Different contribution of muscle and liver lipid metabolism to endurance capacity and obesity susceptibility of mice

Satoshi Haramizu, Azumi Nagasawa, Noriyasu Ota, Tadashi Hase, Ichiro Tokimitsu, and Takatoshi Murase

Biological Science Laboratories, Kao Corporation, Ichikai-machi, Haga-gun, Tochigi, Japan

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Haramizu S, Nagasawa A, Ota N, Hase T, Tokimitsu I, Murase T. Different contribution of muscle and liver lipid metabolism to endurance capacity and obesity susceptibility of mice. J Appl Physiol 106: 871–879, 2009. First published January 8, 2009; doi:10.1152/japplphysiol.90804.2008.—We investigated strain differences in whole body energy metabolism, peripheral lipid metabolism, and energy metabolism-related gene expression and protein levels in BALB/c, C57BL/6J, and A/J mice to evaluate the relationship between endurance capacity, susceptibility to diet-induced obesity, and differences in lipid metabolism in muscle and liver. A high-fat diet significantly increased body weight and fat weight in C57BL/6J mice, but not in BALB/c and A/J mice. The endurance capacity of BALB/c mice was 52% greater than that of C57BL/6J mice and 217% greater than that of A/J mice. The respiratory exchange ratio was lowest in BALB/c mice, higher in C57BL/6J mice, and highest in A/J mice, which inversely correlated with the endurance capacity and fatty acid β-oxidation activity in the muscle. Plasma lactate levels measured immediately after exercise were lowest in BALB/c mice and highest in A/J mice, although there was no difference under resting conditions, suggesting that carbohydrate breakdown is suppressed by enhanced fat utilization during exercise in BALB/c mice. On the other hand, the body weight increase induced by high-fat feeding was related to a reduced whole body energy expenditure, higher respiratory quotient, and lower fatty acid β-oxidation activity in the liver. In addition, β-oxidation activity in the muscle and liver roughly paralleled the mRNA and protein levels of lipid metabolism-related molecules, such as peroxisome proliferator-activated receptor and medium-chain acyl-CoA dehydrogenase, in each tissue. These findings indicate that genetically determined basal muscle and liver lipid metabolism and responsiveness to exercise influence physical performance and obesity susceptibility.

Numerous studies on energy metabolism and exercise have been performed, aimed at preventing and treating obesity. Genetically identical inbred mouse strains are recently established models for studying genetic influences on phenotypes (1, 3, 33), and C57BL/6J and A/J mice are now widely used as biomedical study models. C57BL/6J mice develop severe obesity when fed a high-fat diet, whereas A/J mice become only moderately obese and are classified as an obesity-/diabetes-resistant strain (35, 39).

Several studies have evaluated the mechanisms underlying the differences in the susceptibility to high-fat diet-induced obesity between C57BL/6J and A/J mice. In A/J mice, but not C57BL/6J mice, the expression of uncoupling proteins (UCP) in adipose tissues, which regulate thermogenesis through dissipation of the proton gradient across the inner mitochondrial membrane, is significantly increased in response to a high-fat diet (36). Furthermore, the intestine is highly responsive to dietary fat and high-fat feeding-induced activation of lipid metabolism-related genes that include carnitine palmitoyltransferase I and malic enzyme, which is more pronounced in the intestine of A/J mice than C57BL/6J mice (16). These findings suggest that differences in the basal and upregulated expression levels of energy metabolism-related molecules in various organs are important factors in the susceptibility to diet-induced obesity.

Determining the factors that influence physical performance and developing a methodology for improving performance has attracted increased attention in sports science. Strain differences in intrinsic endurance capacity leading to a two- to fourfold difference have been identified in rats and mice using a treadmill exercise test (2, 19, 20). Lightfoot et al. (21) reported that there is a strong genetic contribution to endurance capacity in mice and that A/J mice cannot run as long as C57BL/6J and BALB/c mice (21). Furthermore, rats with high endurance capacity have lower blood pressure, visceral adipose fat, plasma insulin, and glucose levels than rats with low endurance capacity (40), suggesting that endurance capacity is linked to metabolic disorders, including obesity.

