Intrinsic (Genetic) Aerobic Fitness Impacts Susceptibility for Metabolic Disease

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THYFAULT, J.P. and E.M. MORRIS. Intrinsic (genetic) aerobic fitness impacts susceptibility for metabolic disease. Exerc. Sport Sci. Rev., Vol. 45, No. 1, pp. 7–15, 2017. Low-capacity runner (LCR) and high-capacity runner (HCR) rat strains are divergent for running capacity and aerobic fitness. The LCR rats are susceptible to obesity, insulin resistance, and fatty liver whereas the HCR are protected. We performed studies testing the hypothesis that the divergence in susceptibility for obesity and metabolic dysfunction between HCR/LCR is due to differences in hepatic mitochondrial function that also may drive differences in energy expenditure and substrate usage. Key Words: fitness, aerobic capacity, mitochondria, energy expenditure, obesity, hepatic steatosis, fat oxidation

INTRODUCTION

Aerobic capacity, also termed cardiorespiratory fitness, is the capacity of the body to deliver and use oxygen during maximal-intensity exercise. Aerobic capacity commonly is measured with direct assessments of oxygen consumption (\(\text{VO}_2\text{max}\)) during graded exercise tests to exhaustion in exercise physiology laboratories and clinics. In addition, exercise tests to failure without direct measures of oxygen consumption and submaximal field tests also can provide indirect assessments of maximal aerobic capacity. Thus, aerobic capacity is a physiological outcome measure that can be tested in a variety of settings. Most importantly, aerobic capacity has proven to be critical for health. In fact, it could be argued that aerobic capacity is the most critical health-related outcome measure. Low aerobic capacity increases the risk for the development of multiple disease states and increases the risk for early mortality regardless of disease state (4,18,41). In contrast, high aerobic capacity is linked to lower risk for disease and greater overall survivability (4,18,41).

Aerobic Capacity, Much Is Left To Be Discovered

Factors controlling aerobic capacity are a merging of daily behavior patterns, genetics, and age. Current evidence suggests that aerobic capacity peaks in the second to third decade of human life before it begins to decline slowly through subsequent decades (5). Importantly, however, most individuals can embark on an exercise program at any age and see measurable improvements in aerobic capacity in weeks to months. Studies suggest that approximately 70% of fitness is because of genetics and not solely because of daily activities or exercise training (8). Current evidence in both humans and rodents suggests that there is a lifetime apex for peak aerobic capacity after which the capacity declines across the lifespan despite individuals remaining sedentary or staying highly active (6). This was shown recently in rats where daily wheel running versus sedentary cage-only activity increased \(\text{VO}_2\text{peak}\) but did not change the age at which the apex for \(\text{VO}_2\text{peak}\) occurred (38). This has led to the concept of achieving a high level of aerobic capacity at a young age, so that the decline that occurs with aging will not reach a critical frailty threshold until an older age (6).

Although aerobic capacity is linked clearly to health and mortality, it is surprising that so many basic research questions underlying this fundamental physiological readout largely remain unanswered. For example, we are unaware of research that
clearly has deciphered whether the possession of low aerobic capacity due to genetics or due to low physical activity differently alters disease and mortality. However, it is more likely that low aerobic capacity due to low physical activity patterns is the primary culprit. A recent report cited that physical inactivity and low aerobic capacity provide a similar increase in mortality rate, and that the greatest protection is provided by staying out of the most unfit or most inactive groups (7). The concept that fitness due to daily activity patterns and not genetics is most critical for health is supported by a report that individuals who went from being unfit to fit by increasing physical activity between two doctor visits spread apart by 4.9 years significantly lowered mortality (3). However, evidence in a rat model described later in this article clearly suggests that genetic (intrinsic) fitness also can play an important role.

There are multiple reasons why the mechanistic links between fitness status and disease susceptibility are unknown. First, aerobic capacity status is largely underappreciated by the medical and biomedical science communities (1). Additionally, studies in humans are difficult. Most researchers can obtain only muscle whereas access to other tissues (heart, lungs, liver, etc.) is difficult or impossible. The primary focus of the biomedical community has been on single-gene manipulation within animal models, approaches that cannot mimic the polygenic/multitissue effects required to study the impact of divergent aerobic capacity levels. A final complication is the difficulty in separating the impact of exercise versus intrinsic fitness itself. For example, highly fit humans also are usually regular exercisers, and, thus, it is unknown if the outcomes measured in these individuals are due to fitness or due to the effects of different physical activity or exercise patterns as already mentioned. Because of these limitations, an appropriate model to examine the mechanisms by which high and low aerobic capacity impacts disease was needed.

