ABSTRACT: Divergent selection for heat loss was applied to lines of mice for 15 generations (G) in 3 replicates. Selection resumed at G42 and continued through G51 across all replicates. At the end of G51, differences in heat loss and feed intake per unit of BW were approximately 56 and 34%, respectively, between high heat loss (MH) and low heat loss (ML) lines, as a percentage of the control line (MC) mean. Rates of liver 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 maintenance requirements. Body composition, BW, liver weight, feed intake, and residual feed intake (RFI) were also measured or calculated. Results reported here represent data from 197 mature male mice from all replicates. There were no differences in BW ($P = 0.91$) between the selection lines. Selection had an effect on lean percentage ($P = 0.02$), with MH mice being leaner. Fat percentage differences between the selection lines tended toward significance ($P = 0.13$). Livers of MH mice were approximately 13% larger than livers of ML mice ($P = 0.01$). An effect of selection was observed ($P < 0.01$) in feed intake per unit BW, with MH mice consuming 29% more feed than ML mice in G58. Differences in state 2 and state 4 respiration rates were significant ($P = 0.01$), whereas state 3 rates approached significance ($P = 0.06$). Mitochondria of MH mice respirated at a greater rate than mitochondria of ML mice in all states of respiration; ML mice had respiratory control ratios that were, on average, 8% greater than MH mice ($P = 0.14$). Although this difference only tended toward significance, we suspect a greater degree of coupling of mitochondrial processes exists in ML animals. Mice selected for reduced heat loss had ADP:oxygen ratios that were approximately 20% greater than MH mice ($P = 0.03$). Therefore, greater mitochondrial efficiency was expressed in the ML animals. Within a line-replicate, there was no correlation between ADP:O and feed intake per unit BW ($P = 0.71$). In addition, no correlation of ADP:O and RFI existed ($P = 0.92$). Although the selection lines differed in mitochondrial traits, including overall mitochondrial efficiency (ADP:oxygen), these differences were not a significant underlying cause of variation in feed intake per unit BW or in RFI estimates.

Key words: feed efficiency, heat loss, mice, mitochondria, residual feed intake

INTRODUCTION

The greatest economic inputs in livestock production are the costs of feed, mainly the costs of meeting maintenance requirements of an animal. The existence of variation and the ability to estimate maintenance requirements from energy consumed or heat loss make it possible to select animals for more efficient food utilization. Previously, Nielsen et al. (1997b) 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 3 replicates and carried out the selection process through 15 generations (G). McDonald and Nielsen (2007) continued selection of these lines for an additional 9 generations from G42 through G51. Once selection ceased, the MH and ML lines had diverged by approximately 56 and 34% of the MC mean in heat loss and food intake per unit of BW, respectively, with the MH mice losing more heat and consuming more food than ML mice, although no differences in BW existed. These differences make the heat loss lines of mice an excellent animal model for the study of metabolic systems and
efficiency associated with maintenance energy requirements of animals.

Rolfe and Brown (1997) estimated that approximately 90% of mammalian oxygen consumption occurs in the mitochondria. Therefore, this organelle is a likely place to begin the investigation of the underlying variation in maintenance energy requirements. Mitochondrial efficiency is measured by calculating the rates of oxygen consumption of isolated mitochondria. 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 if divergent selection for heat loss has changed the functions and efficiency of mitochondria isolated from the livers of the selection lines of mice. The relationships of these mitochondrial traits with feed efficiency, residual feed intake (RFI), body composition, and liver size were also determined.

**MATERIALS AND METHODS**

All mice were reared and handled according to a protocol approved by the Institutional Animal Care and Use Committee of the University of Nebraska—Lincoln.

**Experimental Animals**

The lines of mice used for this study were described previously by Nielsen et al. (1997b) and were divergently selected for heat loss based on a single heat loss measurement obtained through direct calorimetry. There were 3 criteria for selection: MH = selection for high heat loss; ML = selection for low heat loss; and MC = no selection. Selection progressed in all lines within 3 independent replicates; thus, there were 9 unique lines. After initial selection for 16 generations, selection ceased, and the lines were maintained through G41 by using parents equally representing 26 litters per line-replicate-generation to slow inbreeding. At G42, selection resumed for 9 generations based on the described criteria through G51 with 16 litters per line-replicate-generation to slow inbreeding. At G42, selection resumed for 9 generations based on the described previously criteria through G51 with 16 litters per line-replicate-generation. When selection ceased at G51, line sizes were expanded to 24 litters in each line-replicate-generation in the MH and ML lines and to 23 litters in the MC lines. For this study, 1 male mouse from each litter was randomly chosen from the MH, ML, and MC lines of G58. A total of 70 male mice were used (24 MH and ML, 22 MC) within each of the 3 replicates.