Thus, although energy metabolism, obesity susceptibility, and endurance capacity seem closely related, few studies have evaluated the relationships between the individual components. In addition, to our knowledge, few studies have examined how differences in energy metabolism in the liver and muscle influence the endurance capacity and susceptibility to obesity in an integrative manner. In the present study, we attempted to clarify the relationship between three factors, energy metabolism, obesity susceptibility, and endurance capacity, at the individual and molecular levels in an integrative manner, by examining whole body energy metabolism, fatty acid β-oxidation activity, and expression of energy metabolism-related molecules in the liver and skeletal muscle in C57BL/6J, A/J, and BALB/c mice. A better understanding of the critical determinants of obesity susceptibility and physical performance at various levels will provide new insights for the management of obesity-related disorders and the improvement of athletic performance.
MATERIALS AND METHODS

Experimental Animals and Diet

Six-week-old male C57BL/6J, BALB/c AnNCrj, and A/J mice were purchased from Charles River Laboratories Japan (Kanagawa, Japan). The rodents were housed in plastic cages in groups of five in a room maintained at 23 ± 2°C with a relative humidity of 55 ± 10% and a light period from 0700 to 1900. Mice were allowed free access to drinking water and a standard diet (CE-2, CLEA Japan, Tokyo, Japan) for maintenance during the preliminary breeding period. In experiment 1, mice were maintained on one of the following synthetic diets: a low-fat diet containing 5% (wt/wt) fat, 20% casein, 66.5% potato starch, 4% cellulose, 1% vitamin mixture, and 3.5% mineral mixture (low-fat group); or a high-fat diet containing 30% fat, 20% casein, 28.5% potato starch, 13% sucrose, 4% cellulose, 1% vitamin mixture, and 3.5% mineral mixture (high-fat group). In experiment 2, mice were maintained on a standard diet. During the experiments, the animals were cared for in accordance with the American Physiological Society’s Guiding Principles for the Care and Use of Animals. This study was approved by the Animal Care Committee of Kao Tochigi Institute.

Experimental Design

Two experiments were conducted in this study. In experiment 1, the effects of a high-fat diet on body weight and fat accumulation were examined. In experiment 2, the endurance capacity and energy metabolism of the mice were determined.

Experiment 1. At 8 wk of age, mice of each strain were divided into two groups (n = 8/group; 4 mice/cage) and maintained on their respective diets for 15 wk. Body weight and cumulative energy intake were monitored throughout the experimental period. On the final day of the experiment, all of the mice were anesthetized with sevoflurane (SEVOFRAN, Maruishi Pharmaceutical, Osaka, Japan) inhalation and killed to measure abdominal fat (epididymal, perirenal, and retroperitoneal) weights.

Experiment 2. At 8 wk of age, mice of the three strains were run on a treadmill with a 7° incline and adapted to running at 25 m/min using the 5-day training program described below. At 8–9 wk of age, energy metabolism studies during exercise and under resting conditions were performed using indirect calorimetry. Blood samples from 9-wk-old mice were collected from the tail vein immediately after the running exercise. At 10 wk of age, running time to exhaustion was measured. On the final day of the experiment (at 11 wk of age), nonfasted mice were anesthetized by sevoflurane inhalation and blood samples were collected from the postcaval vein. The muscles (gastrocnemius and soleus), abdominal fat (epididymal, perirenal, and retroperitoneal), and liver were dissected and the weights of these tissues were measured.

Running Exercise and Evaluation of Endurance

A 10-lane motorized rodent treadmill (MK-680, Muromachi Kikai, Tokyo, Japan) was used to determine the endurance capacity for running. Mice were adapted to running at 25 m/min according to the following 5-day running training program: day 1: 10 m/min for 15 min and 15 m/min for 15 min; day 2: 10 m/min for 10 min and 15 m/min for 20 min; day 3: 15 m/min for 15 min and 20 m/min for 15 min; day 4: 15 m/min for 5 min, 20 m/min for 15 min, and 25 m/min for 10 min; and day 5: 15 m/min for 5 min, 20 m/min for 10 min, and 25 m/min for 15 min.

Running times to exhaustion were measured according to the following program for measurement of endurance: 10 m/min for 5 min, 15 m/min for 5 min, 20 m/min for 30 min, 25 m/min for 300 min.

Indirect Calorimetry Analysis in Exercised and Resting Conditions

Respiratory metabolic rate was measured using an individual open-circuit indirect calorimetric system equipped with a four-lane airtight rodent treadmill (Modular Treadmill system, Columbus Instruments, Columbus, OH), with one mouse per lane. The details of the methods were described in a previous report (32).

