Effects of ovariectomy and intrinsic aerobic capacity on tissue-specific insulin sensitivity

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Running head: Aerobic fitness impacts OVX-induced insulin resistance

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Abstract

High capacity running (HCR) rats are protected against the early (i.e., ~11 weeks post) development of ovariectomy (OVX)-induced insulin resistance (IR) compared to low capacity running (LCR) rats. The purpose of this study was to utilize the hyperinsulinemic-euglycemic clamp to determine: 1) if HCR rats remain protected from OVX-induced IR when the time following OVX is extended to 27 weeks; and 2) whether tissue-specific glucose uptake differences are responsible for the protection in HCR rats under sedentary conditions. Female HCR and LCR rats (n=40; age ~22 weeks) randomly received either OVX or sham (SHM) surgeries and then underwent the clamp 27 weeks following surgeries. $^3$H-3-Glucose was used to determine glucose clearance, while $^{14}$C-2-Deoxyglucose (2DOG) was used to assess glucose uptake in skeletal muscle, brown adipose tissue (BAT), subcutaneous white adipose tissue (WAT), and visceral WAT. OVX decreased the glucose infusion rate and glucose clearance in both lines, but HCR had better insulin sensitivity than LCR ($P<0.05$). In both lines, OVX significantly reduced glucose uptake in soleus and gastrocnemius muscles; however, HCR showed ~40% greater gastrocnemius glucose uptake compared to LCR ($P<0.05$). HCR also exhibited greater glucose uptake in BAT and visceral WAT compared to LCR ($P<0.05$) yet these tissues were not affected by OVX in either line. In conclusion, OVX impairs insulin sensitivity in both HCR and LCR rats, likely driven by impairments in insulin-mediated skeletal muscle glucose uptake. HCR rats have greater skeletal muscle, BAT and WAT insulin-mediated glucose uptake which may aid in protection against OVX-associated insulin resistance.

Key words: ovariectomy, insulin resistance, aerobic fitness, glucose uptake, brown adipose tissue
Introduction

Women are now spending almost half of their lives in menopause, characterized in part by ovarian hormone deficiency. This ovarian insufficiency leads to metabolic dysregulation, ultimately increasing risk for the development of type 2 diabetes (T2D) (3) and other cardiometabolic diseases for which postmenopausal women are particularly susceptible (5). The underlying mechanism(s) of menopause-associated changes in body composition and metabolic function are unclear; however, studies suggest that reduced physical activity (11), rather than increased food intake (10, 30), plays a critical role. Reduced physical activity decreases aerobic fitness, and impaired aerobic fitness is a strong risk factor for all-cause mortality (23).

Conversely, exercise training or increased physical activity attenuates the prevalence of metabolic syndrome and T2D in postmenopausal women (12, 23). Ovariectomy (OVX) in rodents, a model of ovarian hormone depletion, is a good model of human menopause, as it provides insight into some of the menopause-associated changes such as increased adiposity and insulin resistance (IR) (36, 48). At least one rodent study (17) reported that exercise training mitigates OVX-induced metabolic disorders, but the impact of inherent aerobic capacity per se on attenuating OVX-induced metabolic dysfunction is still unclear.

Koch and Britton (22) developed a rat model of contrasting aerobic fitness (high-capacity running (HCR) and low-capacity running (LCR) rats) determined by endurance treadmill running. HCR rats display ~30% greater aerobic fitness measured by VO2max and ~3.5 fold longer run time to exhaustion than the LCR rats. These contrasting phenotypes are evident even in the sedentary condition, so this model allows the investigation of inherent aerobic fitness independent of structured exercise training. Evidence based on investigation of the HCR/LCR model suggests a protective role of high aerobic capacity on a variety of metabolic outcomes (27,
Female HCR rats showed greater mitochondrial aerobic capacity including fatty acid oxidation in skeletal muscle (29, 38), and greater inherent insulin sensitivity compared to LCR rats (50). The greater basal skeletal muscle oxidative capacity in HCR appeared to prevent or attenuate metabolic dysfunction including IR associated with consumption of a high fat diet (29, 31). Recently, we demonstrated that HCR rats are protected from early development (i.e. ~11 weeks post) of OVX-induced IR assessed by HOMA-IR (47) and found that, unlike HCR rats, LCR rats had an exaggerated insulin response to a glucose tolerance test (GTT). In this study, we determined whether HCR rats remain protected from OVX-induced IR when the time following OVX is extended to 27 weeks, and how these rat lines differ in tissue-specific insulin-mediated glucose uptake across tissues in both OVX and ovary-intact (i.e., SHM) conditions.

