Artificial selection for high-capacity endurance running is protective against high-fat diet-induced insulin resistance

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Noland RC, Thyfault JP, Henes ST, Whitfield BR, Woodlief TL, Evans JR, Lust JA, Britton SL, Koch LG, Dudek RW, Dohm GL, Cortright RN, Lust RM. Artificial selection for high-capacity endurance running is protective against high-fat diet-induced insulin resistance. Am J Physiol Endocrinol Metab 293: E31–E41, 2007. First published March 6, 2007; doi:10.1152/ajpendo.00500.2006.—Elevated oxidative capacity, such as occurs via endurance exercise training, is believed to protect against the development of obesity and diabetes. Rats bred both for low (LCR)- and high (HCR)-capacity endurance running provide a genetic model with inherent differences in aerobic capacity that allows for the testing of this supposition without the confounding effects of a training stimulus. The purpose of this investigation was to determine the effects of a high-fat diet (HFD) on weight gain patterns, insulin sensitivity, and fatty acid oxidative capacity in LCR and HCR male rats in the untrained state. Results indicate chow-fed LCR rats were heavier, hypertriglyceridemic, less insulin sensitive, and had lower skeletal muscle oxidative capacity compared with HCR rats. Upon exposure to an HFD, LCR rats gained more weight and fat mass, and their insulin resistant condition was exacerbated, despite consuming similar amounts of metabolizable energy as chow-fed controls. These metabolic variables remained unaltered in HCR rats. The HFD increased skeletal muscle oxidative capacity similarly in both strains, whereas hepatic oxidative capacity was diminished only in LCR rats. These results suggest that LCR rats are predisposed to obesity and that expansion of skeletal muscle oxidative capacity does not prevent excess weight gain or the exacerbation of insulin resistance on an HFD. Elevated basal skeletal muscle oxidative capacity and the ability to preserve liver oxidative capacity may protect HCR rats from HFD-induced obesity and insulin resistance.

fatty acid; lipid metabolism; liver; heart; skeletal muscle

THE INCIDENCE OF METABOLIC DISEASES such as obesity and type II diabetes is increasing dramatically and is strongly linked to the rise in cardiovascular disease. In 2002, ~64% of the population in the United States was classified as overweight or obese (22), and health care costs attributable to these conditions exceeded $78 billion dollars (13). Although type II diabetes afflicts a substantially lower percentage (~6.3%) of the population (9), this disease accounts for $132 billion in annual health care costs (24). With the increase in the incidence of such metabolic diseases reaching epidemic proportions and the threat of health care costs spiraling out of control, much research has been focused toward elucidating the mechanisms involved in the etiology of these conditions in hopes of ultimately discovering better treatments. Several therapies are currently used to alleviate symptoms of these diseases, but other than dietary modifications, endurance exercise is the only universally prescribed treatment.

Enhanced aerobic capacity has long been associated with diminished morbidity and improvements in functional living, yet all the physiological mechanisms underlying these observations are not certain. Furthermore, it is widely accepted that individuals exhibit drastically different aerobic capacities in the absence of a training stimulus and differ vastly in their inherent capacity to adapt to exercise. It is not known whether the genetic components associated with the predisposition to enhanced endurance capacity alone result in protection against metabolic diseases, whether a training stimulus is required, or whether both work synergistically. The recent development of strains of rats that contrast for intrinsic aerobic treadmill running capacity by Koch and Britton (37) allows for the exploration of these questions in the untrained condition.

Wisloff et al. (68) found artificial selection for low-capacity endurance running (LCR) resulted in untrained rats having a higher incidence of risk factors associated with cardiovascular disease than untrained rats selected for high-capacity endurance running (HCR). Of particular interest to the current investigation is the finding that LCR rats were inherently more insulin resistant compared with their HCR counterparts and exhibited lower expression of proteins involved in skeletal muscle fatty acid oxidation. Several lines of evidence suggest impaired mitochondrial function contributes to intramuscular lipid accumulation, peripheral insulin resistance, and hence the predisposition toward the metabolic syndrome (35, 51, 54, 56–58). Although the correlation between diminished mitochondrial oxidative capacity and insulin resistance is strong, it is largely speculative, since most of this information has been collected from individuals with a preexisting condition. It is clinically important to determine how individuals with inherently different oxidative capacities tolerate stresses known to exacerbate the insulin-resistant condition, such as high-fat feeding. Previous studies have attempted to assess the causative role of diminished oxidative capacity on the development of insulin resistance by manipulating genes involved in lipid
oxidation either directly (1, 11, 12, 20, 21, 38, 49, 63) or pharmacologically (15, 19, 41, 52, 64, 67, 71), but efforts have yielded varying results. Additionally, current strategies such as chemical, physical, gene knockout, and mutagenic approaches fail to emulate mechanistically the progression of complex diseases and provide no direct information as to what combination of allelic variants is responsible for a given environment. In contrast, LCR and HCR rats, because of the unique selective pressure by which they were bred, provide an inherent model with disparate oxidative capacities without the confounding effects of exercise training or gene manipulation. As such, experiments were designed to determine whether inherent differences in aerobic capacity result in dissimilar susceptibility to lipid-induced insulin resistance. More specifically, the purpose of the current investigation was to assess the impact of a high-fat diet (HFD) on weight gain patterns, insulin resistance values, which were calculated as fasting insulin (μU/ml) × fasting glucose (mM)/22.5.

MATERIALS AND METHODS

Animal strains. The development of LCR and HCR rats has been described previously in detail (37). Briefly, two-way artificial selection of a heterogenous rat population from the N:NIH stock (National Institutes of Health) produced rat strains differing in inherent aerobic capacity. Endurance running capacity was assessed at 11 wk of age using run time and distance to exhaustion on a treadmill (15 degree incline; initial velocity 10 m/min and increased 1 m/min every 2 min) as parameters. The top 20% of each sex was randomly bred to produce the HCR strain, whereas the bottom 20% was mated to produce the LCR strain. Each subsequent generation was stratified and bred in a similar fashion with precaution being taken to minimize inbreeding (<1%/generation). It is important to note for the purpose of the current investigation that once the rats were phenotyped based on run time/distance to exhaustion they were not exposed to an exercise training stimulus throughout the remainder of the study.