Mice were deprived of food overnight, then allowed access to a standard diet (CE-2) for 1 h to avoid the effects of a time lag and differences in the amount of food eaten by each mouse, and habituated to the treadmill chamber for 2 h (data were not collected). Mice were then run on a 5° incline, with an initial speed of 10 m/min for 5 min, to adapt to running gradually on the treadmill. Running speed was changed to 15 m/min for 5 min, and subsequently to 20 m/min for 20 min. Data were collected continuously every 5 min with a settling time of 45 s during which the chamber sample was purged, and a measurement time of 15 s, with the room air as reference.

Respiratory metabolic rate in the nonexercised condition was also measured using an indirect calorimetric system equipped with an eight-channel airtight metabolic cage (Oxymax Equal Flow 8 chamber/Small Subject System, Columbus Instruments). Each mouse was allowed free access to a standard diet and water and oxygen consumption was measured for 24 h. During this time, the data for each chamber were collected every 18 min with a settling time of 30 s and a measurement time of 90 s, with room air as reference. The respiratory quotient (RQ) and respiratory exchange ratio (RER) were calculated by dividing the CO₂ production by the O₂ consumption.

Blood Components

Blood lactate, ketone bodies, and glucose levels in blood were determined with Lactate Pro (Arkley, Kyoto, Japan), Xtra (Abbott Japan, Tokyo, Japan) and Accu-Check (Roche Diagnostics, Tokyo, Japan), respectively, according to the manufacturer’s instructions. Plasma nonesterified fatty acids (NEFA) and glucose levels were measured with NEFA HA, L-Type Glu2 assay kits (WAKO Pure Chemical Industries, Osaka, Japan).

Glycogen and Triglyceride Measurement

Liver and muscle glycogen levels were determined using a standard enzymatic technique as described previously (27). In brief, frozen liver and gastrocnemius muscle were digested in 30% KOH for 30 min at 100°C. After saturated sodium sulfate was added, the glycogen was precipitated by adding 95% ethanol. The solution was then centrifuged at 1,600 g. The supernatant was collected and the remaining alcohol was vaporized. The pellet was dissolved in H₂O and precipitated with 95% ethanol. The supernatant was decanted after centrifugation at 1,600 g, and the remaining alcohol was vaporized. Purified glycogen was hydrolyzed in 0.6 N HCl at 100°C for 2 h. Glucose residue levels were determined with the Glucose CII test kit (WAKO Pure Chemical Industries).

To analyze triglyceride levels, frozen liver tissue was homogenized and extracted with a chloroform-methanol mixture (2:1 vol/vol) as described previously (41) with minor modifications. In brief, chloroform-methanol was added to the homogenate and shaken overnight. After shaking, 4 mM MgCl₂ (100 μl) was added to the solution. The organic layer was collected after centrifugation at 1,000 g for 60 min. The collected sample was dried and resuspended in 1% Triton X-100/ethanol, and then triglyceride was enzymatically quantified using a TG-E test Wako (WAKO Pure Chemical Industries).

Fatty Acid β-Oxidation Activity

Fatty acid β-oxidation activity was measured as described previously (27). Frozen gastrocnemius muscle and liver from nonfasted, anesthetized mice in experiment 2 were thawed and homogenized on ice using a Physcocon homogenizer (model NS-310E, Microtech, Chiba, Japan) in 250 mM sucrose, 1 mM EDTA in 10 mM HEPES (pH 7.2). Subcellular debris was removed by centrifugation at 600 g for 5 min, and the resulting supernatants were used in the assays. Activity was measured using [1-14C]palmitic acid as the substrate.
Values were expressed as percentages, using the value of C57BL/6J mice as 100%.