It is known that ovary-intact female HCR rats display 30-50% greater insulin-induced glucose uptake and oxidation in skeletal muscle compared to LCR rats (35). Indeed, striking skeletal muscle phenotypic differences have been observed between HCR and LCR rats (29, 35, 38). Additionally, white and brown adipose tissues (WAT, BAT, respectively) are gaining appreciation as also being important tissues in whole body glucose regulation (26), and may influence changes in glucose metabolism associated both with loss of ovarian hormones (39) and low aerobic capacity (44). No studies have investigated if HCR rats possess greater insulin-mediated glucose metabolism in WAT and BAT compared to LCR rats, and/or if OVX affects this intrinsically enhanced phenotype in WAT and BAT.

In the present study, we employed the ‘gold standard’ hyperinsulinemic-euglycemic clamp technique to determine whether rats selectively bred for high aerobic capacity are protected from OVX-induced reduction in whole body insulin sensitivity and glucose clearance,
and if so, what specific tissues may be driving this protection. To this end, insulin-mediated tissue specific glucose uptake was measured and compared among groups using $^{14}$C-2-Deoxyglucose (2DOG) radiolabelled isotope during a clamp procedure. We hypothesized that protection against OVX-induced IR in HCR rats would be largely attributed to greater insulin-stimulated skeletal muscle glucose uptake and also in part to enhanced glucose uptake in WAT and BAT.

**Methods**

**Animals**

Previous research has well described the HCR/LCR rat model (27, 29, 31, 45). Forty female HCR and LCR rats from generation 33 were shipped to the University of Missouri, and singly housed under standard humidity and temperature on a 12h-12h light/dark cycle. All animals were fed standard rodent chow (Harlan Teklad Rodent Diet 8604) and water *ad libitum* throughout the study. Animal procedures were approved by Institutional Animal Care and Use Committee (IACUC) at the University of Missouri-Columbia.

**Experimental design**

At approximately 22 weeks of age, HCR and LCR rats were randomized to receive OVX or sham (SHM) surgeries, generating the following groups: HCR$_{SHM}$, HCR$_{OVX}$, LCR$_{SHM}$, and LCR$_{OVX}$ (n=10/group). All rats underwent a hyperinsulinemic-euglycemic clamp procedure 27 weeks following OVX or SHM surgery. Three to five days following surgical catheterization of arterial and venous lines, rats were fasted for 5 hours and a hyperinsulinemic-euglycemic clamp was initiated to assess whole body insulin sensitivity. During the clamp, $^3$H-3-glucose radiolabeled isotope was used to determine glucose clearance and glycolytic rate, while $^{14}$C-2-deoxyglucose (2DOG) was used to examine the tissue-specific assessment of glucose uptake.
Following the clamp, skeletal muscle (soleus and gastrocnemius), BAT, subcutaneous (SQ), and visceral WAT (i.e. retroperitoneal (RP), perigonadal (PG), and omental (OMEN)) were collected and stored at -80°C until analyzed. Body weight was measured pre-, mid- and post- surgeries.

**Ovariectomy and sham surgeries**

OVX and SHM surgeries were conducted as previously described (18). Briefly, while rats were anesthetized with inhaled 2% isoflurane, a one-inch incision at midline of the dorsal surface was made followed by bilateral incisions through the muscle layer. The whole ovarian bursa was removed for OVX procedure, while it was externalized and located back inside the body for SHM procedure. The skin incision was closed using wound clips, and acetaminophen (500 mg/kg) was given.

**Catheterization surgeries**

Catheterization surgeries were performed as previously described (1). The right jugular vein and the left common carotid artery were catheterized under anesthetization with inhaled 2% isoflurane. The free ends of the catheter lines tunneled subcutaneously to the back of the neck, and were exteriorized and sealed with steel plugs. The clamp experiments were performed when the body weight of the rats reached within 5% of pre-surgery weight (3-5 days). To maintain the proper function of the catheter lines during these recovery days, the lines were flushed with heparinized-saline twice a day.