**Feed Intake and BW Measurements**

At 8 wk of age, males were randomly selected from each litter within a replicate and individually housed in plastic shoebox cages (Ancare Corp., Waupaca, WI). Stainless-steel wire-bar lids with built-in hanging baskets for feed and slots for a water bottle were used. Feed was weighed and placed in the basket with a weighted plexiglass cover to avoid spillage. Feed intake was measured as the amount of feed disappearance for a 3-wk period through 11 wk of age. Throughout the trial, mice were given ad libitum access to water and were fed a maintenance diet (Teklad 8604: 24% CP, 4% crude fat, and 4.5% crude fiber, and 3.93 kcal/g of GE, Harlan Teklad, Madison, WI). Individual BW were recorded at the first and last days of the trial.

**Mitochondrial Isolation and Body Composition Measures**

Before data collection, all glassware was soaked for 24 h in a 10% HCl solution to dissolve any leftover detergent from washing. After soaking in HCl, glassware was rinsed with distilled H2O and then rinsed with deionized, distilled H2O. All glassware was allowed to dry completely before use. On the day of data collection, mice were weighed and killed one at a time by cervical dislocation. The liver was immediately removed and weighed. The liver was then quickly transferred to a small chilled beaker containing isolation buffer A (250 mM sucrose, 10 mM Tris-HCl, 1 mM ethylene glycol-bis-(2-aminoethyl)-N,N,N’,N’-tetraacetic acid (EGTA), and 0.5% BSA, pH 7.4). The liver was washed 3 times using buffer A to remove blood and debris. After the final rinse, 2 mL of buffer A was added to the liver sample. The sample was then minced on ice with scissors and transferred to a cold Potter-Elvehjem homogenizer. A Teflon-coated pestle was attached to an overhead stirrer (Wheaton Science Products, Millville, NJ). The sample was homogenized for 1 stroke with the pestle spinning at 3,000 rpm and then for 1 more stroke with the pestle spinning at 2,000 rpm. The glass mortar was situated in an ice-filled beaker during homogenization to keep the sample chilled and to inhibit proteases.

The homogenate was poured into a chilled centrifuge tube, and the total volume was brought to 11 mL using buffer A. The homogenate was centrifuged at 700 × g for 5 min at 4°C. Using a Kimwipe (Kimberly-Clark, Mississauga, Ontario, Canada), lipids were removed from the top of the supernatant. Lipids form a white foamy substance near the top of the tube, which can cause uncoupling if they come into contact with isolated mitochondria. The supernatant was then transferred to a cold, clean centrifuge tube using a prechilled Pasteur pipette. The supernatant was centrifuged at 7,500 × g for 10 min at 4°C to pellet the mitochondria. The mitochondrial pellet was resuspended in 5 mL of isolation buffer B (250 mM sucrose, 10 mM Tris-HCl, and 1 mM EGTA, pH 7.4) using a chilled glass rod. This procedure was done very gently so that air bubbles were not introduced into the solution. The mitochondria were then centrifuged again at 7,500 × g for 10 min at 4°C. The supernatant was discarded, and the pellet was re-suspended in 500 µL of buffer B. While centrifugation was taking place, body composition was determined on the liver-free body using a PIXI-mus dual x-ray densitometer (Lunar Corp., Madison, WI). This instrument estimated grams of lean and fat tissues of the mouse carcass, which was then expressed as a percentage of whole BW.
**Determination of Mitochondrial Protein Concentration**

A modified Bradford assay was used to determine the protein concentration of the isolated mitochondrial fraction. While keeping the original mitochondrial preparation on ice, 1.2 mL of de-ionized, distilled H2O, 250 µL of Bradford reagent (Sigma, St. Louis, MO) and 5 µL of the sample were combined and allowed to incubate for 5 min. Aliquots of 300 µL were loaded into 4 separate wells on a micro-plate. Absorbance was then measured at 595 nm using a Spectra-MAX spectrophotometer (Molecular Devices Corp., Sunnyvale, CA). Protein concentration of the isolated mitochondria was calculated using the average of the 4 absorbance measurements obtained and compared with values obtained from a standard curve of BSA standards (50, 30, 20, and 10 mg/mL). The standard curve was obtained before the day of data collection. The equation determined by analysis of the BSA standards was

\[
\text{average absorbance of sample} = 0.342 + 0.001 \\
\text{(mg of protein/mL of mitochondrial preparation)}.
\]