RNA Extraction and Real-Time PCR

Total RNA was isolated from the mouse gastrocnemius and soleus muscles, and liver from nonfasted, anesthetized mice in experiment 2 using Isogen (WAKO Pure Chemical Industries) according to the manufacturer’s instructions. Reverse transcription was performed with a SuperScript first-strand synthesis system (Invitrogen, Carlsbad, CA). Transcript levels were examined by real-time PCR with SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA). Real-time PCR was performed with an ABI PRISM 7000 sequence detector using the following protocol: 50°C for 2 min, 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. The mRNA levels were calculated relative to those of the 36B4 mRNA, and the normalized values were expressed as ratios, with the value of C57BL/6J mice defined as 1.0. The primers used were the following: peroxisome proliferator-activated receptor (PPAR)-α (GenBank: NM_011144, nt 1639-1658 and 1711-1730), PPAR-δ (NM_011145, nt 1245-1264 and 1369-1388), PPAR-γ coactivator-1α (PGC-1α; NM_008904, nt 642-661 and 751-770), medium-chain acyl-CoA dehydrogenase (MCAD; NM_007382, nt 1064-1083 and 1164-1184), acyl-CoA oxidase (ACO; NM_015729, nt 1843-1863 and 1942-1963), carnitine palmitoyltransferase (CPT)-1α (NM_013495, nt 4029-4048 and 4205-4229), CPT-1β (NM_009948, nt 2290-2309 and 2371-2390), fatty acid translocase (CD36/FAT; NM_011145, nt 1415-1435 and 1593-1615), fatty acid binding protein (FABP; NM_010174, nt 279-298 and 359-379), cytochrome-c oxidase (COX)-4 (NM_053091, nt 276-295 and 396-415), UC2P (NM_011671, nt 858-878 and 973-993), and UCP3 (NM_009464, nt 1009-1029 and 1067-1087).

Western Blot Analysis

The livers and gastrocnemius muscles were homogenized and lysed with ready-made homogenization buffer, Celllyte: MT Mammalian Tissue Lysis/Extraction Reagent (Sigma, St. Louis, MO) containing a medium of proteins to the membrane in each lane. The membranes were stained with Ponceau S (Sigma) to verify equal loading and transfer of proteins. The membranes were incubated with horseradish peroxidase-labeled anti-rabbit immunoglobulin (GE Healthcare, Buckinghamshire, UK) as a secondary antibody. Blots were visualized with Enhanced Chemiluminescence plus reagent (GE Healthcare) and a ChemiDoc XRS imaging system (Bio-Rad Laboratories, Hercules, CA).

Statistical Analysis

All values were presented as means ± SE. Statistical analysis was conducted using the unpaired t-test and Tukey-Kramer test. Bartlett’s test was used to evaluate whether fatty acid β-oxidations in the muscle and liver of individual mice correlated with the running time and body weight increase. All statistical analyses were performed by using StatView (SAS Institute, Cary, NC). Results with values of $P < 0.05$ were considered statistically significant.

RESULTS

Effects of High-Fat Diet on Body Weight and Fat Accumulation

The body and relative fat weights of each strain are shown in Table 1. Consistent with previous reports (35, 39), chronic consumption of the high-fat diet significantly increased body weight compared with the low-fat diet in C57BL/6J mice. There was no body weight increase in BALB/c and A/J mice. The relative weight of the intra-abdominal fat was significantly increased in C57BL/6J mice but not in BALB/c and A/J mice. These data confirmed that C57BL/6J mice are obesity prone and BALB/c and A/J mice are obesity resistant.

Running Endurance Capacity and Relative Muscle Weight

There was a clear difference in the running endurance capacity among the strains. The running time of 10-wk-old BALB/c mice was 52% greater than that of C57BL/6J and 217% greater than that of A/J mice (Fig. 1). The relative ratio of gastrocnemius muscle to body weight of each strain was 0.94 ± 0.009% in BALB/c, 0.981 ± 0.005% in C57BL/6J, and 0.881 ± 0.022% in A/J mice. The relative ratio of the soleus muscle to body weight was 0.051 ± 0.002% in BALB/c, 0.053 ± 0.002% in C57BL/6J, and 0.052 ± 0.003% in A/J mice. There were no significant differences among the strains.