**Hyperinsulinemic-euglycemic clamps**

Following a ~5 hour fast, hyperinsulinemic-euglycemic clamps were conducted in conscious rats as previously described (1). Following collection of a baseline blood sample, a priming bolus (20 µCi) of $^3$H radiolabeled isotope was infused, followed by a constant infusion (0.2 µCi/min) of $^3$H for 2 hours. A second baseline of blood sample was collected. A priming bolus of insulin (20
mU/kg) was given, followed by a constant infusion of insulin (4 mU/kg/min) and $^3$H-3-glucose (0.4 µCi/min) for 2 hours. Glucose infusion rate (glucose concentration: 50g/100ml) was adjusted for a 2 hour period to maintain euglycemia during the clamp. Blood samples were taken every 10 min for the last 40 min out of 2 hours (80-120 min time points) to assess glucose infusion rate, glucose clearance, and glycolytic rate. At the end of the clamp, a bolus of 2DOG (39 µCi) was infused and 25 min following the bolus targeted tissues were promptly harvested and frozen for assessment of the tissue-specific assessment of glucose uptake. Rates of whole body glucose clearance were determined as the ratio of the $[^3]$H specific activity of plasma glucose to the net glucose levels during the final 40 min. Glycolytic rate was determined by $^3$H$_2$O accumulation, the difference in plasma $[^3]$H radioactivity between non-dried and dried samples during the final 40 min.

**Tissue-specific assessment of glucose uptake**

Glucose uptakes in skeletal muscle (i.e. soleus and gastrocnemius), BAT, subcutaneous WAT, and visceral WAT were determined via the assessment of 2DOG. Briefly, ~60 mg of powdered tissues was homogenized in 1.5 mL of 0.5 % perchloric acid, and centrifuged for 20 min at 2000 g at 4°C. The supernatant was neutralized at 7.5 pH, mixed with 125 µL of each 0.3N BaOH and 0.3N ZnSO$_4$, and analyzed by liquid scintillation counter (Beckman Coulter, Inc., Brea, CA). Glucose uptake was calculated as dpm of $[^{14}]$C radioactivity per mg of tissue per specific activity of the tracers.

**Tracer incorporation into skeletal muscle glycogen**

Incorporation of $^3$H-3-glucose radiolabeled isotopes into skeletal muscle glycogen was measured to determine glucose retention into glycogen. Approximately 30 mg of powdered skeletal muscle tissue was solubilized in 500 µL of 1N NaOH for an hour at 37°C (vortex every 15 min).
Following mixed carrier glycogen (33 µL of 60 mg/mL) and 1.2 mL of 75% ethanol to homogenates, the glycogen was precipitated overnight at 4°C. Samples were centrifuged for 10 min (10,000 g, 4°C), and the supernatant was discarded. The glycogen pellets were dried at the bottom of each tube, resuspended in 600 µL of H₂O, and analyzed by liquid scintillation counter (Beckman Coulter, Inc., Brea, CA). Glucose retention into glycogen was calculated as dpm of [³H] radioactivity of per mg of tissue per specific activity of the tracers.

**Statistical analysis**

Statistical differences were analyzed using a 2 (HCR vs. LCR) x 2 (SHM vs. OVX) ANOVA. We determined if differences occurred between line (HCR vs. LCR) and treatment (SHM vs. OVX), and if a treatment x line interactions existed. Bivariate Spearman’s correlations were performed to determine the tissue-specific association between the whole body glucose clearance and glucose uptake in HCR and LCR rats. All data were analyzed using SPSS 22.0. In all cases, $P<0.05$ was considered statistically significant and data are reported as mean ± SEM.

**Results**

**Animal characteristics and body composition**

Prior to OVX, body weight was ~18% greater in LCR compared to HCR (line main effect, $P<0.001$; Figure 1A), and both HCR and LCR experienced OVX-induced weight gain (treatment main effect, $P=0.003$; Figure 1B). LCR were heavier than HCR at the end of the study (line main effect, $P<0.001$; Figure 1B). The body weight differences paralleled differences in visceral (i.e., RP, PG, and OMEN) and SQ WAT weight, such that both lines increased WAT percentage following OVX (treatment main effect, $P=0.012$; Figure 1C), and LCR had greater adiposity than HCR (line main effect, $P<0.001$; Figure 1C).

**Insulin sensitivity and glycolytic rate**
No significant differences in glucose levels were found between groups at any time points for the last 40 min during the clamp, indicative of a maintained euglycemic state. The HCR rats were more insulin sensitive, as indicated by a greater glucose infusion rate and glucose clearance compared to LCR rats (line main effect, $P<0.001$ and $P=0.037$, respectively), yet OVX decreased glucose infusion rate and glucose clearance in both lines (treatment main effect, $P=0.014$ and $P=0.005$, respectively; **Figure 2B and C**). Glycolytic rate during the hyperinsulinemic-euglycemic clamp was greater in HCR compared to LCR (line main effect, $P=0.002$, **Figure 2D**). OVX also tended to decrease glycolytic rate in both lines (treatment main effect, $P=0.053$; **Figure 2D**).