**A Model to Answer Why Genetic Aerobic Capacity Impacts Health**

To fill the model gap, Lauren Koch and Steve Britton began breeding high- and low-capacity running rats (HCR/LCR) more than 15 years ago with the goal of creating rats with intrinsically high and low aerobic capacity (15,42). Outbred NIH:N strain rats were tested for exercise capacity to exhaustion on treadmills, followed by selective breeding for high (top 10%) or low (bottom 10%) running performance through successive generations. The design and breeding of the model is described in detail elsewhere (15). After several generations of breeding, the HCR rats could run dramatically longer than LCR rats during exercise tests, despite the animals receiving no exposure to exercise training and simply being maintained in cages. Therefore, the strains intrinsically possess the phenotype of high or low running capacity due to permanent genetic changes. Importantly, outcomes in the HCR/LCR model mimic the impact of aerobic capacity on human health. LCR rats fed a normal chow diet show multiple cardiovascular/metabolic syndrome risk factors at a young age, whereas the HCR display no risk factors (42). The LCR rats show early mortality, approximately 6-months less than the HCR rats, studies that were replicated in two different cohorts of rats (17). In addition, the HCR displayed 60% higher VO2peak compared with the LCR rats after exercise testing (42). Therefore, by breeding for endurance capacity, Koch and Britton created rats with truly divergent aerobic capacities and phenotypical characterization that largely mimic health outcomes measured in humans. Recent studies continue to match findings in this model to those in humans. LCR rats have been shown to be more susceptible to a variety of other conditions that also are linked to low aerobic capacity in humans (16,27,40).

**Why Does Aerobic Capacity Status Impact Susceptibility for Disease?**

Our laboratory has been interested primarily in the role of physical activity/exercise and fitness to impact susceptibility for metabolic diseases. The remainder of this article will focus on our studies examining the links between aerobic capacity and metabolic health including a focus on fatty liver disease in the HCR/LCR rat model. The underlying hypothesis of these studies is that intrinsic aerobic fitness impacts hepatic mitochondrial function, which impacts susceptibility for metabolic conditions systemically and within the liver.

Initial studies in the HCR/LCR model established a clear metabolic phenotype between the strains. Wisloff et al. (42) reported that skeletal muscle of the HCR rats displayed higher mitochondrial content and oxidative capacity than the LCR, matching the well-established muscle mitochondrial phenotypes described by multiple studies in exercise-trained versus untrained humans (34). Low skeletal muscle mitochondrial content and fatty acid oxidative (FAO) capacity have been linked to skeletal muscle insulin resistance and obesity for many years, although it remains a controversial topic. Noland et al. (26) used a chronic (8 wk) high-fat diet (HFD, 50% of energy from fat) in male HCR/LCR rats to examine susceptibility for dietary-induced obesity and insulin resistance. HFD in rodents are a commonly used tool to induce insulin resistance and obesity. We expected the LCR to demonstrate greater susceptibility to the HFD than the HCR because of low skeletal muscle mitochondrial content and FAO. As expected, the LCR displayed reduced total FAO in skeletal muscle homogenates and reduced mitochondrial content (citrate synthase enzyme activity) compared with the HCR on a chow diet. Interestingly, both the HCR and LCR increased skeletal muscle FAO on the HFD, resulting in no difference between the strains after the HFD. To compliment this profile, the LCR displayed higher initial body mass, higher fasting glucose and insulin, and higher insulin responses to an oral glucose tolerance test than the HCR on the normal chow diet. After 8 wk of the HFD, the LCR displayed pronounced weight gain and significant increases in both glucose and insulin during the oral glucose tolerance test, evidence of worsening insulin resistance. In contrast, the HCR animals showed protection against these effects as the body weight gain and glucose/insulin responses in the HFD-fed HCR rats mirrored those of the HCR rats fed normal chow. This was the first evidence that the HCR rats were protected against HFD-induced weight gain and insulin resistance, whereas the LCR were susceptible. Similar outcomes were found in female HCR/LCR rats after an HFD in studies from our laboratory (25). At the time of these studies, skeletal muscle mitochondrial oxidative capacity was a prime area of focus linking obesity and insulin resistance. Because of this, and the well-known link between high and low endurance capacity and divergent skeletal muscle mitochondrial content and oxidative capacity, the field largely believed...
the metabolic differences between the HCR and LCR were tied
to divergent skeletal muscle phenotypes (2).