Whole Body Energy Metabolism Under Resting Conditions and During Exercise

To evaluate the interaction between energy metabolism and either endurance capacity or obesity susceptibility, we examined oxygen consumption and RQ (RER) in these strains by indirect calorimetry. Under resting conditions, oxygen consumption in BALB/c and A/J mice was significantly higher than that in C57BL/6J mice (Fig. 2A). On the other hand, RQ values in BALB/c and A/J mice were significantly lower than

Table 1. Body weight and relative abdominal fat weights after high-fat diet feeding

<table>
<thead>
<tr>
<th>Strain</th>
<th>BALB/c</th>
<th>C57BL/6J</th>
<th>A/J</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LF</td>
<td>HF</td>
<td>LF</td>
</tr>
<tr>
<td>Final body weight, g</td>
<td>33.28±0.59</td>
<td>33.19±0.60</td>
<td>33.11±0.64</td>
</tr>
<tr>
<td>Epididymal fat, %</td>
<td>1.45±0.14</td>
<td>1.36±0.15</td>
<td>2.78±0.27</td>
</tr>
<tr>
<td>Perirenal fat, %</td>
<td>0.56±0.06</td>
<td>0.49±0.04</td>
<td>0.54±0.05</td>
</tr>
<tr>
<td>Retroperitoneal fat, %</td>
<td>0.36±0.05</td>
<td>0.43±0.04</td>
<td>0.78±0.11</td>
</tr>
</tbody>
</table>

Values are means ± SE. Tissue weights were measured after 15 wk (experiment 1). LF, low-fat diet; HF, high-fat diet. The relative abdominal fat weight (%) was calculated from the absolute fat weights and the body weight of each mouse. *Significant difference between HF diet and LF diet groups in the respective strain, $P < 0.05$ (unpaired t-test).

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that in C57BL/6J (Fig. 2B), suggesting that a higher level of fat utilization and energy expenditure under resting conditions is related to resistance to high-fat diet-induced obesity.

Under running exercise conditions, oxygen consumption in all strains increased compared with that under resting conditions (Fig. 2C). Unlike in the resting condition, however, oxygen consumption in C57BL/6J mice was significantly greater than that in BALB/c and A/J mice. On the other hand, the RER of BALB/c mice during exercise was significantly lower, whereas the RER of A/J mice was significantly higher than that of C57BL/6J mice (Fig. 2D), suggesting that the increased fat utilization generates running energy in BALB/c mice. These results suggest that a higher level of lipid utilization during exercise is a crucial factor that determines running endurance capacity. There was no difference in the amount of food ingested among the strains.

**Blood Analysis Under Resting Conditions and Immediately After Exercise**

The blood components of each strain under resting conditions and immediately after exercise are shown in Table 2. In the resting condition, plasma glucose and NEFA levels were significantly lower in BALB/c and A/J mice than in C57BL/6J mice.

Immediately after running exercise, plasma glucose levels decreased in all strains, and were significantly lower in BALB/c and A/J mice than in C57BL/6J mice. Conversely, lactate levels increased in all strains; A/J mice had the highest levels and BALB/c mice had the lowest. Serum NEFA levels also increased in all strains; A/J mice had significantly lower levels than C57BL/6J and BALB/c mice. Serum ketone body
levels were higher in BALB/c and A/J mice than in C57BL/6J mice.

Liver and Muscle Glycogen Content and Hepatic Triglyceride Content Under Resting Conditions

The liver glycogen levels were 31.6 ± 2.8 mg/g tissue in BALB/c, 30.1 ± 2.4 mg/g tissue in C57BL/6J, and 36.2 ± 2.4 mg/g tissue in A/J mice. The muscle glycogen levels were 4.4 ± 1.1 mg/g tissue in BALB/c, 4.5 ± 0.8 mg/g tissue in C57BL/6J, and 4.1 ± 1.2 mg/g tissue in A/J mice. No significant differences in either the liver or muscle were detected between the strains. The liver triglycerides were 24.9 ± 1.6 mg/g tissue in BALB/c, 19.4 ± 1.6 mg/g tissue in C57BL/6J, and 21.1 ± 1.9 mg/g tissue in A/J mice and there was no difference between the strains.

Fatty Acid β-Oxidation Activity in Skeletal Muscle and Liver

Fatty acid β-oxidation activity from the skeletal muscle homogenate was highest in BALB/c mice and lowest in A/J mice (Fig. 3A). The activity was significantly higher in BALB/c mice by 41 and 82% compared with that in C57BL/6J and A/J mice, respectively. On the other hand, the β-oxidation activity of A/J and BALB/c mice measured from the liver homogenate was significantly higher by 96 and 49%, respectively, than that in C57BL/6J mice. There was no significant difference between A/J and BALB/c mice. The level of liver β-oxidation activity was well correlated with obesity susceptibility, and the level of muscle β-oxidation activity was correlated with the endurance capacity.

