Intrinsic aerobic capacity impacts susceptibility to acute high-fat diet-induced hepatic steatosis

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Morris EM, Jackman MR, Johnson GC, Liu TW, Lopez JL, Kearney ML, Fletcher JA, Meers GM, Koch LG, Britton SL, Rector RS, Ibdah JA, MacLean PS, Thyfault JP. Intrinsic aerobic capacity impacts susceptibility to acute high-fat diet-induced hepatic steatosis. Am J Physiol Endocrinol Metab 307: E355–E364, 2014. First published June 24, 2014; doi:10.1152/ajpendo.00093.2014.—Aerobic capacity/fitness significantly impacts susceptibility for fatty liver and diabetes, but the mechanisms remain unknown. Herein, we utilized rats selectively bred for high (HCR) and low (LCR) intrinsic aerobic capacity to examine the mechanisms by which aerobic capacity impacts metabolic vulnerability for fatty liver following a 3-day high-fat diet (HFD). Indirect calorimetry assessment of energy metabolism combined with radiolabeled dietary food was employed to examine systemic metabolism in combination with ex vivo measurements of hepatic lipid oxidation. The LCR, but not HCR, displayed increased hepatic lipid accumulation in response to the HFD despite both groups increasing energy intake. However, LCR rats had a greater increase in energy intake and demonstrated greater daily weight gain and percent body fat due to HFD compared with HCR. Additionally, total energy expenditure was higher in the larger LCR. However, controlling for the difference in body weight, the LCR has lower resting energy expenditure compared with HCR. Importantly, respiratory quotient was significantly higher during the HFD in the LCR compared with HCR, suggesting reduced whole body lipid utilization in the LCR. This was confirmed by the observed lower whole body dietary fatty acid oxidation in LCR compared with HCR. Furthermore, LCR liver homogenate and isolated mitochondria showed lower complete fatty acid oxidation compared with HCR. We conclude that rats bred for low intrinsic aerobic capacity show greater susceptibility for dietary-induced hepatic steatosis, which is associated with a lower energy expenditure and reduced whole body and hepatic mitochondrial lipid oxidation.

fatty liver; energy intake; fitness; obesity; energy expenditure

LOW AEROBIC CAPACITY OR FITNESS is a powerful predictor of cardiovascular and all-cause mortality independent of other risk factors, including smoking, obesity, previous cardiovascular disease, and diabetes (18, 19). Importantly, improving fitness also significantly increases survival in previously low-fit individuals (19). Aerobic capacity is also an independent predictor for the development of the metabolic syndrome and type 2 diabetes (12, 34). Low aerobic capacity is also a powerful predictor of nonalcoholic fatty liver disease (NAFLD) prevalence (2) and negatively impacts lifestyle-based treatments for NAFLD (16). However, the mechanism(s) linking low aerobic capacity to metabolic disease processes remains unknown. In particular, there are limited data documenting the whole body and liver-specific energy metabolism phenotypes that exist between groups of differing aerobic capacities and how these different phenotypes may modulate susceptibility for NAFLD.

NAFLD represents a spectrum of disease initially characterized by steatosis that may progress to hepatic inflammation, fibrosis, and cirrhosis. The presence of NAFLD represents a significant health risk, independently predicting increases in liver-related and all-cause mortality (37). NAFLD is the hepatic manifestation of the metabolic syndrome and is reaching epidemic proportions around the globe (1). The etiology of NAFLD is a complex interaction between environmental and genetic factors to produce liver-specific and multisystem pathophysiology resulting in the initial accumulation of hepatic lipid (1). This is emphasized by the strong association of NAFLD with other metabolic disease states and the increased risk of developing cardiovascular disease, type 2 diabetes, and chronic kidney disease in subjects with NAFLD (1). To date, there is no effective pharmacological intervention for NAFLD. The only accepted treatment paradigm for NAFLD is lifestyle modification including weight loss and increased physical activity (33), a modifier of aerobic capacity. As such, it is critical to understand the physiological characteristics by which aerobic capacity impacts the development of NAFLD to better tailor lifestyle modification treatment and target other novel therapeutic approaches.