In 2006, Church et al. (10) reported that low-fit men were at
a greater risk for developing nonalcoholic fatty liver disease
(NAFLD) compared with moderate- and high-fit men. NAFLD
is a clinical condition (also called hepatic steatosis) in which
greater than 5% of liver weight is comprised of fat. NAFLD
commonly occurs with obesity and is linked to elevated hepatic
glucose production and an increased risk for the development of
type 2 diabetes. The study also reported that controlling for
body weight differences did not impact the statistical relation
between fitness and NAFLD. The report by Church et al. (10) led our laboratory to hypothesize that low-fit LCR rats
also may have a greater risk for hepatic steatosis. We further
questioned if the increased risk for NAFLD in the low-fit
LCR would be secondary to reduced hepatic mitochondrial
content and/or reduced hepatic FAO.

Hepatic Mitochondria and Susceptibility For Fatty Liver
Disease: Impact of Fitness

Mitochondrial FAO (specifically β-oxidation) is the domi-
nant oxidative pathway for the complete disposal of long-chain
fatty acids in the liver. Total capacity for hepatic FAO is con-
trolled by a variety of conditions including hepatic mitochon-
drial content or density, enzymatic activity of CPT-1α, the
rate-limiting step for long-chain fatty acid entry into the mito-
chondrial, and a variety of other factors including substrate sup-
ply and energy status. Previous results from our collaborative
research team have shown that reduced hepatic mitochondrial
FAO increased risk for hepatic steatosis. We have shown that hy-
perphagic, obese Otsuka Long-Evans Tokushima fatty (OLETF)
rats display hepatic steatosis in association with reduced hepatic
mitochondrial content and lower hepatic mitochondrial com-
plete FAO (oxidation of 14C-palmitate to 14CO2) (29). We also
have shown that hepatic steatosis is effectively prevented or
treated with exercise effects that track with increased hepatic
FAO and other markers of enhanced mitochondrial content
and function (28,29). In addition, we also showed that the
OLETF possesses lower hepatic mitochondrial content and FAO
in the liver at a young age before the development of he-
aptic steatosis (30).

As expected, we found that the LCR display evidence of
lower hepatic mitochondrial content, and lower hepatic mitochon-
drial FAO compared with high-fit HCR rats (37). The
LCR also displayed mild NAFLD at a young age on a normal
cow diet, marked by higher hepatic triglycerides, steatosis
score, and percent of nuclei associated with lipid droplets in
the LCR versus the HCR. Typically, NAFLD is not witnessed
in young rodents unless the animals are obese after an HFD, hy-
perphagic because of altered satiety signals (OLETF and Zucker
rats), or with specific genetic alterations that negatively impact
lipid metabolism. The LCR is heavier than the HCR rat but is
not grossly obese like OLETF or Zucker models. Body composition
analysis has shown that the 20%–30% greater body mass in
the LCR over the HCR is partially due to a bigger body size
(longer snout to tail), with increases in both lean body mass
and slightly higher body fat mass (21,26). Therefore, young,
normal chow-fed fit LCR rats displayed hepatic steatosis with-
out dietary manipulation or excessive obesity. Most impor-
tantly, the preclinical HCR/LCR data matched the results
from Church et al. (10) in human subjects that fitness was an
independent risk factor for NAFLD.

Why Would Hepatic Mitochondrial Content and FAO
Be Elevated in High- Versus Low-Fit Rats?

As previously mentioned, the health outcome data first col-
clected in the HCR/LCR rats suggested that skeletal muscle mito-
chondrial content and function and cardiovascular adaptations
were the fundamental reason for differences in susceptibility to
metabolic disease in the model (42). This was a logical assump-
tion given that heart and muscle are two highly important tissues
for delivering and using oxygen during exercise, respectively.
However, the liver also plays a critical, but underappreciated,
role in the metabolic demands of endurance exercise capacity.
As stated elegantly by Trefts et al. (39), “The accelerated de-
mands of working muscle cannot be met without a robust re-
source from the liver. If not for the hepatic response, sustained
exercise would be impossible. The liver stores, releases, and recy-
cles potential energy. Exercise would result in hypoglycemia if it
were not for the accelerated release of energy as glucose.” The
needs for liver glucose production are necessitated by the limited
amounts of circulating glucose (~4 g) that would be diminished
in minutes after the onset of exercise.

