

Mitochondrial Efficiency in Lines of Mice Divergently Selected for Heat Loss

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MITOCHONDRIAL EFFICIENCY IN LINES OF MICE DIVERGENTLY
SELECTED FOR HEAT LOSS

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Divergent selection for heat loss was applied to lines of mice for 15 generations (G) in three replicates. Selection resumed at G42 and continued through G51 across all replicates. Selection of this sort resulted in differences of 55.7% and 34% in heat loss and feed intake, respectively, at the end of G51 between high heat loss (MH) and low heat loss (ML) lines. Rates of mitochondrial respiration states, degree of coupling and mitochondrial efficiency were measured in G58 using a Clark-type oxygen electrode to investigate possible causes of underlying variation in feed intake. Body weight, body composition, liver weight, and feed intake were also measured. Results reported here represent data from 197 mature male mice from replicates 1, 2 and 3. There were no significant differences in body weights ($P=0.91$) between the selection lines. Selection had a significant effect on percent lean tissue ($P=0.02$) with MH mice being leaner, while differences in percent fat between the lines approached significance ($P=0.13$) with ML mice being fatter. Livers of MH mice were approximately 13% larger than for ML mice ($P=0.01$). An effect of selection was observed ($P<0.01$) in feed intake/body weight with MH mice consuming 29% more feed than ML mice in G58. Differences in State 2 and State 4 respiration rates were significant ($P=0.01$), while State 3 rates approached significance ($P=0.06$); mitochondria of MH mice respired faster than in ML mice. ML mice had respiratory control ratios that were, on average, 8% greater than MH mice ($P=0.14$), suggesting a greater degree of coupling in ML animals. ML mice had ADP:O ratios that were approximately 20% greater than MH mice ($P=0.03$). Therefore, greater mitochondrial efficiency is expressed in the ML animals. Across all replicates a partial correlation of -0.33 was observed between feed/body weight and ADP:O. However, within a line-replicate this correlation failed to exist. Even though lines differed in ADP:O, it was difficult to detect any part of this difference explaining line differences in feed intake.

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Introduction

PREVIEW

The greatest economic inputs in livestock production are the costs associated with feeding the animals. Among these inputs, the costs of feeding animals to meet their maintenance requirements are the largest, and partial energy costs per unit body size for maintenance exhibit more genetic variation when compared to the partial costs of feeding animals for production purposes (i.e. per unit of milk production or growth). The ability to measure maintenance requirements either in feed energy consumed or as the proxy heat production, and the existence of such variation, makes it possible to select animals to change maintenance requirements in order to produce animals with a more desirable ability to efficiently utilize food.

Previously, Nielsen et al. (1997a) created unique lines of mice that were divergently selected for heat loss. They created high heat loss (MH) lines, low heat loss (ML) lines, and unselected control (MC) lines in three replicates and carried out the selection process through 15 generations. Nielsen et al. (1997a) found that selection for heat loss was in fact possible and that the high and low lines diverged successfully with an overall heritability of 0.28. McDonald and Nielsen (2007) continued selection of these lines from generation 42 through generation 51. Once renewed selection ceased, the MH and ML lines had diverged by approximately 56% of the MC mean in heat loss, with the MH mice losing more heat than the ML. The lines had also diverged by 34% of the control mean in feed intake per metabolic weight with the MH consuming more feed even though there were no significant differences in body weight between the lines. These differences make the heat loss lines of mice an excellent animal model for the study of metabolic systems and overall metabolic efficiency associated with maintenance requirements of animals.

Rolfe and Brown (1997) estimated that approximately 90% of mammalian oxygen consumption, in the steady state, is mitochondrially related. Therefore, this organelle seems to be a likely place to begin the investigation of variation in maintenance requirements on a cellular level. Mitochondrial efficiency is measured by calculating oxygen consumption and rates of respiration of isolated mitochondria using a Clark-type oxygen electrode. Several studies have shown that variation exists in these mitochondrial traits within a species of animal. Therefore, the objective of this study was to determine whether divergent selection for heat loss has changed the functions and efficiency of mitochondria isolated from mouse livers. The relationships of these mitochondrial traits with feed efficiency, body weight gain, body composition, and liver size will also be determined.