The purpose of this investigation was to examine whether and how differences in energy metabolism associated with divergent intrinsic aerobic capacity impact the development of hepatic steatosis following an acute high-fat diet. We utilized the high-capacity runner (HCR)/low-capacity runner (LCR) rat model system that was created to demonstrate divergent intrinsic aerobic capacity in a sedentary condition and results in differing vulnerability to chronic disease conditions (metabolic syndrome, insulin resistance, and steatosis) (39, 42). This model provides a unique opportunity to investigate the role of aerobic capacity on adaptive energy metabolism responses to an acute high-fat diet (HFD), a metabolic challenge known to
induce obesity, steatosis, and insulin resistance (14, 20). Herein, we utilized indirect calorimetry to examine how divergent intrinsic aerobic capacity influences whole body energy intake, energy expenditure, energy balance, and substrate utilization following a 3-day HFD challenge. Additionally, we examined whole body and liver-specific fatty acid oxidation (FAO), mitochondrial respiration, and lipid storage. We hypothesized that low intrinsic aerobic capacity would increase susceptibility for HFD-induced liver steatosis through reduced whole body and hepatic lipid oxidation, whereas high intrinsic aerobic capacity would provide protection against steatosis.

MATERIALS AND METHODS

Animals. The HCR/LCR rat model was developed and characterized as described previously (17, 27, 39, 42). At 25–30 wk of age, animals were singly housed and acclimatized to the low-fat control diet (D12450B, 10% kcal fat; Research Diets, New Brunswick, NJ) for ≥7 days prior to the initiation of the 3-day HFD (D12451, 45% kcal fat; Research Diets). Food intake was monitored daily for at ≥3 days prior to and during the 3-day HFD. The animal protocols were approved by the Institutional Animal Care and Use Committees at the University of Missouri and University of Colorado School of Medicine and the Subcommittee for Animal Safety at the Harry S. Truman Memorial Veterans Affairs Hospital.

Body composition analysis. Body composition was measured by MRI using the EchoMRI-900 (EchoMRI, Houston, TX). Body fat percentage was calculated as the fat mass divided by animal weight. Lean mass is a value analytically determined by the instrument. Fat-free mass (FFM) is the difference between body weight and fat mass. Body composition was determined immediately before euthanization.

Steatosis analysis. Hematoxylin and eosin (H & E), steatosis score, and percent nuclei with lipid were performed as described previously (31). Liver triacylglycerol concentration was determined as described previously using a commercially available kit (F6428; Sigma, St. Louis, MO) (39).

Metabolic monitoring. Energy utilization was determined by monitoring VO2, VCO2, and urinary nitrogen in the metabolic monitoring system developed by the Energy Balance Core Laboratory at the Satellite Facility, as described previously (22, 23, 38). Animals were acclimated to the indirect calorimetry cages for 4 days prior to initiation of HFD and data collection. Metabolic rate was calculated with the Weir equation (MR = 3.941 × VO2 + 1.106 × VCO2 − 2.17 × N) and respiratory quotient (RQ) as VCO2/VO2. Total energy expenditure (TEE) was calculated as the average of all metabolic rate measurements over the 23-h daily data collection and was extrapolated for presentation purposes to reflect that amount of energy expended through 24 h. Resting energy expenditure (REE) was extrapolated from the resting metabolic rate. The resting metabolic rate was determined by calculating the average metabolic rate during a 1-h period occurring in the latter part of the light cycle, when metabolic rate and RQ indicated minimal physical activity and food intake. Non-resting energy expenditure (NREE) was calculated as the difference between TEE and REE. Calorimetry data is represented as the average of the daily values across the 3-day HFD challenge. Energy balance (EB) was calculated as the average of the daily difference between energy intake (EI) and TEE throughout the 3-day period. Percent substrate utilization was calculated as the 3-day average of the daily kilocalories of carbohydrate, lipid, and protein disposal divided by the energy intake. Each metabolic cage was equipped with an animal activity meter (Opto-Max; Columbus Instruments, Columbus, OH). This allows for the determination of total, ambulatory, and nonambulatory activity by monitoring the number of beam breaks within a one-dimensional series of infrared beams. Activity was monitored continuously for 24 h.