Male LCR and HCR rats (n = 40) from generation 13 were used for the purpose of this investigation and were individually housed in a temperature-controlled environment with a 12:12-h light-dark cycle throughout their lifespan. Rodents arrived at East Carolina University at 3 mo of age and were provided standard rat chow and water ad libitum until they reached 6 mo of age. At this time, LCR and HCR rats were divided into four groups (10/group) and fed ad libitum either a chow (14% energy from fat; Purina Mills Prolab RMH 3000 5P00) or HFD (50% energy from fat; Purina Mills Prolab RMH 3000 58A0) between 6 and 9 mo of age. Because run time to exhaustion varies significantly within these strains, animals were assigned to assure similar mean run times between the dietary groups. On experimental days, rats were fasted for 12 h, anesthetized using 0.1 ml/100 g body wt of a mixture containing 90 mg/ml ketamine and 10 mg/ml xylazine, and tissues were harvested. All procedures were approved by the Animal Care and Use Committee at East Carolina University.

Oral glucose tolerance test. Animals were accustomed to the esophageal gavage procedure by administering 1 ml of water over 3 days before performing the oral glucose tolerance test (OGTT). Animals selected to be administered the HFD underwent the OGTT before and following the 3-mo dietary intervention. Following an overnight fast, blood was collected from the tail vein to determine baseline serum glucose and insulin values. A glucose load (2 g glucose/kg body wt) was administered by gavage, and additional blood draws were performed at 30, 60, and 120 min to assess in vivo glucose clearance. Glucose levels were determined on whole blood samples using a glucose oxidase method (OneTouch Ultra glucose analyzer; Lifescan, Milpitas, CA). Remaining blood samples were allowed to clot for 30 min on ice and subsequently centrifuged at 4°C, 2,500 g for 20 min to separate serum. Serum was stored at −80°C until insulin levels could be determined via a rat/mouse ELISA kit (Linco Research, St. Charles, MO). Results from these analyses were used to derive homeostasis model assessment (HOMA) insulin resistance values, which were calculated as fasting insulin (μU/ml) × fasting glucose (mM)/22.5.

Serum profiling. Serum lipid profiling was performed to determine nonesterified fatty acid (NEFA) content (Wako Chemicals, Richmond, VA), and a triglyceride kit (Sigma Chemicals, St. Louis, MO) was used to measure free glycerol, total triacylglycerols, and total triacylglycerol − free glycerol. A rat/mouse endocrine Lincoplex kit (Linco Research) was used to determine serum amylin, leptin, glucagon, and glucagon-like peptide-1 levels. Adiponectin levels were measured using an adiponectin RIA kit (Linco Research).

Glycogen synthesis. Extensor digitorum longus (EDL) and soleus muscles were removed from anesthetized rats (9 rats/group). Each muscle was teased into four individual strips; thus, a total of eight EDL and eight soleus muscle strips were obtained from each animal. These muscle strips were placed in clamps and immediately bathed in preincubination media for 30 min containing gassed (95% O2-5% CO2) Krebs-Henseleit buffer (KHB) with pyruvic acid (1.2 M) and 1% BSA. In the final 5 min of preincubation, 100 nM insulin was added to the appropriate samples (quad replicates for each tissue per rat), whereas the remaining four muscle strips for each tissue were used as basal controls (no insulin treatment). After preincubation, muscle strips were quickly moved to gassed KHB media (1% BSA) containing 0-[14C]glucose (0.5 μCi/ml and 5 mM glucose; American Radio-labeled Chemicals, St. Louis, MO) in the presence or absence of 100 nM insulin, as appropriate, for a 1-h incubation. Tissue was quickly removed, blotted dry, frozen in liquid nitrogen, and weighed. Each sample was then dissolved in 0.5 ml of 1 N NaOH for 1 h at 37°C while vortexing intermittently. Upon dissolving, 1 mg of glycogen (Sigma Chemicals) and 1.5 ml of 75% ethanol were added to each tube, and glycogen was then precipitated overnight at 4°C. The glycogen pellet was then spun down, washed, and dissolved with double-distilled H2O (ddH2O) before counting radioactivity on a liquid scintillation counter.

Tissue homogenization procedure. Mixed gastrocnemius (MG), liver, and heart tissues were quickly excised from anesthetized rats and placed in ice-cold isolation buffer (in mM: 100 KCl, 40 Tris-HCl, 10 Tris base, 5 MgCl2·6H2O, 1 EDTA, and 1 ATP; pH 7.4). Homogenates were prepared according to the methods of Kim et al. (36). Briefly, ~50–100 mg of tissue were thoroughly minced with scissors in 200 μl of buffer containing (in mM) 250 sucrose, 1 EDTA, 10 Tris·HCl, and 2 ATP (pH 7.4), and then the buffer volume was brought up to yield a 20-fold (wt/vol) diluted sample. This was transferred to a 3-ml Potter-Elvehjem glass homogenization vessel. Muscle suspensions were homogenized on ice with a Teflon pestle at 10 passes over the course of 30 s at 1,200 rpm. Homogenates were kept on ice until oxidation experiments were performed.

