Brandsma HA, Kanis E (1982) Heat production of groups of growing pigs as affected by weight and feeding level. In: Ekern A, Sundstol F (eds) Energy metabolism of farm animals. Proc 9th Symp Energy Metabolism. EAAP Publication No. 29, pp 218–221