**Tissue-specific assessment of glucose uptake**

Glucose uptake in slow-twitch predominant soleus muscle tended to be greater in HCR compared to LCR (line main effect, $P=0.072$), while in both lines OVX significantly reduced glucose uptake in this muscle type (treatment main effect, $P=0.023$; **Figure 3**). Compared to LCR, HCR rats showed significantly greater glucose uptake in the fast-twitch predominant gastrocnemius muscle (line main effect, $P=0.002$), and as in the case with soleus, OVX reduced gastrocnemius glucose uptake in both lines (treatment main effect, $P<0.001$; **Figure 3**). In examining adipose tissues, HCR rats exhibited greater glucose uptake in BAT and visceral WAT than LCR rats (1.7- and 2-fold greater uptake, respectively; line main effects, $P=0.005$ and $P=0.003$, respectively; **Figure 3**), and there was a similar trend in subcutaneous WAT (line main effect, $P=0.062$). Unlike the case with skeletal muscle, OVX had no effect on insulin-stimulated glucose uptake in any of the adipose tissue depots.

**Tracer incorporation into glycogen**
Compared to LCR, HCR rats had greater $[^3]$H glucose retention in glycogen in both soleus and gastrocnemius muscles (line main effect, $P=0.014$ and $P=0.003$, respectively), and OVX reduced $[^3]$H glucose retention in glycogen in soleus and gastrocnemius in both lines (treatment main effect, $P=0.013$ and $P=0.016$, respectively; Figure 4A).

**Brown adipose tissue glucose uptake**

BAT mass was greater in LCR than HCR rats (line main effect, $P=0.01$; Figure 4B). The BAT/WAT ratio was higher in HCR than LCR, and OVX reduced it in both lines (line main effect, $P=0.01$; treatment main effect, $P=0.023$; Figure 4C). The total amount of BAT glucose uptake was greater in HCR than LCR (line main effect, $P=0.044$; Figure 4D).

**Tissue-specific relationship between glucose uptake and whole body glucose clearance**

Whole body glucose clearance was strongly associated with glucose uptake by skeletal muscles, BAT, and subcutaneous WAT (soleus: $r=0.441$, $P=0.02$; gastrocnemius: $r=0.680$, $P<0.001$, BAT: $r=0.490$, $P=0.014$; subcutaneous WAT: $r=0.569$, $P=0.005$; Figure 5). In comparing HCR and LCR rats in terms of how specific tissues contributed to whole body glucose clearance, adipose tissue glucose uptake was the strongest correlate to systemic glucose clearance in LCR rats ($r=0.68$ and $r=0.61$ for WAT and BAT, respectively ($P<0.02$ in each case) compared to $r=0.65$ ($P<0.01$) and $r=0.34$ ($P=0.12$) for WAT and BAT, respectively among HCR. Among HCR rats, the strongest correlate to systemic glucose clearance was skeletal muscle ($r=0.72$ and $r=0.56$ for gastrocnemious and soleus muscles, respectively ($P<0.01$ for both)) whereas those correlations among LCR were $r=0.59$ ($P=0.02$) and $r=0.21$ ($P=0.07$), respectively.

**Discussion**

The purpose of this study was to determine: (1) whether high aerobic capacity, independent of exercise training, protects against the development of OVX-induced reduction in
whole body insulin sensitivity and glucose clearance during hyperinsulinemic-euglycemic clamp conditions; and (2) what specific tissues may be driving this protection. By ~7 months following OVX, both high-fit HCR and low-fit LCR rats developed insulin resistance (IR) indicated by reduced glucose infusion rate and glucose clearance during the clamp procedure. However, HCR rats remained more insulin sensitive than LCR in both SHM and OVX conditions. Given the increasing body of evidence utilizing the HCR/LCR rat model to illustrate the robust protection imparted by high aerobic fitness on a wide variety of metabolic outcomes, the fact that the HCR rat is not resilient to OVX-associated IR is quite remarkable. Moreover, the OVX-associated impairment in glucose clearance observed in both rat lines appeared to be due specifically to impairment in skeletal muscle insulin-mediated glucose uptake, rather than impairments in brown or white adipose tissue (i.e., BAT, WAT, respectively). This was especially surprising since a key distinguishing feature between HCR and LCR rats is their divergence in skeletal muscle metabolic phenotype (31). That this enhanced skeletal muscle phenotype of the HCR rat was not sufficient to protect against OVX-mediated impairments highlights the strong physiological effect of OVX on skeletal muscle insulin sensitivity.