Twenty-four-hour dietary free fatty acid tracer. To assess dietary fatty acid oxidation, tissue retention and trafficking of dietary fat, and de novo lipogenesis, an in vivo, 24-h, dual-tracer study was performed as described previously (15, 38). On day 2 of the 3-day HFD, animals received an intraperitoneal injection of [3H2]O (200 µl, 1 mCi/ml) 1 h prior to the start of the dark cycle, which allows for tracer equilibration with total body water, and measurement of incorporation of tritium in extracted lipid serves as an estimate of net retention of carbon via de novo lipogenesis. A 4:1 ratio of [1-14C]oleate and [1-14C]palmitate was blended into both the low-fat diet (LFD) and HFD, resulting in specific activities of 0.45 and 0.92 µCi/g diet (4.82 and 2.04 µCi/g dietary fat), respectively. The labeled diet was given at the start of the final dark cycle. Every 3 h, CO2 from each chamber was collected in 3.0-mL aliquots of a 2:1 mixture of methanol and methylbenzethonium hydroxide (no. B2156; Sigma Chemical). The 14C content of these samples was then measured with a Beckman LS6500 scintillation counter. At the end of the 24-h tracer study, rats were anesthetized with isoflurane and euthanized by exsanguinations as the tissues were extracted.

Tissue dietary lipid analysis. To determine total tissue lipid 14C and 3H incorporation, total lipids were extracted utilizing the Dole extraction (4). Two-hundred milligrams of liver and gastrocnemius and 100 mg of fat pads (retroperitoneal, epidydimal, omental, mesenteric, and inguinal) were homogenized in isopropanol-heptane-1 N H2SO4 (40: 10:1). Phases were separated with normal saline, the upper phase was collected and dried under N2 gas, and 14C and 3H were determined by liquid scintillation counting. 14C per sample was converted to milligrams of dietary fat and normalized to sample weight in grams. Total adipose and liver tissue dietary fat were determined as the grams of dietary fat per gram of tissue times the weight to the tissue in grams. Total skeletal muscle tissue dietary fat was determined as the grams of dietary fat per gram of gastrocnemius times the approximate weight of skeletal muscle for each rat (~38% of body weight) (13). 3H counts per sample were normalized to sample weight in grams.

Mitochondrial isolation. Mitochondria were isolated from rat liver tissue, as described previously (25). Briefly, tissue was homogenized (Teflon on glass) in cold liver mitochondrial isolation buffer (220 mM mannitol, 70 sucrose, 10 mM Tris, and 1 mM EDTA, adjusted to pH 7.4 with KOH) and centrifuged (1,500 g, 10 min, 4°C). The supernatant was centrifuged (8,000/6,000/4,000 g, 10 min, 4°C), with the pellet resuspended (glass on glass) in liver mitochondrial isolation buffer following each centrifugation. The protein concentration was determined by BCA assay.

Hepatocyte isolation, culture, and lipid treatment. Hepatocytes from male HCR/LCR rats were isolated by the two-step collagenase perfusion method, as described previously (11, 25). The liver was blanched with EGTA perfusion solution (HBSS without Ca2+ and Mg2+; Invitrogen), 15 mM HEPES, 100 U·ml penicillin−1·100 µg/ml streptomycin−1, and 0.5 mM EGTA, pH ~7.2, and digested with collagenase solution (HBSS; Invitrogen), 15 mM HEPES, 100 U·ml penicillin−1·100 µg/ml streptomycin−1, 10 mM L-glutamine, 0.1% collagenase (Invitrogen), and 0.1% soybean trypsin inhibitor (Invitrogen), pH ~7.2. Cells were washed in Hepatoxyte Wash Media (Invitrogen) three times, with the cells pelleted at 50 g for 5 min for the first wash and 2 min for subsequent washes. Cells were resuspended in hepatocyte growth medium (Williams E; Invitrogen), 10% FBS, 4 mM L-glutamine, 100 µM penicillin/100 µg streptomycin, 2 ng/ml rat EGF, 100 µM insulin, 100 nM dexamethasone, 0.1% BSA, and 10 mM sodium pyruvate, and viability was determined using trypan blue. Cell viability was never observed to be <90%. The crude cell isolate was preplated on 100 mm2 plates for 1 h. After 1 h, the unattached cells were collected, pelleted, and resuspended in a collagen matrix [DMEM-low glucose (Invitrogen), rat tail collagen] and the cells plated to produce 10 µg/cm2 collagen culture matrix. After the collagen matrix solidified, the cells were fed daily with hepatocyte
growth media. After 4 days of culture, the cells (12-well and 100 mm² plates) were exposed to 250 µM palmitate-250 µM oleate or control in lipid media (hepatocyte growth media, lipids conjugated to BSA, 0.5% final concentration). Following overnight free fatty acid (FFA) exposure, the cells were serum starved for 4 h.