**Respiration Data Collection**

Mitochondrial protein (1 mg) and 5 µM rotenone were added to the electrode chamber with enough oxygen-saturated respiration buffer (100 mM KCl, 20 mM sucrose, 10 mM KH2PO4, 5 mM HEPES, 2 mM MgCl2·6H2O, and 1 mM EGTA, pH 7.2, 37°C) to achieve a final reaction volume of 1 mL. The stopper was adjusted to touch the top of the liquid level within the chamber to avoid the incorporation of atmospheric oxygen into the respiration buffer during the reaction. Once a stable signal was achieved on the recorder, 10 mM succinate was added to the chamber to induce a state 2 rate of respiration, and the addition was marked on the recorder. After several minutes of state 2 respiration, 150 µM ADP was added to the chamber to induce state 3 respiration. The state 3 rate of oxygen consumption was expected to be considerably greater than state 2. If there was no response to ADP, the mitochondria were considered to be completely uncoupled than state 2. If there was no response to ADP, the mitochondria were considered to be completely uncoupled. Ratios of ADP:O ratios (ADP:O) were determined by first calculating the total amount of oxygen consumed during state 3 respiration. The amount of ADP in micromoles (in this study, 150 µmol) was then divided by the micromoles of molecular oxygen consumed in state 3.

**RFI Estimates**

The following model was used to obtain an intercept and regression coefficients used subsequently to predict feed intake:

\[
\text{actual intake}_{ij} = \text{intercept} + \text{rep}_i + \left[ b_m \times (\text{BW}_{ij}) \right] + \left[ b_g \times (\text{BW gain}_{ij}) \right] + \epsilon_{ij},
\]

where rep is the random effect of replicate (1, 2, or 3); BWij and BW gainij are the average of beginning and ending BW and 3-wk BW gain of an individual animal, respectively; the intercept was estimated to be 50.5973; bm is the partial regression coefficient for maintenance requirements and was estimated to be 2.2058; bg is the partial regression coefficient for growth and was estimated to be −2.0469; and ej is the error term. An alternative model fitting BW0.75 was also evaluated; however, the R² was similar (0.2711 vs. 0.2707) for the 2 models, likely due to the similarity in BW among the animals. Predicted feed intake was then estimated by the equation

\[
\text{predicted feed intake} = 50.5973 + 2.2058(\text{BW}) - 2.0469(\text{BW gain}),
\]

where the BW and gain of an individual animal over a 3-wk period were entered for BW and BW gain, respectively. Estimates of RFI were then obtained for each individual mouse by

\[
\text{RFI} = \text{actual intake} - \text{predicted intake},
\]

**Statistical Analyses**

Differences between the lines in average BW (g), BW gain (g), feed intake per BW (g/d·g), RFI, percent lean, percent fat, and percent liver were all analyzed with the same model using the MIXED procedure (SAS Institute Inc., Cary, NC):

\[
y_{ijk} = \text{mean} + \text{line}_i + \text{rep}_j + (\text{line} \times \text{rep}_j) + \epsilon_{ijk},
\]

where yijk was any of the traits listed above; line is the fixed effect of line (MH, MC, or ML); repj is the random effect of replicate (rep 1, 2, or 3); line × repj is the random interaction of line and replicate; and ejk is the
RESULTS AND DISCUSSION

Mitochondrial Traits

Several mitochondrial traits were measured including state 2 respiration, state 3 respiration, state 4 respiration, RCR, and ADP:O ratio. Line means across all 3 replicates for the rates of respiration are given in Table 1. State 2 respiration rate is the basal rate of respiration that occurs immediately after providing the mitochondria with substrate. Divergent selection for heat loss created highly significant differences between the MH and ML lines in state 2 rate of oxygen consumption ($P < 0.001$). The MH mice exhibited state 2 respiration rate that averaged approximately 15% greater than the ML mice when the difference was calculated as a percentage of the MC mean. The MC mice were intermediate to the MH and ML; therefore, symmetry was accepted ($P = 0.98$). Repeatability of state 2 measurements was estimated to be 30%.