High rates of adenosine triphosphate (ATP) production are
required to sustain hepatic glucoseabolic flux during prolonged
exercise. The liver generates ATP by increasing mitochondrial
oxidation of fatty acids that are being delivered to the liver at a
high rate secondary to increased lipolysis of triglycerides from
adipose stores. Therefore, the liver acts as a core energetic con-
verter during prolonged exercise.

Importantly, the graded exercise test to failure used to select
the HCR/LCR rats was designed differently than what is com-
monly used in humans. In human subjects, the goal is to reach
maximal effort with VO2max between approximately 8 and
15 min of duration. However, the goal of the breeding program
designed by Koch and Britton (15) was to breed selectively for
endurance-running capacity. Therefore, they designed a graded
exercise test (increased treadmill speed every 2 min) that was
longer in distance and duration and truly tested endurance
capacity. In the founder population, the rats ran 355 m for approx-
imately 30 min before reaching exhaustion. By generation 6, the
HCR rats were running for an average of 839 m for 42 min
(15). The HCR rats have continued to increase running dis-
tance and duration through several generations of breeding
and are now running an average distance of approximately
2000 m at generation 28. In converse, the LCR are running
only an average of 200 m at generation 28 (31).

Pronounced differences in hepatic mitochondrial content
and function between the HCR and LCR are one of the primary
traits acquired as a result of selectively breeding for high- and
low–endurance exercise capacity. We posit that the higher he-
aptic mitochondrial content and function in the HCR is neces-
sary for fueling the elevated rates of glucogenesis that would
be needed for prolonged endurance capacity. In contrast, we prop-
ose that selectively breeding for reduced endurance capacity led
to decreased hepatic mitochondrial content and function. Perox-
osome gamma coactivator 1-alpha (PGC-1α) is a ubiquitously
expressed transcriptional coactivator that functions as a master
regulator of mitochondrial biogenesis, oxidative phosphoryla-
tion, and fatty acid oxidation (11) and also is a major regulator

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of gluconeogenesis in the liver (43). As expected, the HCR chronically display fourfold higher expression of hepatic PGC-1α compared with the LCR (Fig. 1). PGC-1α is known to be increased by exercise in muscle and liver (11), but differences in hepatic PGC-1α between the HCR and LCR also would indicate that expression patterns are inherited, a factor that is worthy of future exploration. It should be noted that we do not know if hepatic PGC-1α levels between the HCR and LCR rats have continued to become more divergent through several generations of selective breeding. Figure 1 depicts the relation between endurance running capacity, hepatic PGC-1α, hepatic FAO, and hepatic glucose output capacity. Although we have not demonstrated that HCR have greater hepatic glucose output, we do have shown that the LCR have an impaired ability to maintain circulating glucose during prolonged fasting (Morris EM, unpublished data, 2015), data that supports reduced hepatic glucose output in the LCR versus HCR.

As previously mentioned, skeletal muscle of the HCR/LCR rats also display differences in mitochondrial content and FAO (26), an effect that we have found to be less pronounced in females (25). Interestingly, other tissues also have shown similar differences in mitochondrial content, function, or proteins controlling mitochondrial biogenesis between the HCR and LCR including white (36) and brown adipose tissue (20,40). How selective breeding for endurance exercise capacity would necessitate differences in mitochondrial phenotypes in adipose are beyond the scope of this article, but deserve attention and also likely play an important role for disease susceptibility between the HCR and LCR rats.