Mitochondrial respiration. Mitochondrial oxygen consumption was measured using a Clark-type electrode system (Strathkelvin Instruments, North Lanarkshire, Scotland), as described previously (24). Incubations were carried out in a 0.5-ml final volume maintained at 37°C, containing (in mmol/l) 220 mannitol, 70 sucrose, 10 KH₂PO₄, 5 MgCl₂, 2 HEPES, 1 EGTA, 10 glucose, and 0.2% bovine serum albumin, pH 7.4. Mitochondrial respiration of substrates was monitored at the following concentrations: 1 mM malate, 10 mM glutamate, and 10 mM succinate. Mitochondria and substrates were added, and the coupled maximal respiration rate (state 3) was initiated with the addition of adenosine 5'-diphosphate (ADP; 200 µM).

Respiration through complex I was measured with glutamate (+ malate), whereas convergent respiration through complexes I and II of glutamate was examined by the addition of succinate. Maximal uncoupled respiration was determined by titration of carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (1 µM). The consumption of oxygen in nanomoles per minute was normalized to mitochondrial protein in the respirometer cell.

Palmitate oxidation by liver homogenate and isolated mitochondria. The oxidation of [1-¹⁴C]palmitate was measured in fresh liver homogenates and isolated mitochondria, as described previously (25). Fatty acid oxidation was assessed by measuring the production of ¹⁴CO₂ (complete FAO) and [¹⁴C]acid-soluble metabolites (ASM) in a sealed trapping device at 37°C containing 200 µM palmitate, [¹⁴C]palmitate, tissue sample, and reaction buffer [100 mM sucrose, 10 mM Tris-HCl, 10 mM KPO₄, 100 mM KCl, 1 mM, 4 MgCl₂·6H₂O, 1 mM l-carnitine, 0.1 mM malate, 2 mM ATP, 0.05 mM CoA, and 1 mM DTT (pH 7.4)]. To assess the induction of complete FAO in isolated liver mitochondria, ADP (2.5 mM) was added to appropriate wells. ADP induction of complete FAO in isolated mitochondria was graphed as the difference of basal complete FAO and ADP-stimulated complete FAO.

Primary hepatocyte fatty acid oxidation. The rate of FFA oxidation by primary hepatocytes was determined as described previously, with minor modifications (25). Following serum starvation, 12-well plates were washed with warm PBS and the cells incubated with [¹⁴C]FFA FAO reaction medium (DMEM-low glucose (Invitrogen), 0.5 µCi/ml [¹⁴C]palmitate, 200 µM palmitate, 0.5% BSA, 1 mM carnitine, and 12.5 mM HEPES (pH 7.4)) at 37°C for 3 h in triplicate. After 3 h, the medium from each well was collected, and an aliquot of medium was dispensed into the sealed trapping device. The [¹⁴C]CO₂ was driven from the media aliquot by addition of perchloric acid and trapped in NaOH. The NaOH was collected and analyzed by liquid scintillation counting for determination of complete FAO to CO₂.

Statistical analysis. The main effects of phenotype and diet were tested by using two-way ANOVA. Where significant main effects were observed, post hoc analysis was performed using least significant difference to test for any specific pairwise differences. Two-way ANCOVA was performed to test the impact of differences in animal size on the main effects of phenotype and diet for various outcome measures. All statistical analysis was performed with SPSS (Armonk, NY). Statistical significance was set at P < 0.05.

RESULTS

Animal characteristics. Body weight was 30% higher in LCR rats compared with HCR rats, which reflected higher levels of both FFM and fat mass (P < 0.05; Table 1). The 3-day HFD induced weight gain in LCR rats but not HCR rats (Table 1). LCR controls (CON) had ~15% higher percent body fat (%BF) than HCR CON (P < 0.05). The 3-day HFD rats had a higher %BF than CON rats (P < 0.05; Table 1), an effect that was more apparent in LCR rats (20%, P < 0.05) than in HCR rats (P = 0.078).

Steatosis. Representative images of liver stained for H & E are presented in Fig. 1A and show increased lipid vacuolization in the LCR animals compared with HCR on both diets. These observations are supported by the increased steatosis scores.
(~60%) and percent nuclei with lipid (2.2-fold) in LCR vs. HCR rats (Fig. 1, B and C, respectively). HFD did not alter these outcome measures within groups. As observed previously (39), biochemical liver triacylglycerol (TAG) was 40% higher in LCR than in HCR on CON (Fig. 1D). The 3-day HFD resulted in no change in TAG accumulation in the HCR, whereas the LCR rats increased liver TAG by 36%, resulting in the LCR having 2.3-fold higher liver TAG levels than HCR following 3-day HFD. Importantly, the significant increase in TAG, without increase in H & E analysis, indicates that the HFD increased lipid droplet size and not number of lipid-positive hepatocytes in LCR. Overall, these data demonstrate that low intrinsic aerobic capacity increases susceptibility for hepatic steatosis following an acute HFD.