Literature Review

PREVIEW

Maintenance Requirements

Feed costs are the largest economic input that goes into livestock production. These costs include the amount of feed necessary to provide enough metabolizable energy for growth of young animals and reproduction for adults in addition to maintenance requirements. Ferrell and Jenkins (1985) estimated that maintenance requirements alone are responsible for 70-75% of total annual energy requirements of a herd of cattle. Variation among maintenance requirements can be explained by variation in many body components including variation in visceral organ mass, performance capabilities, physiologic state, age, and sex.

Selection to reduce maintenance requirements would be a useful tool in decreasing feed costs of livestock production. Many researchers have approached the idea of changing maintenance requirements through selection in different ways. Some have studied selection on body weight (Dunnington and Siegel 1996; Kownacki et al. 1975; Kownacki and Keller 1978; Canolty and Koong 1976), some on feed intake and feed: gain ratio (Selman et al., 2001; Bishop et al., 1991a, b; Bishop and Hill, 1985), and some have selected on heat loss (Nielsen et al. 1997a, b) to understand possible changes in maintenance requirements, as well as correlated responses associated with those changes.

Variation in Maintenance Requirements

Ferrell and Jenkins (1985) suggested that there is a considerable amount of variation in total energy expenditures among animals. They were able to show that there was a significant difference in energy expenditure among different breeds of

cattle as well as among individuals within one breed type. They stated that variation among maintenance requirements, and thus efficiency of an animal, seems to be associated with the animal's genetic potential for production. Production in this sense equates to genetic potential for growth rate or milk production. Therefore, some cattle may be able to achieve a higher level of production, but these animals may have lower overall efficiency when compared to lower producing animals or breeds. Ferrell and Jenkins (1985) note that animals with potential for high productivity will be at a disadvantage in a more restrictive environment.

Montaño-Bermudez et al. (1990) modelled the efficiencies of three beef cattle crosses with different milking potentials, and confirmed Ferrell and Jenkins (1985) conclusions that efficiency of an animal is related to genetic potential for production. Montaño-Bermudez et al. (1990) investigated Hereford X Angus (low milk = L), Red Poll X Angus (medium milk = M) and Milking Shorthorn X Angus (high milk =H) for economic and biological efficiency. They observed that the H and M cows required 12% more energy per unit metabolic weight than L cows to maintain body weight during both gestation and lactation. They found that differences in milk production explained 23% of the variation in maintenance requirements. This suggests that important differences exist beyond those associated with output potential and further investigation into variation of maintenance requirements is needed

Van Oijen et al. (1993) also modeled the efficiencies of the same three beef cattle crosses as Montaño-Bermudez et al. (1990). They showed that cattle with the lowest potential for milking ability were the most economically and biologically

efficient when compared to high milking breeds. The authors suggested that when considering a breeding program, inputs should be taken into consideration rather than focusing solely on animals with the greatest output. Van Oijen et al. (1993) questioned the use of beef breeds with high genetic potential for milking ability, due to low efficiency in those breeds.

Taylor et al. (1986) conducted a similar experiment in which different breeds were tested on milk performance. The genetic relationship between milk yield and maintenance requirements was then examined. In their research, two beef breeds (Hereford, Angus), two dairy breeds (British Friesians, Jersey), and one dual-purpose breed (Dexter) were used. In all cases, beef breeds were found to have the lowest maintenance requirements and thus the greatest efficiency, defined as mature body weight per unit of food intake, among the five breeds. They demonstrated that Herefords were able to maintain themselves more efficiently than British Friesians. This idea is consistent with previous findings and conclusions of Ferrell and Jenkins (1985), Montaño-Bermudez et al. (1990) and Van Oijen (1993). Even further, Taylor et al. (1986) showed that efficiency has an inverse relationship with lactation ability. Therefore, as milk production of an animal decreases the efficiency of an animal increases.

Ferrell (1988) found that 70% of a mature cow's feed costs are due to maintenance requirements. Ferrell and Jenkins et al. (1985) concluded that a high proportion of maintenance energy requirements are attributed to energy expenditures of visceral organs, especially the liver and gastrointestinal tract. The high rate of energy metabolism of these tissues is associated with high rates of protein synthesis

in these organs of the body. They hypothesized that, like maintenance requirements, visceral organ masses vary within different breeds/types, diets, maturity, and with physiological state. Prior to this paper, Smith and Baldwin (1974) demonstrated that physiological state plays a major role in determining mass of visceral organs. They showed that the liver, heart, mammary glands, stomach, and intestines were all larger in lactating animals than in non-pregnant, non-lactating animals. Jones et al. (1992) also evaluated genetic variation among liver mass and relationships between liver mass and body mass in mice. Jones et al. (1992) found that genetic variation does indeed exist for both liver and body mass, and that the genetic correlation between the two was 0.81. The coefficient of variation for liver mass was found to be 12%, and heritability ranged from 0.43-0.70.