Testing Susceptibility for Dietary-Induced Steatosis in High- Versus Low-Fit Rats: Integrative Responses

We next sought to determine if the divergence in hepatic mitochondrial content and FAO between the HCR and LCR rats would alter susceptibility for fatty liver disease induced by an HFD. Chronic HFDs (45%-60% of energy from fat) often are used to induce hepatic steatosis, but other work had shown that acute 3-d HFD could be used to induce hepatic steatosis. We favored the acute HFD approach to examine the responses to the diet rather than examine responses after a chronic HFD when it is less likely to decipher what is cause or effect. Weight gain in humans can occur incrementally as a result of acute bouts of overconsumption that occur on holidays or weekends, periods that would match a 3-d HFD approach. Our hypothesis was that the high-fit HCR rats would be protected against acute

Figure 1. Theoretical mechanism by which aerobic capacity differences in the high- and low-capacity running rats (HCR/LCR) rats impacts hepatic metabolism through peroxisome gamma coactivator 1-alpha (PGC-1α). Elevated-endurance exercise capacity would depend on the ability to maintain euglycemia through sustained gluconeogenesis. The lipolysis of free fatty acids during prolonged exercise provides necessary substrate for hepatic mitochondrial fatty acid oxidation, which generates adenosine triphosphate (ATP) to fuel gluconeogenesis. Hepatic mitochondrial content, fat oxidation, and gluconeogenesis are regulated transcriptionally by the transcriptional coactivator PGC-1α that we have found to be expressed consistently higher in the liver of HCR vs LCR rats.
HFD-induced weight gain and hepatic steatosis because of their elevated hepatic mitochondrial content and FAO. In contrast, the LCR would be very susceptible to the insult. However, we also suspected that other peripheral factors including metabolic flexibility and energy balancer were implicated.

Mechanism(s) for excessive fat storage in the liver are reduced FAO, increased or excessive fatty acid uptake, reduced triacylglycerol secretion, or increased de novo lipogenesis. Alterations in all of these factors may be involved to one degree or another in the development of hepatic steatosis depending on the model or condition that is being studied. In addition, factors related to energy balance also are likely at play. Conditions in which energy intake is significantly greater than energy expenditure leads to weight gain and expansion of lipid stores in not only adipocytes but also in ectopic storage into muscle and liver. The best evidence for this is that more than 70% of obese and 100% of extremely obese individuals reportedly have fatty liver (9). Therefore, although we suspect the hepatic mitochondrial phenotype impacts susceptibility for hepatic steatosis, there is no doubt that peripheral metabolic factors also play a role. Given that the HCR/LCR already had known differences in peripheral metabolism, we designed 3-d HFD studies to carefully examine integrative metabolism from the whole body to isolated hepatic mitochondria.

**Energy balance and weight gain**

Indirect calorimetry experiments were performed with the HCR/LCR rats before and during the transition to the 3-d HFD so that precise measurements of energy expenditure, energy intake, and energy balance could be made (21). Total energy expenditure was not different between the HCR/LCR rats on either diet condition. However, given that the LCR displayed 30% higher body mass, it was obvious that energy expenditure per unit of mass was not the same. Therefore, we adjusted for body mass differences by covariate analysis, which revealed that the HCR displayed higher basal energy expenditure than the LCR. As was reported previously (37), the HCR showed higher spontaneous activity in their cages than the LCR as measured by X and Y beam breaks. However, the increased spontaneous activity was not the sole reason for differences in total energy expenditure. To eliminate the effects of the differences in spontaneous activity, we approximated resting energy expenditure from periods when the rats had the least amount of movement and displayed a prolonged period of reduced oxygen consumption. Resting energy expenditure, when adjusted for body mass by covariate analysis, was still higher in the HCR than the LCR. Importantly, resting energy expenditure makes up more than 70% of total energy expenditure in most caged rodents and, thus, is a critical driver of total energy expenditure in rodents.

Food and energy intake values also differed between strains after the transition to the HFD (21). Although both groups significantly increased the energy intake of the calorically rich HFD, the increase was twofold higher in the LCR than the HCR. As a result, both groups displayed increases in positive energy balance with the 3-d HFD. However, the HFD-induced positive energy balance was 50% higher in the LCR than the HCR (Fig. 2A).

The explanation for the increased energy intake in the LCR over the HCR rats on transition to the HFD is unknown. However, our subsequent studies (23) found that the HFD induces...
elevated energy intake in the LCR over the HCR for up to 3 wk. We also have found this energy intake to be predictive of weight gain during a 1-wk HFD (Fig. 2B) (20). Existing data from two separate groups may explain these results, and interestingly, it could be related to hepatic mitochondrial FAO and energy state differences. The Friedman group showed that chemical inhibition of hepatic FAO lowered hepatic ATP and acutely increased (up to 4 h) food intake in rats (12). They further showed that this acute increase in food intake was signaled to the brain through vagal afferent signaling (13).