Skeletal muscle is thought to dispose of up to ~80% of the glucose load during hyperinsulinemic clamp conditions (8); thus skeletal muscle insulin sensitivity and oxidative metabolism play an important role in maintaining euglycemia during these conditions (20). Moreover, decreased skeletal muscle mitochondrial content and function associated with low aerobic capacity is thought to cause IR (21, 34). Our group and others using the HCR/LCR model have reported that HCR rats have greater mitochondrial aerobic capacity, increased mitochondrial content in skeletal muscle (29, 38, 47), and 30-50% greater insulin-mediated glucose uptake and oxidation in skeletal muscle (35). Despite that, both the HCR and LCR rats
studied here similarly reduced insulin-mediated skeletal muscle glucose uptake in response to OVX, indicated in part by reduced insulin-mediated $[^{14}\text{C}]-$glucose uptake in soleus muscle (HCR: ~30%; LCR: ~26% decrease compared to each SHM group). The evidence in $[^{3}\text{H}]-$glucose retention in skeletal muscle glycogen confirmed this (HCR: ~35%; LCR: ~32% decrease compared to each SHM group), which paralleled the OVX-induced impairments in whole body glucose clearance (HCR: ~37%; LCR: ~45% decrease compared to each SHM group).

Interestingly, Bergeron et al. showed that OVX rats did not develop systemic IR six weeks following surgery, yet did develop impairments in skeletal muscle insulin signaling (2). This may suggest that OVX-mediated impairment in skeletal muscle insulin sensitivity is an early result of OVX, and perhaps an initiating factor for systemic IR.

An important question is whether lack of ovarian hormones directly or indirectly impairs insulin signaling. Importantly, OVX reduces spontaneous physical activity (SPA) (46), even in HCR rats that we previously demonstrated to show a ~20% reduction in SPA following OVX, consistent with the reduction observed in LCR rats (47). While we did not measure SPA in this particular set of animals, it is possible that an OVX-mediated drop in SPA is mechanistically responsible for the OVX-mediated reduction in skeletal muscle insulin sensitivity noted here. It might be that the emergence of IR in the HCR rat after several months is the result of the cumulative effect of reduced SPA over this extended time frame. This hypothesis needs to be addressed in future studies. Bergeron’s group did not assess the OVX-mediated reduction in physical activity, but did find that voluntary wheel running rescued the muscle IR observed in sedentary OVX rats (2). Future work should elucidate whether OVX-mediated impairments in insulin sensitivity are fully or partially explained by an OVX-associated reduction in SPA. Alternatively, the OVX-mediated skeletal muscle impairments may have been directly attributed
to ovarian hormone (e.g., loss of estrogen signaling in skeletal muscle) loss, which has been
associated with reduced skeletal muscle mitochondrial aerobic capacity (6). Others have shown
that OVX in rats with normal intrinsic aerobic capacity led to ~50% attenuation in skeletal
muscle insulin-stimulated $[^{14}C]$-glucose uptake and incorporation into glycogen (24); and, a
study investigating skeletal muscle ex vivo of OVX female rodents showed a ~30% reduction in
insulin-mediated $[^{14}C]$-glucose uptake compared to that of SHM rodents (33).

Rivas et al. (35) hypothesized that skeletal muscle mitochondrial content differences in
HCR/LCR rats is fiber-type specific, reporting greater mitochondrial content in HCR compared
to LCR, but only in fast-twitch predominant white muscle (i.e. extensor digitorum longus),
whereas slow-twitch predominant soleus was not different between lines. Although we did not
measure skeletal muscle mitochondrial aerobic capacity or content in the present study, we found
that glucose uptake into both muscle types tended to be greater in HCR compared to LCR,
although the line difference for soleus did not reach statistical significance ($p=0.072$), whereas
that in gastrocnemious did. Soleus displayed ~3-fold greater insulin-stimulated glucose uptake
than gastrocnemius muscle in both HCR and LCR rats. Red skeletal muscle (e.g. slow-twitch
predominant soleus) is thought to be more insulin sensitive than white skeletal muscle (e.g. fast-
twitch predominant gastrocnemius) (16) due to its higher oxidative capacity and mitochondrial
density. That OVX affected both muscle types similarly may suggest that the effects of OVX on
skeletal muscle insulin sensitivity are not fiber-type specific. More research is certainly needed
to determine precise mechanisms by which OVX affects skeletal muscle metabolic function.