**Energy balance.** Daily average EI, TEE, and EB (EB = EI − TEE) during the 3-day HFD challenge are presented in Fig. 2 and Table 1. Despite the differences in body weight, HCR and LCR rats consumed the same amount of food while on the CON diet (65.12 ± 3.97 and 65.70 ± 2.87 kcal/day, respectively). Consumption of a HFD resulted in greater EI in both HCR and LCR rats compared with CON (73.83 ± 3.16 and 96.86 ± 2.03 kcal/day, respectively, P < 0.05; Fig. 2A), but this increase was significantly greater in LCR vs. HCR rats (P < 0.05). To assess the potential impact of the difference in body size between the HCR/LCR animals, EI was adjusted for body weight by ANCOVA. LCR rats had lower adjusted EI for body weight compared with HCR on CON (58.85 ± 3.46 and 71.89 ± 3.29 kcal/day, respectively, P < 0.05; Fig. 2B), whereas acute HFD resulted in increased adjusted EI in both phenotypes (88.23 ± 3.73 and 81.06 ± 3.43 kcal/day, LCR and HCR respectively) compared with CON (P < 0.05). However, no difference in EI between HCR/LCR on HFD was observed following adjustment for body weight. Consistent with their larger body mass, LCR rats tended to have a higher TEE compared with HCR on HFD (57.02 ± 2.80 and 54.17 ± 1.34 kcal/day, respectively, P < 0.05; Table 1). Three-day HFD increased TEE in both HCR and LCR (56.87 ± 0.98 and 62.96 ± 1.62 kcal/day, respectively, P < 0.05; Table 1), resulting in higher TEE in LCR. No difference in EB occurred between strains on CON (8.55 ± 2.98 and 12.93 ± 1.61 kcal/day, HCR and LCR, respectively; Fig. 2C), whereas the HFD increased EB in both HCR (2-fold, 18.78 ± 2.03 kcal/day) and LCR (3-fold, 39.34 ± 1.75 kcal/day) compared with CON (P < 0.05). During the HFD, the LCR displayed a twofold higher positive EB compared with the HCR (P < 0.05).

**Component analysis of TEE.** To pursue a better understanding of the contribution of TEE in the differential response to HF feeding, TEE was dissected in resting (REE) and nonresting (NREE) components (Table 1). During homeostatic conditions, REE represents the majority of TEE and provides a reasonable estimate of basal energy requirements, whereas NREE is comprised primarily of activity-related expenditure and the thermic effect of food. On the CON diet, REE tended to be higher in LCR rats compared with HCR (40.02 ± 2.08 and 37.37 ± 0.79 kcal/day, respectively; Table 1), which is consistent with their higher body weight. Following correction for the difference in body size by ANCOVA (Table 1), resting metabolism was lower in LCR rats compared with HCR (37.90 ± 1.20 and 42.75 ± 1.29 kcal/day, respectively, P <
0.05), suggesting that LCR are more metabolically efficient than their HCR counterparts prior to the HFD challenge. The ANCOVA analysis of REE can be further extended to assess the approximate contribution of two components of the difference in body weight, fat mass, and FFM. In these studies, FFM represents ~3.5 times the relative contribution to the difference in REE between HCR and LCR rats compared with fat mass (Table 1). NREE was similar for the two groups (17.08 ± 0.60 and 17.00 ± 1.10 kcal/day, HCR and LCR, respectively) on CON diet despite the lower levels of cage activity in LCR rats (P < 0.05; Fig. 2D), which has been observed previously (39). These data suggest that activity-associated energy expenditure is similar for the two groups. This can be explained by the fact that LCR rats use more energy to move their larger mass. This is supported further by the observation that NREE comprises the same percentage of TEE (27–30%) in both animal phenotypes, regardless of diet. Taken together, these data suggest that LCR rats are more metabolically efficient and are inherently less active than HCR rats prior to the HFD challenge.