Fatty acid oxidation. The liberation of 14CO2 from [1-14C]palmitate was used to assess mitochondrial fatty acid oxidation as previously described (7). Briefly, palmitate (200 μM) was bound to 0.5% BSA (final concentration) and brought up in reaction buffer to yield the following final concentrations (in mM): 100 sucrose, 10 Tris·HCl, 10 KPO4, 100 KCl, 1 MgCl2·6H2O, 1 l-carnitine, 0.1 malate, 2 ATP, 0.05 coenzyme A, and 1 dithiothreitol (pH 7.4). Aliquots (40 μl) of the appropriately diluted tissue homogenates were plated in quadruplicate in a modified 48-well cell culture plate (Costar, Cambridge, MA). A small groove was engineered between adjacent wells so that CO2 could freely diffuse between the incubation and trap wells. Reactions were started by the addition of 160 μl reaction buffer. Culture plates were sealed with parafilm and a siliconized rubber gasket and allowed to incubate in a shaking water bath at 37°C. Reactions were terminated after 30 min by addition of 100 μl of 70% perchloric acid to the incubation wells. The incubation plate was
transferred to an orbital shaker, and $^{14}$CO$_2$ was trapped in 200 $\mu$l of 1 N NaOH for 1 h at room temperature. Radioactivity of CO$_2$ was determined by liquid scintillation counting using 4 ml Uniscint BD (National Diagnostics, Atlanta, GA).

Citrate synthase assay. Citrate synthase activity was determined using the methods of Sere (60). Briefly, homogenates were incubated in the presence of oxaloacetate, acetyl-coenzyme A, and 5,5’-dithiobis (2-nitrobenzoic acid) (DTNB). Spectrophotometric detection of reduced DTNB at a wavelength of 412 nm served as an index of enzyme activity.

**SDS-PAGE Western blotting and immunodetection.** Skeletal muscle protein content for cytochrome c oxidase complex 4, subunit-1 (CytOX-I) and uncoupling protein-3 (UCP-3) was determined on MG from 20 animals (HCR and LCR chow fed, HCR and LCR high fat fed; $n=5$/group). Upon death, skeletal muscle was snap-frozen with liquid nitrogen-cooled tongs and stored at −80°C until analysis.

Protein was extracted from 20 mg of frozen, pulverized MG by homogenization at 20,500 rpm for 15 s (Ultra-Turrax-T25; Janke & Kunkel, Staufen, Germany) in 1 ml/10 mg of radioimmunoprecipitation assay buffer/protease inhibitor cocktail. The samples were solubilized for 20 min on ice and then centrifuged at 10,000 g for 30 min. The supernatant was assayed for protein concentration by the bicinchoninic acid method (Pierce, Rockford, IL). Results were used to calculate volumes for equal protein loading before SDS-PAGE. Samples were treated with Laemmli buffer (Bio-Rad Laboratories, Hercules, CA) and then boiled for 10 min to ensure optimal epitope exposure. All 20 samples were loaded on a single precast 10.5–14% Tris⋅HCl criterion gel (Bio-Rad Laboratories) with a well volume of 15 $\mu$l to prevent potential effects of gel-to-gel variability. Proteins were separated at room temperature at 200 volts for 1 h by electrophoresis and then transferred for 1 h at 90 volts at 4°C on a methanol-activated polyvinylidene fluoride membrane (Millipore, Bedford, MA) in transfer buffer (25 mM Tris base, 192 mM glycine, 20% methanol). Visual verification of transfer and equal protein loading among lanes was accomplished by Ponceau S staining (Sigma Aldrich) of the membranes. For immunodetection, blots were blocked overnight at 4°C in blocking buffer [10% nonfat dry milk in 20 mM Tris base, 150 mM NaCl, and 0.1% Tween 20 (TBS-T); pH 7.6], serially washed in TBS-T at room temperature, and then probed with the primary antibody (UCP-3 from ABCAM, Cambridge, MA; CytOX-I from Santa Cruz, Santa Cruz, CA) for 1 h at room temperature with both primary antibodies diluted to 1:2,000. Serially washed membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (Santa Cruz) diluted to 1:5,000 in blocking buffer for 4 h at room temperature and again serially washed in TBS-T. The HRP activity was detected using enhanced chemiluminescence reagent (Amersham ECL plus, Buckinghamshire, UK) and exposure to autoradiographic film (Classic Blue Sensitive; Midwest Scientific, St. Louis, MO). The film was scanned, and antigen concentration was calculated by quantification of the integrated optical density of the appropriate band using Gel Pro Analyzer software (Media Cybernetics, Silver Spring, MD). Antibody specificity was confirmed by a competition assay using appropriate blocking peptide, and comparisons of protein bands were matched for correct molecular weight against a KaleidoScope marker (Bio-Rad Laboratories).

Lipid density. Approximately 20 mg of tissue were fixed in a 4% paraformaldehyde/0.1% glutaraldehyde solution for 3.5 h. Following fixation, tissues were rinsed two times for 10 min each in 0.1 M NaPO$_4$ (pH 7.3) and then stored at 4°C for 24 h in a 30% sucrose and 0.1 M NaPO$_4$ solution. Tissues were subsequently frozen in an optimal-cutting temperature compound and stored at −80°C. Serial sections were cut at 11 $\mu$m, mounted on slides, and stored at −80°C until immunofluorescence was performed. Lipid accumulation was assessed using an Oil red-O 36% triethyl phosphate working solution as described by Koopman et al. (39). Slides were incubated in Oil red-O working solution for 30 min at room temperature. Thereafter, slides were rinsed (3 × 30 s) in ddH$_2$O and then for 10 min with running tap water. Sections were then covered with a cover slip using 10% glycerol in 10 mM Tris-HCl (pH 8.5) containing 0.1 $\mu$g/ml 4’,6-diamidino-2-phenylindole.

Images were digitized with a Nikon E600 fluorescence microscope fitted with an Orca II CCD camera (Hamamatsu, Bridgewater, NJ). A 10 × 10 grid reticle (Klarmann Rulings, Litchfield, NH) numbered 1–100 was placed in the eyepiece to determine the sampling regions (grids) for images to be taken for all microscopy sessions. Determination of the sampling regions was done before experimentation and held constant during all microscopy sessions for their respective stains. Images for all slides were captured in grids 23, 28, 73, and 78. The initial field of view was determined using the ×10 objective to position the slide where tissue “filled” the entire grid reticle. Images were subsequently obtained within each predetermined grid using a ×20 objective.