Muscle and Liver Gene Expression and Protein Contents Associated With Energy Metabolism

We further examined the mRNA expression of genes involved in energy metabolism in liver, and in the gastrocnemius and soleus muscles (Tables 3 and 4). The mRNA expression of lipid metabolism-related genes in the liver tended to be higher in BALB/c and A/J mice than in C57BL/6J mice. The PPAR-α and MCAD mRNA levels in liver were significantly higher in BALB/c and A/J mice than in C57BL/6J mice. The ACO mRNA and CPT1-α mRNA levels were significantly higher in A/J mice than in C57BL/6J mice. UCP2 in BALB/c mice was significantly higher than that in C57BL/6J mice. PPAR-α, ACO, and FABP mRNA expression levels were significantly lower in A/J mice than in C57BL/6J mice. In the soleus muscle, PPAR-α, PPAR-δ, MCAD, and ACO mRNA expression levels were higher in BALB/c mice than in C57BL/6J mice.

We also examined the protein levels of PGC-1α, PPAR-α, and PPAR-δ in gastrocnemius muscle, and of PPAR-α in the liver. Consistent with the mRNA levels, BALB/c mice had higher PGC-1α, PPAR-α, and PPAR-δ protein levels in the gastrocnemius muscle (Fig. 4A). In contrast, A/J mice had the highest PPAR-α protein levels in the liver (Fig. 4B).

Table 2. Blood and plasma component concentrations under resting conditions and immediately after running exercise

<table>
<thead>
<tr>
<th>Condition</th>
<th>BALB/c</th>
<th>C57BL/6J</th>
<th>A/J</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dl</td>
<td>Resting 172.31±6.20a</td>
<td>219.66±14.71b</td>
<td>160.48±9.21c</td>
</tr>
<tr>
<td></td>
<td>After exercise 125.75±6.41a</td>
<td>195.88±6.13b</td>
<td>65.00±10.44c</td>
</tr>
<tr>
<td>Lactic acid, mmol/l</td>
<td>Resting 1.81±0.21a</td>
<td>2.46±0.34</td>
<td>2.87±0.49</td>
</tr>
<tr>
<td></td>
<td>After exercise 2.61±0.21a</td>
<td>4.20±0.32</td>
<td>10.24±0.47</td>
</tr>
<tr>
<td>NEFA, meq/l</td>
<td>Resting 0.73±0.04a</td>
<td>0.92±0.07b</td>
<td>0.67±0.07a</td>
</tr>
<tr>
<td></td>
<td>After exercise 1.13±0.06a</td>
<td>1.03±0.05a</td>
<td>0.76±0.04b</td>
</tr>
<tr>
<td>Ketone body, mmol/l</td>
<td>Resting 0.39±0.08a</td>
<td>0.20±0.03b</td>
<td>0.60±0.05c</td>
</tr>
</tbody>
</table>

Values are means ± SE. Blood was collected at the age of 9 wk immediately after running exercise and at the age of 11 wk under nonfasting resting conditions (experiment 2). NEFA, nonesterified fatty acid. a,b,c Values with different superscripts are significantly different, P < 0.05 (Tukey-Kramer’s test).
There was a significant correlation between endurance capacity and fatty acid β-oxidation in the gastrocnemius muscle (r = 0.80, P < 0.001; Fig. 5A), whereas that in the liver was not significantly correlated with running time (r = −0.17, P = 0.47; Fig. 5B). On the other hand, there was no significant correlation between the body weight increase of mice during experiment 1 and fatty acid β-oxidation in the gastrocnemius muscle (r = −0.21, P = 0.34; Fig. 5C). The body weight increase, however, significantly correlated with fatty acid β-oxidation in the liver (r = −0.68, P < 0.001; Fig. 5D).

**DISCUSSION**

In the present study, we examined the contribution of energy metabolism in the muscle and liver to endurance capacity and susceptibility to high-fat diet-induced obesity in three inbred mouse strains. The results indicated that obesity susceptibility was mainly determined by the level of liver β-oxidation activity, the mRNA levels of related molecules, and resultant whole body oxygen consumption (energy expenditure) level under resting conditions. On the other hand, endurance capacity was mainly determined by the level of β-oxidation activity in skeletal muscle and lipid utilization during exercise, but not by oxygen consumption. The different contribution of lipid metabolism in each organ to obesity susceptibility and endurance capacity was genetically determined by the levels of lipid-metabolizing enzymes and upstream transcription factors.