State 3 respiration rate is the maximum rate of oxygen consumption that occurs after the mitochondria are given exogenous ADP to drive the reaction. It appears that selection for heat loss created differences in state 3 respiration rate between the MH and ML lines ($P = 0.06$). That is, MH mice exhibited rate of state 3 oxygen consumption that was, on average, 8% greater than that observed in ML mice when calculated as a percentage of the MC mean. Although MC mice were intermediate to the MH and ML, MC rate of state 3 respiration was <1% greater than the rate of ML mice. This indicates that selection for heat loss in the high direction resulted in an increase in the maximum rate of respiration. Even so, symmetry of selection was still accepted ($P = 0.40$). Repeatability of state 3 respiration measurements was only 22%.

State 4 rate of respiration is the basal rate of oxygen consumption that occurs once all of the exogenous ADP is exhausted. State 4 rate is often referred to as the proton leak-dependent respiration rate. Selection effects were observed ($P = 0.02$), with MH rate of state 4 respiration being approximately 17% greater than ML state 4 rate, when the difference was calculated as a percentage of the MC mean. The MC mice exhibited a rate that was intermediate to MH and ML respiration rates, and therefore symmetry was accepted ($P = 0.72$). Repeatability of state 4 rates was only 25%. A visual representation of state 2, state 3, and state 4 respiration is given in Figure 1.

Bottje et al. (2002) studied respiration rates (states 2, 3, and 4) of mitochondria isolated from leg and breast muscles from high (i.e., improved) and low feed-efficient broilers. The authors also investigated the effects of different substrates on mitochondrial respiration. When succinate was used as a substrate (as in the current study), Bottje et al. (2002) were not able to detect dif-
ference among any of the respiration rates in muscle or liver tissues, even though the high feed-efficient birds had rates of respiration that tended to be slower than the low feed-efficient birds.

Ojano-Dirain et al. (2004) observed that the broilers in the high feed-efficiency group had greater rates of respiration in state 2 and state 3 than the low feed-efficiency group. The high efficiency group exhibited state 2 rate of oxygen consumption that was, on average, 25% greater than the low efficiency group, and state 3 rate of oxygen consumption that was approximately 19% greater. This is different than what was observed by Bottje et al. (2002) and in the current study where low feed-efficiency animals exhibited greater rates of mitochondrial respiration. Ojano-Dirain et al. (2004) also observed that the high feed-efficiency birds exhibited state 4 rate of respiration that was, on average, 32% slower than that of the low efficiency birds. These results agree with those of Bottje et al. (2002) and the current study. Lutz and Stahly (2003) also observed that decreased state 4 respiration rate was associated with greater G:F in rats.

Kolath et al. (2006) reported relationships between mitochondrial function and RFI in Angus steers. Kolath et al. (2006) used 9 low RFI and 8 high RFI steers to investigate differences in LM mitochondrial function. Kolath et al. (2006) showed that the low RFI (more efficient at feed utilization) animals exhibited state 2 rates of respiration that were approximately 56% greater than the high RFI steers. The authors also noted that the low RFI steers exhibited state 3 respiration rates that were, on average, 50% greater than the rates of the high RFI steers. No differences between the high and low groups in rate of state 4 respiration existed. Thus, Kolath et al. (2006), using cattle, and Ojano-Dirain et al. (2004), using broilers, reported greater respiration rate in more efficient animals; this is in contrast to our results with mice in which ML mice, our most efficient line, had the greater respiration rate.

Efficiency at the mitochondrial level can be expressed in various forms. One measure is the quantitative relationship between electron transport and oxidative phosphorylation, denoted as RCR. Another measure is the ADP:O ratio, which indicates the number of ADP molecules synthesized per atom of oxygen consumed. Line means across replicates for both of the measures are given in Table 1. Selection for heat loss perhaps had some effect on coupling, given that differences in RCR between MH and ML mice approached significance ($P = 0.09$). The ML mice demonstrated RCR that averaged 11% greater than MH mice, when the difference was calculated as a percentage of the MC mean, indicating a greater degree of coupling and thus greater mitochondrial efficiency among ML mice. The MC mice exhibited the least RCR at 16 and 5% less than the ML and MH mice, respectively. But, average RCR observed in this study were low across all lines, indicating a large degree of uncoupling. This may be due to the presence of other organelles or broken membranes within the mitochondrial pellet. The methods employed were consistent across mice of all lines; thus, there should be no bias in meeting our objective of comparing the mitochondrial activities of different lines. Asymmetry of selection approached significance ($P = 0.08$). Repeatability of the RCR measurement was only 20%.