Image analysis was performed using ImagePro Plus 4.5.1 software (Media Cybernetics). Oil red-O image analysis was performed on three slides from each tissue for each rat ($n=3$/model). Two images were taken within each of the four sampling regions (grids 23, 28, 73, and 78) for each slide and quantified for lipid density per square micrometer of tissue. Thus a total of 72 images per tissue was analyzed for each model.

**Statistical analysis.** All data are presented as means ± SE and were analyzed using SigmaStat software (Systat Software, Point Richmond, CA). A three-way (strain × diet × time) repeated-measures ANOVA was used to analyze weight gain throughout the course of the study, with serum variables and glucose tolerance test results with the repeated-measures variable being the time point before vs. after the HFD intervention. Additionally, a three-way (strain × diet × condition) repeated-measures ANOVA was used to analyze insulin-stimulated glycogen synthesis rates in isolated skeletal muscle strips with the repeated-measures variable being basal vs. insulin stimulation. Differences between LCR and HCR rats in run distance to exhaustion and femur length were assessed via one-way ANOVA, whereas palmitate oxidation, citrate synthase activity, and lipid density were determined using a two-way ANOVA (strain × diet). A Tukey’s post hoc test was performed where appropriate. Significance level was established a priori at $P<0.05$.

**RESULTS**

Weight gain patterns. Demographic data in Fig. 1 showed HCR rats from generation 13 had a ninefold greater run distance to exhaustion than LCR rats ($P<0.001$). LCR rats were generally larger animals (Fig. 1), and their femurs were significantly longer than HCR rats (43.1 ± 0.3 vs. 39.9 ± 0.7 mm; $P<0.005$). At 3 mo of age, LCR rats were already 100 grams heavier than HCR rats ($P<0.001$; Fig. 2A). At 6 mo, LCR rats were still heavier than HCR rats, but weight gain from 3 to 6 mo was similar between these groups (LCR = 157 ± 11 g vs. HCR = 136 ± 6 g; Fig. 2A). Animals were in energy balance at 6 mo, since neither chow-fed LCR nor HCR rats exhibited significant weight gain over the subsequent 3–4 mo (LCR = 17 ± 8 g and HCR = 6 ± 9 g; Fig. 2A). Importantly, animals were not introduced to an HFD until they reached 6 mo of age to avoid confounding the results of diet-induced weight gain with differences in their normal pattern of developmental growth between strains. Feeding an HFD resulted in an interaction effect ($P<0.005$) indicating LCR rats gained excess weight compared with HCR rats (LCR = 90 ± 12 g vs. HCR = 24 ± 6 g; Fig. 2A). Upon further inspection, Fig. 2B shows weekly weight gain patterns of HCR rats were generally higher than their prediet level, but repeated-measures ANOVA indicated none of these time points reached statistical significance. Alternatively, LCR rats
exhibited dramatically higher weight gain within the first 4 wk of the HFD than both their prediet levels and the levels exhibited by HCR rats, yet between weeks 5 and 10, these values were not different from prediet values or those found in HCR rats. Figure 2C shows epididymal fat pads in the chow-fed condition were significantly heavier in LCR than HCR rats ($P < 0.01$). The HFD intervention increased fat pad weight and the percent of total body mass represented by adipose tissue (Fig. 2D) only in LCR rats. As a result, following the HFD, LCR rats had greater fat pad mass and percent body fat than their HCR counterparts. Table 1 shows daily dietary consumption for rats on both chow and the HFD during the time of the dietary intervention. Animals on the HFD consumed more absolute gross energy than their chow-fed counterparts ($P < 0.005$), but there were no differences between LCR and HCR rats that were fed similar diets. Absolute metabolizable energy consumption (gross energy intake × %total digestible nutrients) was similar between all groups, regardless of strain and/or dietary intervention. When expressing energy consumption relative to body mass, regardless of diet, the HCR rats consumed more gross and metabolizable energy than LCR rats on a similar diet. Additionally, only HCR rats on an HFD consumed more relative gross energy than their chow-fed controls.

**Insulin sensitivity parameters.** Serum variables linked to insulin resistance are listed in Table 2. Regardless of diet, LCR rats had higher blood triacylglycerol levels compared with HCR rats ($P < 0.05$). In response to the HFD, mean serum triacylglycerol levels were higher in both strains, but the changes did not reach statistical significance. Serum NEFA and free glycerol levels were not different between strains on either diet. A significant main effect for diet was detected for both NEFA ($P < 0.05$) and free glycerol ($P < 0.05$) levels, indicating both decreased in response to the HFD. However, when post hoc analysis was performed, results revealed that the decline within each strain following the HFD was not statistically significant for either serum variable. Amylin, a satiety signal cosecreted from the pancreas with insulin, was not different between strains in the chow-fed state. Alternatively, following an HFD, a statistically significant increase in serum amylin levels was detected only in LCR rats ($P < 0.001$), resulting in significantly greater values than those seen in the HCR rats that were fed an HFD ($P < 0.05$). Leptin, an adipocyte-derived hormone involved in signaling satiety and

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**Fig. 2.** Effect of a high-fat diet on total body and adipose tissue mass. 
*A*: all animals were weighed upon arrival (3 mo), before the dietary intervention (6 mo), and upon death (9 mo). 
*B*: rats that were fed the high-fat diet were weighed weekly throughout the course of the diet. Individual epididymal fat pad weight (C) and the %total body mass represented by this fat pad (D) were obtained upon death, $P < 0.05$ for LCR vs. HCR within same diet category (*), for within-group weight gain from previous time point (*), for chow vs. high-fat diet within a given strain (#), and greater weight gain in LCR rats on a high-fat diet than any other group ($*$). Data are expressed as means ± SE.
improving insulin sensitivity, was lower in HCR than LCR rats on the chow diet ($P < 0.05$). Following an HFD, serum leptin levels in HCR rats increased ($P < 0.005$), whereas these levels did not change in LCR rats. Serum levels of adiponectin, another adipokine known to enhance insulin sensitivity, were not different between chow-fed HCR and LCR rats. In response to the HFD, adiponectin concentrations decreased in LCR rats ($P < 0.01$), whereas these levels remained unaltered in HCR rats.