C57BL/6J mice are susceptible to high-fat diet-induced obesity, hyperglycemia, and hyperinsulinemia. In contrast, A/J mice become only moderately obese on a high-fat diet and are classified as an obesity- and diabetes-resistant strain (35, 39). The present results are consistent with these previous reports. In addition, our results indicated that, like A/J mice, BALB/c mice are less susceptible to becoming obese from ingesting a high-fat diet than C57BL/6J mice. Interestingly, running endurance capacity was highest in BALB/c mice and lowest in A/J mice among the strains, although both BALB/c and A/J mice are obesity resistant.

**Table 4. mRNA expression in the gastrocnemius and soleus muscle from BALB/c, C57BL/6J, and A/J mice**

<table>
<thead>
<tr>
<th></th>
<th>Gastrocnemius</th>
<th>Soleus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BALB/c</td>
<td>C57BL/6J</td>
</tr>
<tr>
<td>PPAR-α</td>
<td>1.01 ± 0.06^a</td>
<td>1.00 ± 0.05^b</td>
</tr>
<tr>
<td>PPAR-δ</td>
<td>0.96 ± 0.03^b</td>
<td>1.00 ± 0.04^a</td>
</tr>
<tr>
<td>PGC-1</td>
<td>1.39 ± 0.13^b</td>
<td>1.00 ± 0.03^a</td>
</tr>
<tr>
<td>MCAD</td>
<td>1.10 ± 0.07</td>
<td>1.00 ± 0.06</td>
</tr>
<tr>
<td>ACO</td>
<td>1.11 ± 0.05^a</td>
<td>1.00 ± 0.05^a</td>
</tr>
<tr>
<td>CPT-1β</td>
<td>1.42 ± 0.08^b</td>
<td>1.00 ± 0.10^a</td>
</tr>
<tr>
<td>CD36/FAT</td>
<td>1.42 ± 0.14</td>
<td>1.00 ± 0.13</td>
</tr>
<tr>
<td>FABP</td>
<td>1.37 ± 0.04^b</td>
<td>1.00 ± 0.05^a</td>
</tr>
<tr>
<td>COX-4</td>
<td>1.25 ± 0.06^b</td>
<td>1.00 ± 0.04^b</td>
</tr>
<tr>
<td>UCPI</td>
<td>3.41 ± 0.20^a</td>
<td>1.00 ± 0.08^a</td>
</tr>
<tr>
<td>UCPIII</td>
<td>0.82 ± 0.06</td>
<td>1.00 ± 0.07</td>
</tr>
</tbody>
</table>

Values are means ± SE. Each tissue was dissected from nonfasted, resting mice on the final day of experiment 2. Gene expression of the muscles is expressed as the ratio of the value in C57BL/6J mice. PPAR-α, peroxisome proliferator-activated receptor-α; PPAR-δ, peroxisome proliferator-activated receptor-δ; CPT-1β, carnitine palmitoyltransferase-1β; CD36/FAT, fatty acid translocase; FABP, fatty acid binding protein; COX-4, cytochrome-c oxidase subunit 4; UCPI, uncoupling protein 3. Values with different superscripts are significantly different, P < 0.05 (Tukey-Kramer’s test).
During endurance exercise, muscle relies mainly on fat and carbohydrate as energy sources (31). Enhanced fatty acid oxidation during exercise reduces the rate of glycogen depletion, resulting in improved endurance capacity (11, 12, 25). The decreased RER in BALB/c mice indicates that an increase in fat utilization during exercise leads to higher endurance. Conversely, the increased RER in A/J mice indicates an increase in carbohydrate-derived energy. Immediately after exercise, NEFA levels were significantly higher in BALB/c mice than in A/J mice, although there was no difference under resting conditions (Table 2). An increased supply of free fatty acids during exercise might induce increased fat oxidation in muscles of BALB/c mice. In addition, plasma lactate levels measured immediately after exercise were lower in BALB/c mice and higher in A/J mice, indicating that carbohydrate utilization is suppressed by enhanced fat utilization in BALB/c mice and accelerated by lower fat utilization in A/J mice.