Effects of selection for gain and growth rate on maintenance requirements

Canolty and Koong (1976) studied mice previously selected for rapid post-weaning growth rate for forty-one generations, comparing the responses in the selection line to that of a randomly selected control line. It was concluded that maintenance requirements and efficiency of lean energy deposition were not affected by selection of this sort. However, the rapid growth line became more efficient over time at utilizing metabolizable energy available for gain (MEA) by altering fat energy deposition coefficient.

Dunnington and Siegel (1996) selected inbred white Plymouth Rock Chickens for high (HWS) and low (LWS) body weight at 8-wk of age. Divergence of selected lines occurred gradually, but resulted in an eight-fold difference between high and

low lines at the last generation. Realized heritability estimates for 8-wk body weight were between 0.22-0.28 for HWS line, and between 0.23-0.28 for LWS line. On average, chickens selected for greater 8-wk body weight consumed more feed, grew faster, had an increased percentage of fat, and performed more efficiently. There was no significant difference in thermoregulation between the two lines.

Kownacki et al. (1975) studied effects of selection for post-weaning growth rate on basal metabolic rate in mice. It was concluded that growth rate of young mice and basal metabolic rate have an inverse relationship. In a related study, Kownacki and Keller (1978) continued selection for post-weaning growth and showed that selected mice had basal metabolic rates that were, on average 8.5% lower than the unselected control group. The second study confirmed the hypothesis that selection for post-weaning gain is related with some physiological mechanism which influences basal metabolic rate. They concluded that animals that are selected for weight gain are able to use their energy more efficiently than animals used in other selection situations.

In a review article by Roberts (1979), data were summarized on several studies dealing with selection for growth rate or post-weaning weight gains. His findings were that, in general, selection for increased body weight in laboratory animals leads to increases in food intake, gross efficiency and fat deposition. However, selection for increased body weight often impairs certain aspects of fertility. Roberts et al. (1979) suggested that fat deposition may be an alternative to heat output, which explains why increases in fat deposition and gross efficiency may

not be incompatible. It was concluded that many of the adverse side effects of selection for growth are the physiological consequences of increased fatness.

Effects of selection for feed intake and/or conversion ratio on maintenance

Bishop and Hill (1985) selected mice based on three criteria: 1) food intake, 2) lean mass deposition, and 3) fat content. High and low levels were selected using each of the criteria. Several implications came from this study. First, it was shown that variation exists in maintenance requirements of mice, as well as in feed intake above that needed to meet maintenance requirements. Second, high intake lines of mice became leaner even though they were consuming larger amounts of feed which suggests that animals maintain lean mass with greater priority as opposed to total body mass. Due to the fact that the fat lines were able to consume less food and maintain their body weight while the high intake lines consumed more but were leaner supports the conclusion of Russell and Wright (1983) that maintenance requirements may be more closely related to lean mass rather than overall body weight. Third, there are two points relating to lack of correlations that are very important to note. Within the fat content and lean mass deposition lines, there was a lack of a correlation between lean tissue deposition and fat deposition. Also, there was no correlation between maintenance requirements and intake in excess of maintenance requirements.

Bishop et al. (1991a) selected bull calves for higher and lower feed conversion (feed: gain). Their findings showed that animals with lower feed: gain ratios, thus greater efficiency, had greater levels of subcutaneous fat than those animals selected

for higher feed: gain ratios. It was concluded that differences among lines may be due to lower metabolizable energy requirements for maintenance of fat than for maintenance of lean tissue. One last conclusion drawn from these results was that selection for feed conversion in beef cattle shows response after just one generation.

In a continuation of the previously discussed study, a second publication by Bishop et al. (1991b) reports genetic and phenotypic correlations to feed conversion, as well as realized heritability estimates for high and low feed conversion lines. They looked at unadjusted feed conversion ratios (feed/gain) as well as feed conversion ratios adjusted for differences in maintenance requirements by expressing intake related to metabolic weight ($BW^{0.75}$) as recommended by Beef Improvement Federation (BIF, 1986). The heritability of unadjusted feed conversion was 0.26, whereas the adjusted estimate was 0.46. The correlation between unadjusted feed conversion ratio and adjusted ratio was 0.97. Bishop et al. (1991b) concluded that because animals with more desirable feed conversion ratios had a larger percentage of subcutaneous fat, selection for improved feed conversion may be beneficial if selection pressure is also applied against excessive accumulation of fat.