Similar to postmenopausal women, OVX rodents increase adiposity along with their
development of IR (42, 51). Moreover, adipose tissue is heavily influenced by female sex
hormones and we have previously demonstrated that OVX leads to adipose tissue inflammation,
which precedes the development of systemic IR in that model (46). Female ovary-intact HCR
rats are protected against diet induced IR (29, 31), which is thought to be driven by their
increased skeletal muscle substrate oxidation (29, 38) and mitochondrial content (47) as
mentioned above. Our group recently demonstrated that HCR rats are also protected from OVX-
induced increases in adiposity and systemic IR assessed by fasting glucose and HOMA-IR,
unlike LCR rats who did experience impairments in those variables (47). Thus, we wanted to
compare adipose tissue and skeletal muscle insulin sensitivity following OVX in HCR and LCR
rats. Counter to our original hypothesis, HCR rats are not resilient to developing OVX-induced
IR and/or OVX-associated changes in body composition over the long-term (i.e. 27 weeks). We
found that both HCR and LCR rats ultimately develop OVX-associated increased adiposity and
IR, as assessed by the reduced glucose infusion rate during a glucose clamp. In our previous
study (47), HCR rats exhibited a gradual (albeit non-significant) increase over time in body
weight during the 11-week period, but were protected from the overt metabolic manifestations of
OVX observed in LCR. Perhaps the attenuation of weight gain and adiposity in HCR delays, but
does not prevent, the development of OVX-associated metabolic syndrome; increased adiposity
does often precede the development of metabolic syndrome (13). The reason why HCR rats were
not fully protected from OVX-induced IR despite their enhanced aerobic capacity and skeletal
muscle mitochondrial content and function compared to LCR is not clear, but may involve the
fact that HCR rats had significantly better adipose tissue insulin sensitivity, at least under clamp
conditions, than LCR rats across both brown and white depots.

Importantly, HCR rats in the present study did have > 30% greater insulin sensitivity and
glucose clearance compared to LCR rats in both ovarian conditions, supporting that high intrinsic
fitness does at least lessen the metabolically damaging effects of OVX. While OVX did not
affect adipose tissue insulin sensitivity in either line, the markedly greater glucose uptake in both BAT and WAT of HCR rats may have ‘buffered’ their OVX-associated systemic IR. Adipose tissue metabolism, which is gaining more and more appreciation as being vital to systemic metabolism (14), is disrupted in obesity and IR (15). Impaired adipose tissue mitochondrial regulation may contribute to the metabolic dysfunction of adipose tissue (7) supported by, for example, reduced mitochondrial gene expression in adipose tissues from individuals with T2D (49) and insulin resistant rodents (37).

BAT is a unique adipose depot in part because of its strikingly greater mitochondrial density compared to WAT depots. For the last several years, BAT has been intensively studied due to its possibility of being an anti-obesity target, as one study (32) calculated that ~50 g of BAT can burn ~125 kcal per day in humans. Emerging evidence also implicates the BAT as an important contributor to systemic insulin sensitivity in both rodents (43) and humans (41). Thus, we assessed the unique roles of intrinsic fitness and/or OVX on insulin sensitivity of BAT. We observed that, although BAT mass was less in HCR, the total amount of BAT glucose uptake in response to insulin (Figure 4D) was actually much greater compared to LCR, and was very strongly associated with their greater whole body glucose clearance (Figure 5 C). Thus, the greater insulin-mediated BAT glucose uptake associated with high aerobic capacity reported here may help explain the enhanced whole body glucose clearance observed in HCR rats. Given the fact that, among the tissues observed, BAT showed the greatest capacity of glucose uptake per tissue weight (even greater than skeletal muscle; Figure 3), its contribution to whole body glucose homeostasis should be emphasized. Interestingly, one recent human study demonstrated a positive relationship between physical activity and BAT activity as assessed via PET scans (9). While we did not assess BAT activity per se, greater insulin-mediated glucose uptake in this
tissue may indicate higher activity; nevertheless, the effects of intrinsic fitness on BAT activity needs to be more precisely tested in future studies. Since research has reported that OVX alters BAT mitochondrial oxidative capacity assessed by uncoupling protein (UCP)-1 and PGC-1 protein expression (28, 47) which is associated with changes in insulin sensitivity (25), we were interested in investigating the effect of OVX on BAT insulin sensitivity. While we found no OVX effect on BAT mass and/or glucose uptake, the BAT/WAT ratio was reduced following OVX in both lines. In our first study with the shorter follow-up time period, the relative amount of BAT based on this ratio was reduced following OVX only in LCR rats, and correlated significantly with resting energy expenditure (47). New evidence suggests that increased adiposity is associated with ‘whitening’ of BAT (40). The increase in adiposity observed here even in the HCR rats may have been associated with some whitening of BAT resulting in an increase in mass. As we did not assess BAT whitening in this present study, that hypothesis could not be tested but should be addressed in future studies.