Basal glucose (Fig. 3A) and serum insulin (Fig. 3B) values were significantly higher in LCR than HCR rats in both the Chow and HFD conditions ($P < 0.05$). Interestingly, although the HFD did not alter resting glucose values in either strain, serum insulin levels were elevated exclusively in LCR rats ($P < 0.005$). These data were used to calculate HOMA values (Table 2), an index of insulin resistance. These data suggest that LCR rats were less insulin sensitive when compared with their HCR counterparts and that HOMA levels were increased in response to the HFD only in LCR rats. In response to an oral glucose challenge, chow-fed LCR rats generally had higher blood glucose values than their HCR counterparts and that HOMA levels were not different between chow-fed LCR and HCR rats. In response to the HFD, adiponectin concentrations decreased in LCR rats ($P < 0.01$), whereas these levels remained unaltered in HCR rats.

Glucose tolerance tests (OGTT) were performed both pre- and post-HFD conditions, and the response to both glucose challenges were used in conjunction with their HCR counterparts and that HOMA levels were increased in response to the HFD only in LCR rats. In response to an oral glucose challenge, chow-fed LCR rats generally had higher blood glucose values than their HCR counterparts and that HOMA levels were not different between chow-fed LCR and HCR rats. In response to the HFD, adiponectin concentrations decreased in LCR rats ($P < 0.01$), whereas these levels remained unaltered in HCR rats.

**Table 2.** Energy consumption

<table>
<thead>
<tr>
<th>Group</th>
<th>Dietary intake, g/day</th>
<th>Gross energy</th>
<th>Metabolizable energy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>kJ/day</td>
<td>kJ/day $^{-1}$ g body wt $^{-1}$</td>
</tr>
<tr>
<td>LCR Chow</td>
<td>19.7±1.1</td>
<td>339.2±19.0</td>
<td>0.68±0.04</td>
</tr>
<tr>
<td>HCR Chow</td>
<td>19.7±1.0</td>
<td>338.8±12.5</td>
<td>0.88±0.03*</td>
</tr>
<tr>
<td>LCR HFD</td>
<td>17.0±1.9</td>
<td>432.6±48.1†</td>
<td>0.79±0.20</td>
</tr>
<tr>
<td>HCR HFD</td>
<td>17.5±1.2</td>
<td>445.6±29.4†</td>
<td>1.25±0.15†</td>
</tr>
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</table>

Data are expressed as means ± SE. LCR and HCR, low- and high-capacity endurance running, respectively; HFD, high-fat diet. $P < 0.05$, LCR vs. HCR within same diet category (*) and chow vs. HFD within a given strain (†).

suggest that LCR rats were more prone to the exacerbation of an insulin-resistant condition when exposed to an HFD, whereas HCR rats were protected against the development of impaired glucose tolerance on an HFD.

At the tissue level, HCR skeletal muscle displayed a trend for higher glycogen synthesis (Fig. 4), but there were no significant differences in either basal or insulin-stimulated glycogen synthesis between chow-fed groups. The HFD did not affect rates of glycogen synthesis in skeletal muscle obtained from HCR rats. Conversely, absolute rates of insulin-stimulated glycogen synthesis were lower in both the soleus (red) and EDL (white) from LCR rats on the HFD vs. the chow-fed condition ($P < 0.05$; Fig. 4, A and B).

**Measures of fatty acid oxidation.** Measures of fatty acid oxidative capacity are shown in Fig. 5. MG from HCR rats on a Chow diet had higher citrate synthase activity ($P < 0.005$; Fig. 5B) and palmitate oxidation rates ($P < 0.05$; Fig. 5A) than LCR rats. Skeletal muscle fatty acid oxidation rates from HFD-fed rats were higher than their chow-fed controls in both strains ($P < 0.005$), although citrate synthase was elevated only in LCR rats ($P < 0.05$). Hepatic citrate synthase activity (Fig. 5D) and palmitate oxidation (Fig. 5C) were similar between Chow-fed LCR and HCR rats. Exposure to an HFD resulted in diminished liver palmitate oxidation in LCR rats ($P < 0.05$) without changes in citrate synthase activity, whereas no changes in either variable were detected in HCR rats. Cardiac citrate synthase activity (Fig. 5F) and palmitate oxidation (Fig. 5E) were similar between Chow-fed LCR and HCR rats. An HFD did not induce any detectable changes in these parameters in either strain for this tissue.