Selman et al. (2001) investigated thermoregulatory responses in mice that were selected for high and low feed intake. They also examined resting metabolic rate through indirect calorimetry at 30°, 25°, and 5° C. Their findings were slightly different than that of previously discussed papers. On average, mice of the high intake line had greater body mass than those in the low intake line in both sexes. However, the increased intake was associated with increased basal metabolic rates at

30° C. The greater intake mice also showed greater thermal conductance, which together with greater intake, accounted for 45% of elevated energy expenditures.

Effects of Selection for Heat Loss on maintenance

Nielsen et al. (1997a) selected mice for high (MH) and low (ML) heat loss per unit metabolic size ($\text{kcal/kg}^{0.75}/\text{d}$) based on direct calorimetry for fifteen generations (G1-15). Three lines (MH = high heat loss, ML = low heat loss, and control MC) in each of three replicates were used, for a total of nine unique lines. They showed that selection of this sort was very effective in changing the maintenance requirements of a mouse. McDonald and Nielsen (2007) reported on divergent selection of the heat loss lines for a total of 9 new generations (G42-51) following a 26-generation period of no selection. Previously, Hotovy et al. (1991) obtained an estimate of heritability for heat production (indirect calorimetry) that was 0.75 ± 0.13 using twin cattle. Their findings agree with the findings of Nielsen et al. (1997a) that selection to change heat loss is quite possible. This was supported by Shuey et al. (1993). They found that maintenance requirements were closely related to fasting heat production ($r^2 = 0.73$) and that heat production could be used as an indicator of maintenance energy requirements.

Heritability estimates, cumulative selection differentials, and cumulative standard selection differentials for the MH and ML lines from Nielsen et al. (1997a) and McDonald and Nielsen (2007) are listed in Table 1. During the initial selection, heat loss was moderately heritable, both in the up and down directions. During renewed selection, heritability for heat loss was moderately heritable in the up

direction, but lowly heritable in the down direction. Cumulative realized selection differentials were similar to cumulative intended selection differentials in both data sets. The amount of selection achieved in G1-15 ranged from 97%-100% and averaged 98% of the selection intended. This means that 98% of the time, on average, the selected males from each line and replicate successfully produced

Line	Realized Heritability		Cumulative Intended Selection Diff. (kcal/kg ^{0.75} /d)		Cumulative Realized Selection Diff. (kcal/kg ^{0.75} /d)		Cumulative Standardized Selection Diff. (kcal/kg ^{0.75} /d)	
	G1-15	42-51	G1-15	42-51	G1-15	42-51	G1-15	42-51
MH	31.0 ± 0.01	0.16 ± 0.05	147.90	91.40	145.10	91.78	10.06	5.46
ML	0.28 ± 0.01	0.07 ± 0.006	-106.70	-54.04	-105.00	-54.75	-9.51	-5.04

Table 1. Realized heritability estimates, cumulative intended, cumulative realized, and cumulative standardized selection differentials for the MH and ML lines in the first 15 generations of selection and for 9 generations of renewed selection (Nielsen et al. 1997a, McDonald 2007).

offspring for the next generation. Selection achieved during G42-51 ranged from 99%-102% and averaged 100%. A graphical representation of the response to selection for heat loss in each generation is given in Figure 1. Variation of response was evident across generations. During initial selection, response in the up direction was greater in some generations, whereas in other generations the large response was in the down direction. During renewed selection, response was in the up direction only. The average change per generation (MH minus ML), when estimated by regression, was 4.70 ± 0.09 (kcal/kg^{0.75}/d) for G1-15 and 2.49 ± 0.03 (kcal/kg^{0.75}/d) for G42-51.