A limitation of this study is that HCR rats are habitually more physically active than their LCR counterparts in their cages, preventing us from being able to separate the effects of aerobic capacity from those attributed to higher SPA. Indeed, there is a close relationship between physical activity level, aerobic capacity, and metabolic function making investigation of each independent factor extremely difficult. However, since we previously demonstrated that both HCR and LCR rats are equally affected by OVX in that they both experience a ~20% reduction in SPA, one would expect OVX to reduce BAT and WAT insulin sensitivity in both lines. This was not the case, as neither HCR nor LCR rats were affected by OVX in terms of any changes in BAT or WAT insulin sensitivity. The fact that skeletal muscle insulin sensitivity was affected similarly with OVX in HCR and LCR rats may suggest that this effect may have been attributed
to the OVX-associated reduction in SPA. Another limitation was the fact that liver insulin
sensitivity was not measured; the possibility exists that the differences in systemic insulin
sensitivity between HCR and LCR rats were due to differences at the level of the liver. In fact,
the liver is known to play a significant role in glucose disposal under clamp conditions (19).
Importantly, it has been shown that estradiol can prevent IR in OVX mice specifically by
improving hepatic as well as skeletal muscle IR (4). How fitness may affect liver insulin
sensitivity in OVX rodents is an important question to be addressed in future studies.

In summary, both high and low running capacity rats experience OVX-induced
reductions in whole body and skeletal muscle insulin sensitivity. Albeit, in both SHM and OVX
conditions, HCR rats display greater insulin sensitivity compared to their respective LCR
controls, demonstrating greater skeletal muscle, BAT and WAT insulin-stimulated glucose
uptake. The systemic IR induced by OVX in both lines appeared to be due to a deficit in skeletal
muscle insulin sensitivity associated with OVX, but not adipose tissue. In conclusion, in the
absence of exercise training, rats selectively bred for high running capacity experience greater
tissue-specific glucose uptake compared to LCR rats. This intrinsically higher insulin-mediated
glucose uptake in skeletal muscle, BAT and WAT may help buffer OVX-induced systemic IR in
HCR rats.

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References


subcutaneous adipose tissue in exercise-induced improvements in glucose homeostasis.


Figure Legends

**Figure 1. Body weight and fat percentage.** (A) body weight gain curves pre-, mid-, and post-experiment, (B) final body weight, and (C) white adipose tissue (WAT) weight. Values are means ± SE (n=7-10 per group). † *P*<0.05, line main effect, HCR vs. LCR; *P*<0.05, treatment main effect, SHM vs. OVX.

**Figure 2. Insulin sensitivity and glucose clearance.** (A) glucose levels for the last 40 min during the clamp (80-120 min time points), (B) glucose infusion rate, (C) glucose clearance, and (D) glycolytic rate. Values are means ± SE (n=6-8 per group). † *P*<0.05, line main effect, HCR vs. LCR; *P*<0.05, treatment main effect, SHM vs. OVX.

**Figure 3. Tissue-specific glucose uptake.** Glucose uptake in skeletal muscle (i.e. soleus and gastrocnemius), brown adipose tissue (BAT), and white adipose tissue (WAT; subcutaneous and visceral WAT). Values are means ± SE (n=5-8 per group). † *P*<0.05, line main effect, HCR vs. LCR; *P*<0.05, treatment main effect, SHM vs. OVX.

**Figure 4. Glucose retention as skeletal muscle glycogen and contribution of brown adipose tissue (BAT) glucose uptake.** (A) [³H] glucose retention in glycogen, (B) BAT mass, (C) BAT/WAT ratio, and (D) total BAT glucose uptake. Values are means ± SE (n=5-8 per group). † *P*<0.05, line main effect, HCR vs. LCR; *P*<0.05, treatment main effect, SHM vs. OVX.