**Skeletal muscle proteins.** Because the most profound differences in oxidative capacity, both between strains and in response to the HFD, were found within the skeletal muscle, we probed this tissue for protein markers associated with mito-

**Table 2.** Serum variables collected in the fasted state before and upon completion of the 12-wk high-fat diet intervention

<table>
<thead>
<tr>
<th>Serum Variable</th>
<th>Chow Diet LCR</th>
<th>Chow Diet HCR</th>
<th>High-Fat Diet LCR</th>
<th>High-Fat Diet HCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEFA, meq/l</td>
<td>1.24±0.18</td>
<td>1.32±0.13</td>
<td>0.76±0.16</td>
<td>0.97±0.13</td>
</tr>
<tr>
<td>Free glycerol, mg/dl</td>
<td>33.3±5.5</td>
<td>32.6±4.4</td>
<td>28.4±2.2</td>
<td>22.4±3.3</td>
</tr>
<tr>
<td>True triacylglycerols, mg/dl</td>
<td>58.8±11.0</td>
<td>28.4±2.4*</td>
<td>33.1±2.4</td>
<td>33.1±2.4*</td>
</tr>
<tr>
<td>Glucagon, pM</td>
<td>35.7±3.9</td>
<td>40.5±3.0</td>
<td>27.6±2.6</td>
<td>34.7±3.9</td>
</tr>
<tr>
<td>GLP-1, pM</td>
<td>7.5±0.4</td>
<td>7.4±0.5</td>
<td>7.3±0.3</td>
<td>7.2±0.5</td>
</tr>
<tr>
<td>Amylin, pM</td>
<td>3.6±0.2</td>
<td>3.6±0.2</td>
<td>6.0±0.6†</td>
<td>4.8±0.4*</td>
</tr>
<tr>
<td>Leptin, pM</td>
<td>468.3±49.9</td>
<td>312.6±34.1*</td>
<td>485.5±44.0</td>
<td>564.9±66.6†</td>
</tr>
<tr>
<td>Adiponectin, µg/ml</td>
<td>9.5±0.6</td>
<td>9.0±1.3</td>
<td>6.7±0.3+†</td>
<td>8.7±1.3</td>
</tr>
<tr>
<td>HOMA</td>
<td>9.7±1.1</td>
<td>6.2±0.4*</td>
<td>19.2±2.5+†</td>
<td>8.1±0.9*</td>
</tr>
</tbody>
</table>

Data are expressed means ± SE. NEFA, nonesterified fatty acid; GLP-1, glucagon-like peptide-1. $P < 0.05$, LCR vs. HCR within same diet category (*) and chow vs. HFD within a given strain (†).
Mitochondrial function. CytOX-I was significantly higher in HCR than LCR rats on the chow diet ($P < 0.05$; Fig. 6A). In response to the HFD, CytOX-I levels were increased in both strains ($P < 0.001$), yet even in this condition the levels detected in HCR rats were significantly greater than those found in LCR rats ($P < 0.001$). This suggests that the LCR group does exhibit metabolic flexibility to a similar extent as the HCR strain when given a stressor such as high-fat feeding. Regardless, CytOX-I protein content does support the results from fat oxidation experiments which demonstrated that palmitate oxidation was lower in the chow-fed state in LCR animals but increased significantly following the HFD treatment.

UCP-3 levels were higher in HCR than LCR rats in the chow-fed condition ($685.5 \pm 270.41$ vs. $204.1 \pm 42.14$), but, because of variability, this did not reach statistical significance (Fig. 6B). These levels remained unaltered in LCR rats following the HFD, whereas a significant and robust increase in UCP-3 protein content was noted in HCR rats ($P < 0.05$). This differential response resulted in HCR rats having significantly greater UCP-3 protein levels than LCR rats on the HFD ($P < 0.005$).

**Lipid density.** Figure 7A indicates LCR rats had higher mean intramuscular lipid densities than HCR rats in the chow-fed condition, but differences did not reach statistical significance. Both strains exhibited significant elevations in intramuscular lipid content following an HFD ($P < 0.001$), and the content detected in LCR rats under this dietary manipulation was significantly greater than HCR rats ($P < 0.05$). Intrahepatic lipid content (Fig. 7B) was similar between HCR and LCR rats on chow. Although both strains exhibited slight increases in lipid accumulation following the HFD, this value reached statistical significance only in HCR rats ($P < 0.005$). Intramyocardial lipid accumulation (Fig. 7C) was not different between strains regardless of diet, and this parameter remained unaffected in both strains following an HFD.

**DISCUSSION**

One of the hallmarks of peripheral insulin resistance is an imbalance in lipid metabolism favoring storage, rather than oxidation, of fatty acids. Lipid accumulation is known to play a causative role in the pathogenesis of insulin resistance (27), and several investigations suggest this effect is secondary to impaired lipid disposal pathways (35, 51, 54, 56–58). Alternatively, endurance exercise training increases whole body fatty acid oxidation, which is linked, in part, to enhanced skeletal muscle mitochondrial content and hence elevated oxidative capacity, an adaptation believed to play a major role in the exercise training-mediated alleviation of insulin resistance (10, 18). Although these studies provide strong evidence indicating a positive relationship between oxidative capacity and improved insulin action, it is largely speculative, since most of the information has been collected from subjects with a pre-existing insulin-resistant condition. Obscuring the issue further is the fact that previous investigations aimed at elucidating the
causative role of oxidative capacity in the development of insulin resistance that have manipulated genes involved in lipid disposal pathways either directly or via pharmacological intervention have yielded inconsistent results. The development of LCR and HCR rats provides an animal model with inherent differences in oxidative capacity, thereby circumventing the confounding parameters involved in selective gene manipulation. This is the first study to use such an animal model to directly test the effects of oxidative capacity on the development of HFD-induced insulin resistance.

On a chow diet, LCR rats were heavier, hypertriglyceridemic, and hyperleptinemic compared with HCR rats. Additionally, despite the absence of significant differences in skeletal muscle insulin-stimulated glycogen synthesis detected between these strains on the chow diet, dynamic in vivo measures of glucose clearance indicated LCR rats were less insulin sensi-

Fig. 5. Palmitate oxidation and citrate synthase activity in mixed gastrocnemius (A and B), liver (C and D), and heart (E and F) homogenates. P < 0.05 for LCR vs. HCR within same diet category (*) and chow vs. high-fat diet within a given strain (#). Data are expressed as mean ± SE.