Nielsen et al. (1997b) and McDonald and Nielsen (2007) used these same lines of mice (MH, ML, and MC) to research correlated responses to selection for

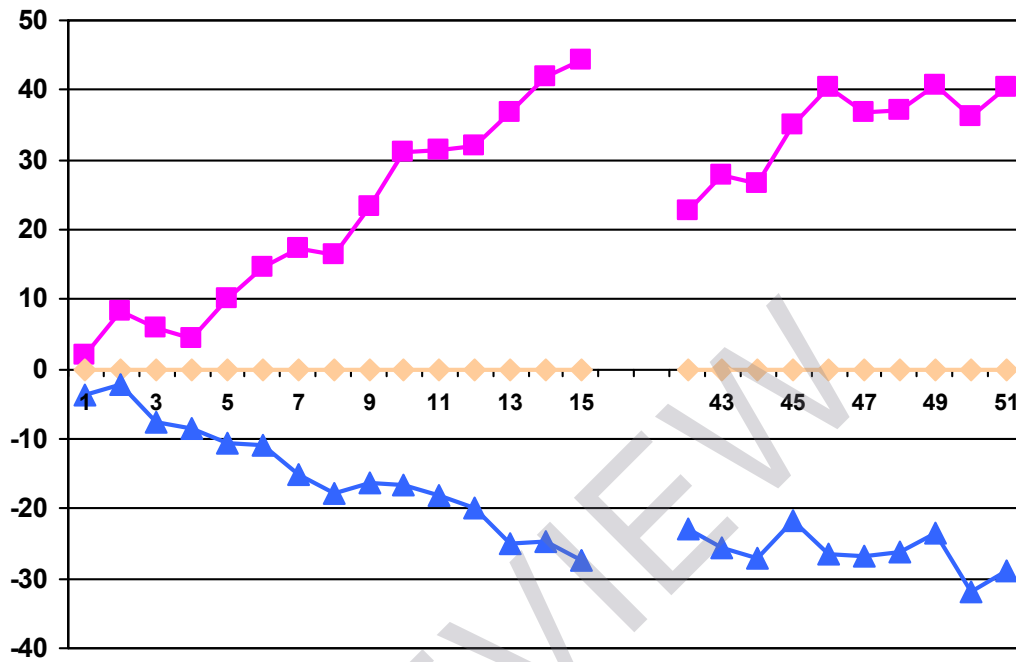


Figure 1. Line (MH = high, ML = low, MC = control, set to zero) means for heat loss ($\text{kcal} \cdot \text{kg}^{-0.75} \cdot \text{d}^{-1}$) averaged across all three replicates of selection. Selection ceased between Generations 16 and 42 and then was renewed.

increased and decreased heat loss. Responses in feed intake, body mass, body composition, and number of pups born were investigated by Nielsen et al. (1997b) while responses in feed intake, body mass, number of pups born, and conception rate were investigated by McDonald and Nielsen (2007). Feed intake, adjusted for metabolic size, changed according to the direction of selection. As heat loss increased in the MH line, feed intake increased for the MH line. The opposite was true for the ML line. By G15, the difference between MH and ML feed intake had reached 20.6%, the difference expressed in relation to the mean of the MC lines. By the end of renewed selection at G51, this difference had reached 34.0%.

Estimates of the genetic correlation of feed intake with heat loss were obtained during initial selection and ranged from 0.27-0.40. This correlation estimate assumed that the heritability of feed intake, adjusted for metabolic size, was between 0.20-0.30. Body composition differences among the two lines were not significant in early generations, but by Generation 14, MH mice showed significantly less body fat ($P < 0.01$) than the ML mice even though there was no difference in overall body mass between the two lines during the initial 15 generations of selection. Mice of the MC lines were intermediate for fat percentage. The fact that these mice consumed more and deposited less fat agrees with the results of Bishop and Hill (1985) and Bishop et al. (1991a, b). Following renewed selection, analysis of average body weights showed that the MC mice were on average 2.86 ± 0.12 g heavier than the MH mice ($P < 0.01$) and 3.10 ± 0.12 g heavier than the ML mice ($P < 0.01$).

Nielsen et al (1997b) reported that selection for heat loss had a significant effect on litter size in these lines of mice. Dams of the MH lines produced larger litters than the dams of the ML lines, and this difference was shown to be due to differences in ovulation rate. Similar litter size results were observed by McDonald and Nielsen (2007) during the last 9 generations of selection. McDonald and Nielsen (2007) also found that ML dams, on average, had a 6.7% greater conception rate than MH dams. The test for selection response (MH vs ML) tended toward significance ($P = 0.08$); there was no evidence for asymmetry of selection response ($P = 0.24$). Nielsen et al. (1997b) noted that a selection program that selects animals based on heat loss with the aim of decreasing feed intake, should take into consideration the effects on reproduction so that the overall economic merit is not reduced.