Whole body substrate utilization. RQ and nonprotein RQ (NPRQ) were used to estimate substrate utilization (Fig. 3, A and B). No differences were observed in RQ or NPRQ between strains on CON. As expected, 3-day HFD increased reliance on lipid utilization (lower RQ) in both groups (P < 0.05). However, the HCR displayed a larger HFD-induced reduction in RQ and NPRQ than the LCR (P < 0.05). These data suggest that the LCR utilize less fat than the HCR following a transition to HFD. Figure 3D highlights these differences by showing substrate disappearance as a percent of EI. Very little difference is observed in the partitioning of EI in HCR/LCR rats on CON. However, 3-day HFD led to an ~20-fold increase in fat disposal in HCR, whereas the LCR displayed a minimal increase (~35%). Furthermore, HFD-fed LCR displayed an ~10-fold increase in the percent of EI allotted to storage, whereas HFD-fed HCR increased storage only ~2.5-fold. Notably, the elevated positive energy state during the HFD makes it possible that metabolic pathways such as lipogenesis affect the calculation of macronutrient specific oxidation; therefore, the substrate disappearance data are presented only qualitatively to provide a framework for assessing macronutrient utilization.

Dietary fat trafficking. Radiolabeled tracers were incorporated into the diet to track dietary FAO/storage over the last 24-h period of the 3-day HFD or CON. No HCR/LCR dietary tracer incorporation differences were observed in liver on CON (Fig. 4A). However, the HFD caused a robust (>3-fold) increase in the total dietary fatty acids retained in the hepatic lipid fractions of both HCR and LCR (P < 0.05). Furthermore, 3H2O incorporation into liver lipid as a marker of de novo lipogenesis was not different between HCR/LCR on the CON or HFD. The HFD caused lipogenesis to be significantly decreased in both strains (P < 0.05; Fig. 4B). Thus, the hepatic steatosis found in LCR is not due to increased dietary fatty acid incorporation into liver TAG or greater rates of de novo lipogenesis.

We also tracked dietary lipid storage into adipose and skeletal muscle. There was no difference between HCR/LCR for dietary lipids trafficked to the epididymal fat on CON (Fig. 4C). HFD increased dietary fatty acids trafficked to epididymal fat in both groups; however, the LCR had a 75% higher net retention of dietary fat than HCR (P < 0.05). Comparable
findings were observed in additional fat pads (retroperitoneal, mesenteric, omental, and inguinal; data not shown). Similar to the fat pads, total dietary fat incorporation into skeletal muscle lipid was not different between HCR/LCR on the CON but was elevated by HFD in both HCR and LCR ($P < 0.05$; Fig. 4D). Interestingly, HCR had a twofold higher dietary fat incorporation into muscle lipids compared with the LCR.

Whole body dietary FAO was also determined by measuring $^{14}$C tracer emerging as exhaled CO$_2$ (Fig. 3C). HFD resulted in a significant increase in cumulative dietary FAO in both HCR and LCR strains ($P < 0.05$). The HCR had higher cumulative dietary FAO on both the CON (46%) and the HFD (43%) compared with the LCR rats ($P < 0.05$). Thus, the higher steatosis found in LCR rats is associated with decreased whole body lipid oxidation. Together, these data demonstrate that divergent aerobic capacity influences trafficking of excess dietary fat between oxidative and nonoxidative tissues, which impacts whole body lipid utilization and storage and ultimately susceptibility for steatosis.

Liver FAO. The liver accounts for $\leq 20\%$ of TEE (28), of which FAO represents a large portion. Reduced hepatic FAO is associated with increased hepatic TAG accumulation in rodent models (30, 31, 39). Therefore, we measured FAO in liver homogenate, primary hepatocytes, and isolated mitochondria to determine whether there were differences in lipid catabolism in the livers of HCR and LCR rats. In liver homogenate, complete FAO to CO$_2$ was significantly lower in LCR rats on CON and HFD compared with HCR (41 and 64%, respectively, $P < 0.05$; Fig. 5A). Incomplete FAO as ASM production was 25% higher in LCR compared with HCR irrespective of diet ($P < 0.05$; Fig. 5B). Acute HFD increased ASM production in HCR (19%, $P < 0.05$) but not in LCR rats. Additionally, FAO was performed in isolated primary hepatocytes from HCR/LCR rats to remove any influence of peripheral factors on hepatic FAO. We have shown previously that increased FAO in rat hepatocytes is associated with decreased TAG accumulation (25). Complete FAO was significantly lower in LCR primary hepatocytes under CON and following overnight FFA exposure compared with HCR (60 and 66%, respectively, $P < 0.05$; Fig. 5C).