Fig. 6. Skeletal muscle mitochondrial proteins. Western blots were performed on mixed gastrocnemius (MG) tissue homogenates for the mitochondrial membrane-associated proteins cytochrome c oxidase complex IV, subunit I (CytOX-I; A) and uncoupling protein-3 (UCP-3; B). P < 0.05, LCR vs. HCR within same diet category (*) and chow vs. high-fat diet within a given strain (#). Data are expressed as means ± SE.
tive than HCR rats, which supports previous findings (68). It should be noted, however, that, when compared with other animal models, LCR rats would not be classified as an overtly insulin-resistant strain.

Assessments of palmitate oxidation rates while the animals were on the chow diet (baseline condition) indicated a heightened oxidative capacity in skeletal muscle from HCR rats, but differences were not detected in the liver and heart. Knowing that fat pad weights and tissue lipid accumulation were significantly greater in LCR rats makes it tempting to argue that the larger body mass in LCR rats is the result of excess adiposity developed secondary to diminished skeletal muscle lipid oxidation; however, there is only a trend for the percent of total body mass represented by adipose tissue to be higher in LCR rats. Therefore, given the finding that femur lengths were significantly greater in LCR rats, it appears that the physiological relevance of the differences in body weight developed within the first 3 mo of life is primarily to impart a larger general body morphology than HCR rats, rather than the development of overt obesity.

The data collected in this investigation are novel in that we demonstrate HCR rats were resistant to the development of HFD-induced obesity, whereas LCR rats gained significantly more body weight and adipose tissue mass than HCR rats. Interestingly, the differences in weight gain between these strains occurred within the first 4 wk of the dietary intervention, but beyond this time period both strains had similar weight gain patterns. Previous investigations comparing alternative animal models that are either prone or resistant to the development of obesity indicate much of these differences can be attributed to variable dietary consumption (30, 32, 44, 45, 55). In opposition to these findings, the differences in weight gain found between HCR and LCR rats cannot be explained by differences in energy intake, since both strains were consuming similar absolute amounts of energy. In fact, relative to body mass, HCR rats actually consumed more energy than LCR rats. Two possible differences between the strains could help to explain our findings. First, it may be that the energy expenditure was greater in the HCR animals because of differences in spontaneous activity levels. Whereas this could, at least in part, explain the lower body mass in the HCR group while on the chow diet, it is less likely to contribute to the observed differences in body/fat mass while on the HFD, since HCR animals consumed significantly greater relative energy during this period of the study yet did not gain in body/fat mass compared with LCR subjects. Therefore, for spontaneous physical activity to account for the differential response to the HFD detected in the current investigation, activity patterns would have had to been altered differently in both strains in response to the diet so as to be decreased in LCR rats and increased in HCR rats. Novak et al. (53) established such a precedent in a study using a different model of obesity-prone vs. obesity-resistant rats, but results from Levin (43) counter this supposition, indicating the differences in weight gain between obesity-prone and obesity-resistant rats is not related to a differential effect of high-fat feeding on activity patterns. Because the effect of an HFD on activity patterns in obesity-prone vs. obesity-resistant rats is currently an unresolved issue, the absence of this data represents a possible limitation of the present study and is worthy of future investigation.

A second explanation for the current results may pertain to HCR rats being inherently more proficient in utilizing lipids as an energy substrate. This is supported by current findings demonstrating that only LCR rats on an HFD gained excess weight and adiposity despite consuming similar amounts of metabolizable energy vs. chow-fed controls. The present research design provides biochemical evidence supporting this possibility, since chow-fed LCR rats had lower basal skeletal muscle palmitate oxidation rates, citrate synthase activity, and CytOX-I protein content than HCR rats. Interestingly, in response to an HFD, both strains exhibited significant increases in palmitate oxidation rates and CytOX-I protein levels when compared with their respective chow-fed controls (although, when expressed in absolute terms, these values remained higher in the HCR vs. LCR rats). Thus, ultimately, it appears that the LCR animals were able to adapt to high-fat feeding, since they exhibited no substantial changes in body weight over the last 6–8 wk of the diet treatment. This suggests that the difference in overall weight gain between these strains is not primarily because of an inability of skeletal muscle from LCR rats to eventually adapt to an HFD but rather is related to an impaired capacity to utilize lipids at the onset of the dietary intervention. Accordingly, lower baseline skeletal muscle oxidative capacity in LCR rats, in combination with the inability of this strain to preserve hepatic oxidative capacity on an HFD, may result in greater amounts of dietary lipids being diverted toward adipose tissue and thus contribute to the excess weight gain detected within the first 4 wk of the dietary intervention in these animals. In contrast, a higher initial capacity for skeletal muscle to oxidize lipids, in conjunction with the ability to maintain hepatic oxidative capacity when challenged with an HFD, likely protects the HCR strain from the development of obesity. Such a paradigm is supported by previous investigations indicating obesity-resistant rats typically have higher in vivo oxidation rates (31, 32, 55) and are better capable of utilizing, rather than storing, fatty acids after 5 days of high-fat
feeding vs. obesity-prone counterparts (28). In summary, these findings stress the potential importance of the ability to handle fatty acids during the early phases of dietary lipid exposure in male HCR and LCR rats. It is also worthy to point out that performing similar experiments in female HCR and LCR rats may be important, since Jackman et al. (29) recently showed distinct sex-related differences in whole body lipid handling in response to dietary fatty acid exposure. Such experiments would provide a more global perspective regarding the handling of fatty acids in these animal strains.

The strain-specific changes in body weight paralleled differences induced by the HFD on whole body glucose clearance, since the insulin-resistant condition was further exacerbated in LCR rats, yet, remarkably, insulin sensitivity was maintained in HCR rats. These findings are corroborated by Levin et al. (44) who showed an exaggerated insulin response following an intravenous glucose tolerance test in obesity-prone rats. Changes in whole body insulin sensitivity are supported at the tissue level, since the HFD reduced insulin-stimulated glucose synthesis rates in skeletal muscle from LCR rats, yet had no effect on muscle from the HCR strain. Diminished skeletal muscle insulin sensitivity in high-fat-fed LCR rats may be linked to the greater amount of intramuscular lipids in this strain, which may in turn be secondary to lower basal oxidative capacity. The changes in skeletal muscle insulin sensitivity likely contribute to the exacerbation of whole body insulin resistance but are not likely to be the sole factor. It is also very likely that the inability of this strain to maintain hepatic lipid oxidation perturbs this condition further, since previous investigations have shown fatty acid oxidation in the liver is diminished in tissue homogenates taken from rodent models of insulin resistance (3, 6).