The only other polygenetic rat model to show a protection and susceptibility to HFD-induced obesity is the obese-prone and obesity-resistant rat model developed by Dr. Barry Levin. Sprague-Dawley rats were bred through several generations to be prone or resistant to HFD-induced weight gain (32). A primary driver of the phenotype for weight gain in the obese prone is greater energy intake on transition to the HFD. Interestingly, Friedman et al. found the obese-prone rats to also show lower hepatic FAO and ATP levels, which he hypothesized to be a primary driver of their energy intake (14). Clearly, more studies are needed to determine the role of fitness on hepatic mitochondrial FAO and links to energy intake. Of note, obese humans have been reported repeatedly to display lower hepatic ATP than lean counterparts (24), suggesting that this same phenomenon could occur in humans. However, we are unaware of studies that have tested this hypothesis, or have even examined if sedentary low-fitness humans display different ATP levels than high-fit humans.

The acute HFD caused both groups to gain total body mass and fat mass, but again, the LCR displayed a greater weight gain and a larger increase in total body fat after the HFD. As expected by the whole body fat mass data, the HFD-induced changes in fat pad weights were altered differently between the HCR and LCR rats. The percent increase in fat pad mass in the HFD versus low-fat control diet groups was double in the LCR than what it was in the HCR. This increase occurred in the omental, retroperitoneal, mesenteric, and epididymal fat pads providing evidence that the LCR display higher risk for visceral fat pad expansion on a short-term HFD.

Metabolic flexibility and dietary fat trafficking

We also used two separate approaches to examine metabolic flexibility and whole-body FAO between the LCR and HCR rats after transition to the 3-d HFD. Metabolic flexibility is the ability to switch between a reliance on fat or carbohydrate depending on nutrient conditions. Metabolic flexibility is commonly measured by respiratory quotient using indirect calorimetry whereby an RQ of 0.7 is 100% fat usage, whereas an RQ of 1.0 is 100% carbohydrate usage according to stoichiometry. Metabolic flexibility can be assessed in transition from a low-fat diet to an HFD where the RQ should be reduced quickly after the onset of the HFD. The HCR and LCR displayed clear differences in metabolic flexibility according to RQ results (21). The lowering of RQ induced by the HFD was significantly greater in the HCR than the LCR. Using the RQ data, it is possible to calculate the percentage of energy usage from the substrates of carbohydrate, fat, or protein. Examining the data in this manner revealed clear differences in substrate usage between the HCR and LCR. On the control diet, there were no differences between strains, but on transition to the 3-d HFD, the HCR were able to increase fat usage by approximately 20-fold whereas the LCR could only increase it by 1.35-fold (Fig. 2B). Therefore, intrinsic fitness dramatically impacts the ability to switch between fuel sources acutely.

Figure 3. High- and low-capacity running rats (HCR/LCR) partition dietary lipids differently after transition to a 3-d high-fat diet (HFD). A. HCR and LCR show similar increases in dietary lipid deposition into the liver after 3-d HFD. B. Both groups show suppressed de novo lipogenesis after 3-d HFD. B, C. Transition to 3-d HFD increases dietary lipid deposition into adipose and skeletal muscle of both strains; however, the LCR primarily increase deposition in adipose, whereas the HCR primarily increase deposition into skeletal muscle. Figures 3A–D (Reprinted from (20). Copyright © 2016 John Wiley & Sons. Used with permission.)
As a second method for analyzing fat usage, we also measured whole-body dietary FAO. Both the low-fat control diet and the HFD were labeled with radio-labeled lipids (14C-labeled oleate and palmitate) (21) allowing us to measure whole-body FAO by measuring 14C tracer in expired CO2. Both groups displayed increased dietary FAO on the HFD, but the HCR response was 46% greater than the LCR (Fig. 2C). Interestingly, the HCR also displayed 43% greater dietary FAO on the low-fat control diet. In conclusion, the HCR displayed significantly more robust responses in changing whole-body fat usage in response to the HFD compared with the LCR. Interestingly, these dramatic differences occurred even though the LCR consumed a greater quantity of the HFD containing more tracer than the LCR.