The direct measure of mitochondrial efficiency is the ADP:O. This measure is expressed as an output divided by an input; therefore, ADP:O will be used to discuss overall mitochondrial efficiency. Repeatability of the ADP:O measure was 28%. Divergent selection proved to be a significant effect on mitochondrial efficiency ($P < 0.01$), with the ML mice having a mean ADP:O ratio that was 19% greater than the ratio in MH mice, when the divergence was calculated as a percentage of the MC mean. Therefore, mitochondria isolated from ML livers were 19% more efficient at creating ATP from the phosphorylation of ADP than mitochondria isolated from MH livers. The MC mice were intermediate to the MH and ML lines, and symmetry was accepted ($P = 0.20$). Figure 2 provides a graphical representation of the line means for RCR and ADP:O.

Bottje et al. (2002) reported that there were no differences between high and low feed-efficiency broilers for ADP:O ratio in the breast muscle, leg muscle, or liver when succinate or glutamate/malate were used as substrates. Bottje et al. (2002) also reported no differences between feed efficiency groups in RCR when succinate was used as a substrate. Bottje et al. (2002) did, however, observe significant differences in RCR in the breast and leg muscles when glutamate/malate was used as the substrate. Birds exhibiting greater efficiency of feed utilization also exhibited greater RCR ($P < 0.01$), which suggests a greater degree of coupling between electron transport and oxidative phosphorylation of the mitochondria. The substrate glutamate/malate provides electrons to the electron transport chain through the first complex of the chain, whereas succinate donates electrons through the second com-
plex. The fact that RCR differed between feed efficiency groups when glutamate/malate was used as opposed to when succinate was used suggests that important differences in mitochondrial efficiency that may be related to efficiency of feed utilization are probably resulting from the operations of the first complex of the electron transport chain. In the current study, succinate was the only source of substrate. Therefore, differences in mitochondrial efficiency between the high and low heat loss lines of mice do not reflect differences in the activities of complex I.

Kolath et al. (2006) reported ADP:O and RCR values for high and low RFI steers. The authors used a combination of glutamate and succinate to stimulate electron transport through the first and second complexes of the electron transport chain simultaneously. There were no observed differences in ADP:O between the high and low RFI groups. The authors reported that steers from the low RFI group had RCR values that were greater than those of the high RFI group. This indicates that a greater degree of mitochondrial coupling exists in improved efficiency of feed utilization. These results agree with the findings of Bottje et al. (2002).

**BW, BW Gain, and Composition**

Body weights were obtained at the start and end of the 3-wk feed intake trial. Data presented here represent the averages of the 2 time points. Line means across all replicates for BW, BW gain, and lean and fat as percentages of BW are presented in Table 2. No selection effects (MH vs. ML) were observed for overall BW ($P = 0.91$), whereas when all lines were compared, the effect of line approached significance ($P = 0.06$). Asymmetry of selection was observed ($P = 0.02$) for BW due to the large BW of MC mice compared with ML and MH mice (Figure 3). A graphical representation of average BW is shown in Figure 3. McDonald and Nielsen (2007) reported correlated responses to renewed divergent selection in the MH and ML lines; when renewed selection ceased at G51, the MC males were approximately 9% heavier than the average of the MH and ML lines, which is similar to the results observed in the current study.

The mice in this study were over 12 wk of age. At this age, mice are approaching maturity and are eating mainly for meeting their maintenance requirements, and thus, only small BW gains were observed. Even so, selection had an effect on 3-wk BW gain ($P = 0.03$) such that ML males gained approximately 46% more from 8 to 11 wk of age than the MH mice, when the difference between MH and ML was expressed as a percentage of the MC mean. The null hypothesis of a symmetric response was accepted ($P = 0.91$).

Significant line and selection effects existed for lean percentage ($P = 0.02$). Difference in MH and ML was approximately 6% of the MC mean, with MH mice having a greater percentage of lean tissue than ML. There were no notable differences between the ML and MC lines (<1.0% difference) in lean tissue mass, causing asymmetry of selection to approach significance ($P = 0.08$). Divergent selection for heat loss tended ($P = 0.13$) to result in correlated responses in fat percentage. The MH and ML male mice differed by approximately

**Table 2.** Least squares means and SE for BW, BW gain, lean percentage, and fat percentage for high heat loss (MH), control (no selection; MC), and low heat loss (ML) lines across all 3 replicates