Skeletal muscle and liver are the two major glycogen storage sites. Although several reports demonstrated that manipulating carbohydrate metabolism influences endurance in humans and mice (7, 22), the benefits of increasing glycogen for endurance are still controversial (10). Recent studies demonstrated that endurance capacity is not altered in mice overexpressing a hyperactive form of glycogen synthase (23, 29) or in mice lacking muscle glycogen synthase (30). In agreement with these findings, liver and muscle glycogen contents under resting conditions did not correlate with endurance capacity, indicating that basal glycogen level is not a primary determinant of endurance capacity. Rather, the difference in endurance capacity might be explained by the capacity and balance of carbohydrate and lipid metabolism.

In BALB/c mice, fatty acid β-oxidation activity was high in both the muscle and liver tissue homogenates. In contrast, A/J mice had high β-oxidation activity only in the liver. Muscle and liver are major regulatory sites of whole body fatty acid and glucose metabolism. The energy expenditure in muscle at rest is nearly equal to that in the liver, and the sum of the proportional contribution of muscle and liver is ~45% of the total resting energy expenditure in humans (8). Muscle uses lipids to a much greater extent during moderate-intensity exercise than during resting conditions (15). We attribute the lower RQ in the resting condition in BALB/c and A/J mice to the higher metabolic activity of lipids in the liver, which suggests that higher levels of lipid oxidation in the liver are closely related to resistance to obesity. Moreover, lower RER during exercise in BALB/c mice is attributed to the higher metabolic activity of lipids, particularly in the muscle, suggesting that muscle lipid oxidation greatly affects endurance. In
fact, the muscle β-oxidation activity significantly correlated with the running time and liver β-oxidation activity significantly correlated with the body weight increase (Fig. 5). A/J mice might use more carbohydrate as energy during exercise because of the lower β-oxidation activity in their muscle. Greater use of carbohydrate during exercise induced hypoglycemia concomitant with the increased lactate concentration after exercise. Moreover, liver β-oxidation activity in A/J mice was the highest among the three strains, which may lead to a lower RQ at rest and greater use of lipids during exercise and a subsequent increase in ketone bodies.

In this study, the expression of lipid metabolism-related genes in the muscle and liver was higher in BALB/c mice than in C57BL/6J mice, whereas it was higher in the liver and lower in the muscle in A/J mice than in C57BL/6J mice. These findings corresponded with the levels of fatty acid β-oxidation activity in both tissues. Most of the molecules necessary for fatty acid uptake and oxidation are regulated by several transcription factors, including the PPAR isoforms PPAR-α, PPAR-γ, and PPAR-δ and their coactivator PGC-1α. PPAR-α is predominantly expressed in the liver (14), and its activation upregulates liver β-oxidation enzymes, resulting in reduced body fat accumulation (24, 34). Wang et al. (38) reported that overexpression of PPAR-δ in skeletal muscle increases endurance capacity together with the upregulation of lipid metabolism-related molecules such as CPT-1 and cytochrome c (38). Furthermore, PGC-1α interacts with various transcription factors, including PPARs and nuclear respiratory factor 1 (18, 28), and regulates the genes involved in energy metabolism (26), mitochondrial biogenesis (6), and exercise performance (4, 9). These transcriptional factors are highly expressed both in the muscle and liver of BALB/c mice; therefore, the different levels of energy metabolism-related gene and the resultant metabolic capacity might be determined, at least in part, by the expression levels of PPARs and PGC-1α in each tissue.

UCPs have important roles in thermogenesis and body weight control (5, 13, 17). The expression level of UCP3, which is abundant in muscle (37), however, was not necessarily correlated with obesity-resistance among the strains (Tables 3 and 4). Similarly, UCP2 and UCP3 expression levels were not correlated with endurance capacity. The involvement of UCPs in thermogenesis, obesity-resistance, and endurance capacity of BALB/c and A/J mice remain to be elucidated.

In summary, we examined energy metabolism in three mouse strains with marked phenotypic differences, and the results revealed that liver and muscle lipid metabolism differentially contribute to physical performance and susceptibility to diet-induced obesity. The results suggest that tissue-directed activation of lipid metabolism is preferable for improving physical performance and managing obesity-related disorders. Furthermore, our results provide additional insight for understanding the mouse models of obesity and endurance capacity.

REFERENCES