Mitochondrial FAO represents $\leq 90\%$ of liver FAO capacity (5). FAO was performed in isolated liver mitochondria to remove extramitochondrial sources and under ADP stimulation to better assess the impact of energy demand on induction of mitochondrial FAO. LCR rats displayed impaired ADP-induced complete mitochondrial FAO compared with HCR regardless of diet ($P < 0.05$; Fig. 6D). To assess whether decreased complete mitochondrial FAO was due to reduced mitochondrial respiratory capacity, mitochondrial respiration studies utilizing glutamate were performed. LCR liver mitochondria displayed decreased basal, ADP-coupled, and maximal respiration regardless of diet ($P < 0.05$; Fig. 6E). Overall, these data demonstrate that low-aerobic capacity LCR rats...
display impaired hepatic complete FAO capacity, which is associated with increased vulnerability to steatosis.

**DISCUSSION**

The underlying mechanisms by which aerobic capacity impacts whole body and tissue-specific energy metabolism and thus predisposition for metabolic disease states remain unknown. Here, we report that rats bred for low intrinsic aerobic capacity display increased steatosis that is worsened after 3-day HFD, whereas rats bred for high aerobic capacity are completely protected from this pathology. Overall, the results show that increased susceptibility for steatosis in low aerobic capacity rats is associated with a greater increase in energy intake and lower weight-adjusted energy expenditure, resulting in a greater positive energy balance following transition to HFD, decreased adaptability to substrate utilization, and decreased whole body and liver-specific lipid oxidation capacity.

Obesity and factors that modulate adiposity directly influence hepatic steatosis in human patients and rodent models (2, 31, 40). As such, the primary treatment for NAFLD is lifestyle-induced weight loss (33). Therefore, understanding the role of systemic EI, EB, and substrate utilization is critical for understanding the mechanisms by which divergent aerobic capacities impact susceptibility for hepatic steatosis following a transition to acute HFD, a known initiator of steatosis (13, 36). In this study, the LCR rats demonstrated a significant increase in hepatic lipid content following the 3-day HFD, whereas the HCR rats were protected despite both groups having the same weight-adjusted EI. However, LCR rats did demonstrate a greater increase in EI during the acute HFD, evoking a greater 3-day weight gain, increased adiposity, and a twofold higher EB in the LCR vs. HCR. Previous findings show that increased EI on a HFD is due to a predisposition to store dietary calories in adipose and decreased whole body (6) and liver-specific FAO (7), resulting in liver-derived afferent signals to increase food intake (21). Therefore, we posit that the phenotype of the low aerobic capacity rat (increased trafficking of fatty acids to adipose and reduced whole body and hepatic fatty acid oxidation) may drive subsequent elevated acute EI in a feed-forward process that increases EB and worsens steatosis outcomes.

In addition to EI, EB is also dependent on energy expenditure. Previous findings in transgenic/knockout rodents have shown an inverse relationship between TEE and sensitivity for HFD-induced steatosis (32, 35, 40, 43). Importantly, much like sedentary humans, we observed that REE of our caged animals represented the vast majority of TEE (67%). Despite their larger size, LCR rats demonstrate the same EI and EB as HCR on the control diet, suggesting that the LCR rats have greater metabolic efficiency. This hypothesis is supported by the observed lower weight-adjusted REE in the LCR compared with HCR regardless of diet. Further support for this assertion comes from studies demonstrating that LCR rats have increased expression of genes involved in energy conservation (10). Overall, these data suggest that an enhanced metabolic efficiency inherent to the LCR phenotype contributed to their greater HFD-induced EB and greater susceptibility for hepatic steatosis in LCR.

Adaptability of fuel utilization following a change in nutrient availability is also critical for the maintenance of energy homeostasis (8). Loss of adaptive substrate utilization is termed metabolic inflexibility. Metabolic inflexibility profoundly impacts storage of excess energy (13, 14) and is a hallmark feature of obesity and the metabolic syndrome (9). When switched to a HFD, LCR rats displayed a minimal
increase in whole body lipid disappearance (34%) compared with the dramatic 20-fold increase witnessed in the HCR. Thus, an inability to dramatically increase lipid utilization is a key finding in the LCR rat. Instead, the LCR display a greater reliance on carbohydrates, as shown by a higher 24-h RQ on HFD. As mentioned previously, the accentuated positive energy state of the animals on HFD can complicate the calculation of macronutrient oxidation, and caution is warranted in interpreting the data. Nevertheless, the suggested lower utilization of lipid during the HFD in the LCR rats is supported by the significantly lower whole body dietary fat oxidation compared with HCR. This is a human relevant finding because obese subjects with NAFLD were observed to have lower whole body fat oxidation compared with lean, healthy controls (3). Additionally, obese subjects with NAFLD display metabolic inflexibility and a significantly reduced level of aerobic fitness compared with lean subjects (3). These data suggest that low aerobic capacity is associated with decreased substrate utilization adaptability following the transition to HFD.