<table>
<thead>
<tr>
<th>Line</th>
<th>Average BW, $^1$ g</th>
<th>BW gain, $^1$ g</th>
<th>Lean, $^1$ %</th>
<th>Fat, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>MH</td>
<td>31.91</td>
<td>1.53</td>
<td>74.77</td>
<td>12.13</td>
</tr>
<tr>
<td>MC</td>
<td>35.35</td>
<td>1.96</td>
<td>70.55</td>
<td>14.44</td>
</tr>
<tr>
<td>ML</td>
<td>32.04</td>
<td>2.44</td>
<td>70.64</td>
<td>13.72</td>
</tr>
<tr>
<td>SE</td>
<td>0.96</td>
<td>0.21</td>
<td>1.75</td>
<td>0.76</td>
</tr>
</tbody>
</table>

$^1$Difference between the MH and ML lines was significant ($P < 0.05$).
11% of the MC mean, with ML having a greater fat percentage than MH mice. However, MC males had the greatest percentage of fat, probably related to their greater BW. An asymmetrical response in fat percent to selection for heat loss tended toward significance (\(P = 0.11\)). These results are similar to those reported earlier in these selection lines by Moody et al. (1997), Nielsen et al. (1997a), and Kgwalala and Nielsen (2004), in that ML mice (greater feed efficiency) were fatter than MH mice.

**Feed Intake per BW and RFI Estimates**

Line means across all 3 replicates of intake (g/d) as a percentage of average BW and average RFI estimates are given in Table 3. Highly significant effects of line (\(P < 0.001\)) and selection (\(P < 0.001\)) on intake per BW existed. Differences between the MH and ML mice were approximately 27% of the MC mean, with the MH mice consuming more feed than ML mice. However, a significant asymmetric response to selection was observed (\(P = 0.03\)) because most of the response was in MH (Figure 4). As expected, selection had a significant effect on RFI estimates (\(P = 0.001\)), and symmetry was accepted (\(P = 0.26\)).

Table 3. Least squares means and SE for intake per BW and residual feed intake (RFI) for 3 wk for the high heat loss (MH), control (no selection; MC), and low heat loss (ML) lines across 3 replicates

<table>
<thead>
<tr>
<th>Line</th>
<th>Intake/average BW,1 %</th>
<th>RFI,1 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>MH</td>
<td>19.86</td>
<td>15.03</td>
</tr>
<tr>
<td>MC</td>
<td>16.43</td>
<td>−2.87</td>
</tr>
<tr>
<td>ML</td>
<td>15.40</td>
<td>−12.87</td>
</tr>
<tr>
<td>SE</td>
<td>0.31</td>
<td>3.28</td>
</tr>
</tbody>
</table>

\(^1\)Difference between the MH and ML lines was significant (\(P < 0.05\)).

Nielsen et al. (1997a) reported correlated responses in feed intake to the initial divergent selection for heat loss. They observed that the average difference between the MH and ML lines was approximately 23% with the MH consuming more than ML and no differences in BW at generation 15. After renewed selection, McDonald and Nielsen (2007) reported differences in feed intake; MH mice consumed approximately 39% more than ML (G51). Although a genetic trend of decreasing BW existed in the ML line across G42 to G51 (renewed selection), there were no differences between BW of MH and ML at generation 51.

**Liver Size**

Line means for liver size as a percentage of total BW were 5.45, 5.25, and 4.83 g for MH, MC, and ML mice, respectively. Divergent selection for heat loss has created differences in liver sizes between the MH and ML lines (\(P = 0.01\)). The difference between the MH and ML was, on average, 12% with MH mice having larger livers than ML mice. Mice of the control line (MC) were intermediate to the MH and ML for liver percentage; thus, the null hypothesis of symmetry was accepted (\(P = 0.45\), Figure 5).

Moody et al. (1997) also investigated differences in liver size between the MH and ML lines of mice. Moody et al. (1997) concluded that, like in the current study, the MH mice had significantly larger livers than the ML mice. However, Kgwalala and Nielsen (2004) found no differences in liver weights (\(P > 0.20\)) among these selection lines. Ferrell and Jenkins (1985) reported that variation among maintenance requirements in cattle can be explained by variation in many body components that include variation in visceral organ mass. Therefore, animals with greater maintenance requirements would be expected to have larger visceral organ size. Due to the conclusions of Ferrell and Jenkins (1985), we would expect the observed differences
in liver size between these selection lines of mice that differ greatly in maintenance requirements.