With the above discussion in mind, UCP-3, the predominant uncoupling protein in skeletal muscle, was assayed for protein content in this tissue for two important reasons. First, because of its high sequence homology to UCP-1, UCP-3 has been suggested to possess mitochondrial uncoupling activity (5), and, second, under conditions of elevated lipid load, mitochondrial UCP-3 activity may be related to generation of reactive oxygen species (ROS; see Refs. 4 and 7). With respect to the former, there is the potential for upregulation of this protein to reduce coupling efficiency in the mitochondria and hence to be a metabolic protection against weight/fat gain despite excess energy/fat intake (8). In the present study, we noted that UCP-3 protein content was severalfold higher in HCR vs. LCR animals while maintained on a chow diet; however, because of variability, the differences did not reach statistical significance. Hence it is not yet possible to attribute the differences (lower in weight and fat mass in HCR vs. LCR animals to increased UCP-3 activity in skeletal muscle. However, large and statistically significant increases in UCP-3 protein content were noted following exposure to an HFD, and importantly, only in the HCR strain, a discovery that is consistent with previous findings comparing obesity-prone vs. obesity-resistant rats (66). This observation could explain two of the significant findings of the present study. First, an increased UCP-3 protein content, if possessive of uncoupling activity, could serve to increase energy turnover and hence help explain the lack of weight/fat gain in the HCR strain on the HFD, even despite the larger relative energy intake during this period of the study. However, if UCP-3 is not linked to skeletal muscle energy expenditure per se but could maintain mitochondrial electron flux by acting to attenuate an excessive rise in transmembrane proton potential (i.e., a high ΔΨ leads to electron leak), the mitochondria could theoretically reduce the generation of superoxide radicals. Accordingly, evidence is accumulating indicating that UCP-3 may facilitate fatty acid handling under elevated lipid load conditions by catalyzing the transport of surplus fatty acid anions out of the mitochondrial matrix while simultaneously lowering the membrane potential and ROS production (4). This may be relevant to the present findings, since elevated ROS production has recently been linked to the development of insulin resistance (25). Thus the significant rise in UCP-3 content observed in the HCR but not LCR strain could help to explain the “resistance” to insulin observed in the former but not the latter animals following high-fat feeding. These are intriguing speculations that are worthy of investigation by future, mechanistically designed studies.

Profiling of several serum variables associated with insulin action was measured to elucidate potential mechanistic variables linked with the insulin resistance induced by the present experimental design. Amylin is cosecreted from pancreatic β-cells with insulin and has a role in signaling satiety, diminishing gastric emptying, inhibiting glucagon secretion, and stimulating growth of pancreatic β-cells (48, 69). However, supraphysiological levels of amylin have inhibitory effects on insulin secretion and action (14, 17, 33, 40, 42, 50, 59) and aggregate to form amyloid plaques that result in pancreatic β-cell destruction (47). Therefore, it is tempting to suggest that the significantly greater increase in serum amylin levels in LCR rats resulting from the consumption of an HFD might have contributed to the development of insulin resistance in this strain, but regression analysis did not reveal a significant correlation between fluctuations in amylin levels and alterations in glucose tolerance (data not shown). In addition to amylin, leptin and adiponectin, adipokines (adipocyte-derived hormones) that mediate insulin-sensitizing effects on peripheral tissues (34, 72), were assayed. Previous investigations have shown that serum adiponectin levels tend to fall (2, 26, 70), and peripheral tissues become less responsive to the effects of leptin in models of obesity and/or insulin resistance (16, 23, 46, 61, 62, 65). Interestingly, the adipokine response in HCR rats is consistent with a protective phenotype, since serum concentrations of leptin increased and adiponectin remained unaltered in response to an HFD. Alternatively, a deleterious profile was found in LCR rats, since they exhibited no change in leptin and a decrease in adiponectin levels in response to an HFD. This is especially interesting considering the fact that these hormones are derived from adipose tissue, and only LCR rats had an increase in fat pad mass. Therefore, it could be argued that the selective breeding for differences in endurance running capacity has also elicited inherent differences in adipose tissue function that protects HCR rats from, yet predisposes LCR rats to, the development of excess adiposity and insulin resistance when exposed to an HFD. Although the current investigation offers no clear molecular mechanism concerning the role of adipose tissue and adipokine pathways in regulating peripheral insulin sensitivity in HCR and LCR rats, these data do provide sufficient evidence to warrant further research in this area.

In summary, these data suggest that animals with inherently higher oxidative capacity do not gain excess weight and do
preserve insulin sensitivity when exposed to an HFD, thus conferring an intrinsic metabolic benefit during conditions that promote obesity and/or diabetes. Alternatively, the fact that LCR rats gained excess weight and became more insulin resistant following an HFD, despite consuming similar amounts of metabolizable energy as chow-fed controls, indicates animals with a low inherent aerobic capacity are particularly intolerant to dietary lipid exposure. The difference between these strains appears to be linked to HCR rats exhibiting higher baseline skeletal muscle oxidative capacity and an ability to maintain hepatic lipid oxidative capacity when fed an HFD. Therefore, the development of a novel animal model established by selective breeding for high- vs. low-endurance capacity provides researchers with the means to address genetic factors related to aerobic capacity that protect or predispose individuals to the metabolic syndrome and could prove immensely valuable in terms of detecting potential therapeutic targets.

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REFERENCES