The radio-labeled dietary lipids also allowed us to quantify the trafficking of dietary lipids into liver, adipose, and skeletal muscle. The HFD resulted in a robust threefold increase in dietary lipids trafficked to the liver in both groups suggesting that the greater steatosis found in the LCR rats is not simply due to greater trafficking of dietary lipids to liver. However, both skeletal muscle and adipose displayed differential lipid trafficking between the strains on an HFD. The HCR increased dietary lipid trafficking into skeletal muscle twofold higher than the LCR. Although the HFD increased dietary lipid trafficking to epididymal fat in both groups, the LCR had a 75% greater net retention of dietary fat than the HCR. Similar patterns of lipid retention between the HCR and LCR were observed in other fat pads (retroperitoneal, mesenteric, omental, and inguinal).

In addition to the robust differences in whole-body dietary FAO, the differential trafficking of dietary fat into skeletal muscle or adipose provides additional insight. Figure 3 depicts the dietary lipid trafficking differences between the HCR and LCR rats. The high-fit HCR primarily traffics lipids to muscle in which they eventually will be oxidized and cannot be rereleased into circulation, whereas in contrast, the LCR primarily traffic dietary lipids to adipose pads where the lipids will be stored for a period, but will then be mobilized and subsequently oxidized in other metabolic tissues (liver, muscle, heart). Therefore, the HCR seem to have an advantage in trafficking lipids to muscle over liver, but the role of this trafficking on the steatosis outcomes and overall differences in susceptibility or protection to obesity is associative and further mechanistic studies are required.

The central mechanisms driving the differences in whole-body metabolic flexibility and substrate trafficking between the HCR/LCR are unknown, but we have reported previously evidence that the differences in hepatic-PGC-1α and mitochondrial oxidative capacity may play a role (22). We overexpressed hepatic PGC-1α in obesity-prone rats before a 3-d high-fat diet. The 3-d HFD lowered hepatic mitochondrial respiratory capacity and reduced metabolic flexibility in rats receiving a control virus, however, rats with hepatic PGC-1α overexpression maintained both hepatic respiratory capacity and whole-body metabolic flexibility despite the HFD. In addition, we found that dietary lipid trafficking to skeletal muscle was increased significantly in the rats with PGC-1α overexpression (22). All told, these findings suggest that hepatic mitochondrial content and function can impact not only hepatic metabolism, but also impact energy intake and substrate use at the whole-body level.

Mitochondrial-respiratory capacity and fat oxidation

As had been previously reported, we found the HCR to have higher complete FAO to CO2 in both isolated hepatic mitochondria (Fig. 4A) and liver homogenates on the control diet (21). However, the LCR displayed higher incomplete FAO (14C to acid soluble metabolites) in liver homogenate on control diet, suggesting that they likely divert more acetyl-CoA to ketogenesis after β-oxidation of long-chain fatty acids. In response to the 3-d HFD, the HCR retained a higher complete FAO but were also able to increase incomplete oxidation (21). Hepatic mitochondrial respiration showed similar results because the HCR displayed higher rates of respiratory capacity than the LCR under basal, ADP-stimulated, and uncoupled conditions using glutamate as a substrate (Fig. 4B). Similar results were found with lipid (palmitoyl carnitine) as a substrate (Morris EM and Thyfault JP, unpublished data, 2016). Assays performed in the presence of maximal concentrations of ADP and substrates assess the maximal capacity of the OXPHOS system. In vivo, however, FAO and mitochondrial respiration are driven by the energy turnover rate (i.e., energy demand). Mitochondrial density and function typically adapt to and reflect the local chronic level of energy turnover with a cell or tissue. Given that the HCR displays greater energy expenditure, it is logical that their mitochondria are characterized by higher maximal functional capacity. The important question is, how does selective breeding for running capacity lead to differences in mitochondrial functional capacity and susceptibility for hepatic steatosis? Are the livers of the HCRs protected simply by virtue of the greater energy

![Figure 4](image-url)
excise training can induce metabolic changes in rodents or humans that were reported here or if the differences in the HCR/LCR rats are due to selective breeding for fitness through several generations. These questions also could largely be tested in humans who are matched for age and body weight but have dramatic differences in aerobic capacity. In conclusion, the HCR/LCR model is beginning to provide important insight into the mechanism(s) by which aerobic capacity impacts aerobic capacity. Moreover, the HCR/LCR rat model is further proof to emerging human data that aerobic capacity is important for long-term metabolic health.

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