**Regression Coefficients of Feed per BW on Various Traits**

Regression coefficients of feed per BW and RFI on each individual mitochondrial trait and some of the body measures are given in Table 4. The table provides the coefficients calculated from 2 different models that included replicate, or replicate and line. These models were used to calculate the percentage of variation in feed intake that can be explained by variation in the given mitochondrial trait. In every case, when only replicate and mitochondrial traits were fitted in the model, regression coefficients that were obtained indicated that the observed differences in mitochondrial traits explained a large portion of the differences in feed intake per BW and RFI. However, when line was added to the model to exclude its confounding effect, the regression coefficients became very small, thus pointing to little or no detectable relationship between feed intake per BW or RFI and the mitochondrial trait; this was unexpected. From the model ignoring line effects, about 18% of the variation in feed intake per BW could be explained by variation in ADP:O ratios, whereas this variation accounted for approximately 17% of the variation in RFI estimates. However, when line was fitted in the model the amount of variation in feed intake per BW and RFI estimates explained by variation in ADP:O dropped to approximately 1.1 and <1.0%, respectively. The same is true for all of the mitochondrial traits, such that a strong relationship with feed efficiency existed within replicate but there was little or no relationship within a line of mice.

A more revealing picture emerged with the regression on liver percentage. On a within line basis, differences between MH and ML mice in liver percentage accounted for ~8% of the differences in feed intake per BW and for 7% of the differences in RFI. Differences in the percentages of lean and fat tissues accounted for approximately 6 and 4% of the variation in feed intake per BW, respectively, while accounting for roughly 2% of the variation in RFI estimates.

The unexpected lack of relationship within a line between feed intake measures and the mitochondrial traits may be due to 2 main factors: 1) a large amount of variation in measures of mitochondrial traits within a line-replicate and 2) a small amount of variation in feed intake per BW or RFI estimates within a line-replicate, although a large amount of variation exists across all lines. Repeatabilities of all of the mitochondrial traits

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### Table 4. Regression coefficients of feed intake:BW and residual feed intake (RFI) on each trait calculated from 2 separate models

<table>
<thead>
<tr>
<th>Trait</th>
<th>Feed:BW = trait + replicate</th>
<th>Feed:BW = trait + replicate + line</th>
<th>RFI = trait + replicate</th>
<th>RFI = trait + replicate + line</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver, %</td>
<td>1.443</td>
<td>0.545</td>
<td>9.562</td>
<td>3.337</td>
</tr>
<tr>
<td>Lean, %</td>
<td>0.000</td>
<td>0.066</td>
<td>0.790</td>
<td>−0.614</td>
</tr>
<tr>
<td>Fat, %</td>
<td>−0.269</td>
<td>−0.167</td>
<td>−1.500</td>
<td>−0.449</td>
</tr>
<tr>
<td>State 2</td>
<td>0.504</td>
<td>0.089</td>
<td>3.461</td>
<td>0.847</td>
</tr>
<tr>
<td>State 3</td>
<td>0.250</td>
<td>0.167</td>
<td>1.565</td>
<td>0.719</td>
</tr>
<tr>
<td>State 4</td>
<td>0.430</td>
<td>0.119</td>
<td>2.923</td>
<td>0.947</td>
</tr>
<tr>
<td>RCR1</td>
<td>−0.066</td>
<td>0.177</td>
<td>−1.346</td>
<td>0.956</td>
</tr>
<tr>
<td>ADP:O2</td>
<td>−1.867</td>
<td>−0.120</td>
<td>−11.581</td>
<td>0.274</td>
</tr>
</tbody>
</table>

1Respiratory control ratio.
2ADP:oxygen ratio.

---

### Table 5. Partial correlation coefficients (with P-value listed in parentheses) between traits measured across and within lines