As part of substrate utilization, key differences in energy storage patterns were also revealed. Although we observed increased energy storage in both HCR and LCR rats during the transition to a 3-day HFD, the estimated rate of storage in the LCR was twofold higher than in the HCR. This is supported further by the observed greater 3-day weight gain and increase in percent body fat in the LCR compared with HCR following the HFD challenge. Also, although there was no difference in the amount of dietary lipid retained in the liver, the LCR rats were observed to have significant increases in dietary lipid storage in metabolically inactive adipose pads. This is in opposition to the observed storage of dietary lipid in highly oxidative skeletal muscle in HCR rats. These opposite dietary lipid trafficking patterns are consistent with the observed differences in whole body fatty acid oxidation and the previously highlighted differences in metabolic efficiency related to divergent aerobic capacity. In summary, these data highlight that differences in intrinsic aerobic capacity greatly impact energy storage patterns following a transition from a LFD to HFD, factors that track with differences in susceptibility for steatosis.

Hepatic steatosis develops as a result of one or more of the following factors: increased FFA delivery, elevated de novo lipogenesis, decreased TAG secretion, and decreased lipid catabolism. Our results suggest that FFA trafficking to the liver was not different between HCR/LCR, nor would differences in serum TAG (data not shown) suggest that TAG secretion is a primary determinant. However, it is clear that differences in hepatic fatty acid catabolism between the HCR and LCR played a primary role, as the LCR displayed dramatically reduced complete hepatic FAO compared with HCR. Significantly, hepatic FAO plays an important role in whole body fat utilization, representing ~20% of whole body fat oxidation (28). Previously, increased or decreased complete FAO in muscle or liver has been associated with positive or negative metabolic phenotypes, respectively (26, 41). Elevated complete FAO represents increased coupling of lipid-derived acetyl-CoA flux from β-oxidation through the TCA cycle and

![Graphs](http://ajpendo.physiology.org/doi/10.1152/ajpendo.00093.2014/fig)
away from potentially pathological incomplete oxidation products (26). These results fit with our previous findings that complete hepatic FAO capacity (25, 30, 31, 39) influences liver lipid content and whole body metabolic health (24), although the underlying mechanisms for these effects remain unknown. Furthermore, LCR primary hepatocytes had decreased complete FAO compared with HCR cells, showing that these phenotype differences were intrinsic to the liver and not influenced by peripheral factors. In the liver, the bulk of FAO occurs in the mitochondria (5), and defects in mitochondrial FAO have been suggested by our group as a possible mechanism for the development and progression of NAFLD (29). Isolated liver mitochondria from LCR animals had 30% decreased capacity to increase complete FAO in response to simulated energy demand (ADP). Furthermore, the observed LCR impairments in mitochondrial FAO were associated with decreased mitochondrial respiratory capacity. These data demonstrate that the selection for divergent intrinsic aerobic capacity in rats has produced divergent capacities for hepatic FAO, effects that are inversely associated with susceptibility for HFD-induced steatosis.

In summary, we demonstrate that reduced aerobic capacity in the LCR results in increased susceptibility for augmented steatosis following an acute HFD, an effect associated with greater increase in energy intake, reduced weight-adjusted REE, increased energy balance, dramatic inability to adapt to greater lipid utilization at the whole body and liver level, and increased storage of dietary fat in the adipose. In contrast, HCR rats with intrinsically elevated aerobic capacity are protected from HFD-induced steatosis, a protection that is associated with increased relative energy expenditure and fat utilization both in vivo and in the liver. Intriguingly, a similar protected metabolic phenotype has been observed in human subjects that are resistant to the development to of obesity (8), a major covariant of NAFLD. In conclusion, these results highlight the complex interaction between intrinsic aerobic capacity and whole body and liver energy metabolism and their influence upon susceptibility for NAFLD.

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DISCLOSURES
The authors have no conflicts of interest, financial or otherwise, to disclose for this research.

AUTHOR CONTRIBUTIONS

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22. MacLean PS, Higgins JA, Johnson GC, Fleming-Elder BK, Donahoo WT, Melanson EL, Hill JO. Enhanced metabolic efficiency contributes