<table>
<thead>
<tr>
<th>Trait</th>
<th>F/BW</th>
<th>RFI</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>RCR</th>
<th>ADP:O</th>
</tr>
</thead>
<tbody>
<tr>
<td>F/BW</td>
<td>1.00</td>
<td>0.94 (&lt;0.001)*</td>
<td>0.28 (&lt;0.001)*</td>
<td>0.28 (&lt;0.001)*</td>
<td>0.30 (&lt;0.001)*</td>
<td>−0.05 (0.493)</td>
<td>−0.33 (&lt;0.001)*</td>
</tr>
<tr>
<td>RFI</td>
<td>0.85 (&lt;0.001)*</td>
<td>1.00</td>
<td>0.30 (&lt;0.001)*</td>
<td>0.28 (&lt;0.001)*</td>
<td>0.31 (&lt;0.001)*</td>
<td>−0.06 (0.407)</td>
<td>−0.32 (&lt;0.001)*</td>
</tr>
<tr>
<td>S2</td>
<td>0.11 (0.142)</td>
<td>0.12 (0.089)</td>
<td>1.00</td>
<td>0.72 (&lt;0.001)*</td>
<td>0.76 (&lt;0.001)*</td>
<td>−0.02 (0.829)</td>
<td>−0.27 (&lt;0.001)*</td>
</tr>
<tr>
<td>S3</td>
<td>0.24 (0.001)*</td>
<td>0.20 (0.006)*</td>
<td>0.72 (&lt;0.001)*</td>
<td>1.00</td>
<td>0.72 (&lt;0.001)*</td>
<td>0.18 (0.013)</td>
<td>−0.24 (&lt;0.001)*</td>
</tr>
<tr>
<td>S4</td>
<td>0.17 (0.021)*</td>
<td>0.14 (0.047)*</td>
<td>0.74 (&lt;0.001)*</td>
<td>0.72 (&lt;0.001)*</td>
<td>1.00</td>
<td>−0.42 (&lt;0.001)*</td>
<td>−0.45 (&lt;0.001)*</td>
</tr>
<tr>
<td>RCR</td>
<td>0.04 (0.630)</td>
<td>0.04 (0.606)</td>
<td>0.02 (0.785)</td>
<td>0.21 (0.005)*</td>
<td>−0.40 (&lt;0.001)*</td>
<td>1.00</td>
<td>0.21 (0.003)*</td>
</tr>
<tr>
<td>ADP:O</td>
<td>−0.03 (0.712)</td>
<td>−0.01 (0.920)</td>
<td>−0.18 (0.130)</td>
<td>−0.21 (0.005)*</td>
<td>−0.38 (&lt;0.001)*</td>
<td>0.17 (0.210)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

1Values above the diagonal are partial correlation coefficients within replicates; values below the diagonal are partial correlation coefficients within line-replicate.
2F/BW = feed:BW; RFI = residual feed intake; S2 = state 2 respiration; S3 = state 3 respiration; S4 = state 4 respiration; RCR = respiratory control ratio; ADP:O = ADP:oxygen ratio.
*Asterisks indicate correlations between 2 traits that met or exceeded the significance criterion of α = 0.05.
were relatively small, which contributes to an extremely low relationship between feed intake and mitochondrial efficiency within a line of mice. To overcome this problem, one would have to obtain more measurements per animal (>3), which may be hard due to the fragility of isolated mitochondria and the increased activation of detrimental proteases with time. To solve this problem, one would need to have multiple oxygen electrode systems operating at once. Alternatively, measuring more animals would give a better insight as to what is taking place within these lines.

**Partial Correlation Coefficients**

Partial correlation coefficients of the single feed intake per BW and RFI measures with the repeated mitochondrial measures were calculated on a within replicate basis as well as a within line-replicate basis. These correlation coefficients are given in Table 5. On a within replicate basis, significant correlations between mitochondrial efficiency and feed intake per BW and RFI were found. Also, significant relationships among all of the mitochondrial traits were indicated. On a within line-replicate basis, we were unable to show correlations of mitochondrial efficiency with nonmitochondrial traits, which was, as expected, consistent with the regression results. Strong relationships still existed between all of the mitochondrial traits on a within line-replicate basis. Very few studies have reported relationships between mitochondrial traits and feed efficiency. Bottje et al. (2002) reported that breast and leg muscle mitochondria RCR values were significantly correlated to feed efficiency \( r^2 = 0.72, \ P < 0.001 \) and \( r^2 = 0.37, \ P < 0.01 \), respectively. But, no relationship was found between liver mitochondrial efficiency and feed efficiency within a line of broilers, which agrees with the results presented here. Lutz and Stahly (2003) reported that decreased state 4 rates of respiration were significantly correlated to improved G:F ratios \( r^2 = 0.42, \ P < 0.01 \). Lutz and Stahly (2003) also found a significant correlation between RCR and feed efficiency \( r^2 = 0.33, \ P < 0.05 \). In the current study, no relationship was observed between RCR of liver mitochondria and feed efficiency.

The goal of this study was to measure mitochondrial efficiency as a means of explaining some of the variation in maintenance requirements between the MH and ML lines of mice that resulted from divergent selection for heat loss. Although no relationship between mitochondrial efficiency and feed intake was detectable, it is evident that divergent selection has resulted in differences in the rates of hepatic mitochondrial respiration and efficiency. Future research should focus on the possible causes of these differences as well as investigation of mitochondrial efficiency in other tissues. To explain more of the variation in maintenance requirement created by divergent selection for heat loss, several routes could be taken such as investigating differences in digestion and possibly whole-body protein turnover.

**LITERATURE CITED**