**Figure 5. Relationships between tissue-specific glucose uptake and whole body glucose clearance.** (A) association between soleus glucose uptake and whole body glucose clearance,
(B) association between gastrocnemius glucose uptake and whole body glucose clearance, (C) association between BAT glucose uptake and whole body glucose clearance, (D) association between subcutaneous glucose uptake and whole body glucose clearance, and (E) association between visceral glucose uptake and whole body glucose clearance. Values are means ± SE (n=5-8 per group).
Table 1. Tissue weights

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<thead>
<tr>
<th></th>
<th>HCR SHM</th>
<th>O VX</th>
<th>LCR SHM</th>
<th>O VX</th>
<th>2-way ANOVA statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>RP (g)</td>
<td>1.12 ±</td>
<td>2.24 ±</td>
<td>2.58 ±</td>
<td>5.68 ±</td>
<td>Line, P=0.001</td>
</tr>
<tr>
<td></td>
<td>0.30</td>
<td>0.38</td>
<td>0.48</td>
<td>1.37</td>
<td>Treatment, P=0.005</td>
</tr>
<tr>
<td>PG (g)</td>
<td>1.87 ±</td>
<td>2.15 ±</td>
<td>3.26 ±</td>
<td>5.16 ±</td>
<td>Line, P=0.003</td>
</tr>
<tr>
<td></td>
<td>0.52</td>
<td>0.52</td>
<td>0.71</td>
<td>1.10</td>
<td></td>
</tr>
<tr>
<td>OMEN (g)</td>
<td>0.72 ±</td>
<td>1.24 ±</td>
<td>1.37 ±</td>
<td>2.39 ±</td>
<td>Line, P=0.014</td>
</tr>
<tr>
<td></td>
<td>0.15</td>
<td>0.27</td>
<td>0.29</td>
<td>0.61</td>
<td>Treatment, P=0.034</td>
</tr>
<tr>
<td>SQ (g)</td>
<td>1.46 ±</td>
<td>2.47 ±</td>
<td>3.65 ±</td>
<td>7.72 ±</td>
<td>Line, P&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>0.27</td>
<td>0.36</td>
<td>0.71</td>
<td>1.32</td>
<td>Treatment, P&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Line x Treatment, P=0.038</td>
</tr>
<tr>
<td>Liver (g)</td>
<td>7.88 ±</td>
<td>7.90 ±</td>
<td>8.61 ±</td>
<td>8.75 ±</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>0.37</td>
<td>0.37</td>
<td>0.47</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>Heart (g)</td>
<td>0.948 ±</td>
<td>0.933 ±</td>
<td>0.873 ±</td>
<td>0.913 ±</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>0.046</td>
<td>0.047</td>
<td>0.025</td>
<td>0.040</td>
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</tr>
<tr>
<td>Uterus (g)</td>
<td>0.483 ±</td>
<td>0.074 ±</td>
<td>0.528 ±</td>
<td>0.129 ±</td>
<td>Treatment, P&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>0.037</td>
<td>0.013</td>
<td>0.043</td>
<td>0.028</td>
<td></td>
</tr>
</tbody>
</table>

Body composition was analyzed. Two-way ANOVA for line (i.e., HCR vs. LCR), treatment (i.e., SHM vs. OVX) and line x diet interaction effects were determined; values are means ± SE (n=8-10 per group). RP = retroperitoneal; PG = perigonadal; OMEN = omental; SQ = subcutaneous; BAT= brown adipose tissue; WAT = white adipose tissue.
Figure 1

A. Graph showing body weight (g) over time (Pre, Mid, Post) for different groups:
- HCR SHM
- HCR OVX
- LCR SHM
- LCR OVX

B. Bar graph showing final body weight (g) for:
- SHM
- OVX

C. Bar graph showing WAT (%) for:
- SHM
- OVX

Statistical significance indicated by:
- * p < 0.05
- † p < 0.01
Figure 2

A) Glucose level over time in different groups (HCR SHM, HCR OVX, LCR SHM, LCR OVX).

B) Glucose infusion rate in HCR and LCR groups.

C) Glucose clearance rate in HCR and LCR groups.

D) Glycolytic rate in HCR and LCR groups.
Figure 3

- **Soleus muscle**
- **Gastrocnemius muscle**
- **Brown adipose tissue**
- **Subcutaneous WAT**
- **Visceral WAT**

Glucose uptake (μg/min/mg)

- **HCR SHM**
- **HCR OVX**
- **LCR SHM**
- **LCR OVX**

* indicates significant difference compared to the respective control group.
† indicates significant difference compared to the other group within the same tissue type.
Figure 4

A. 

- **3H-glucose retention in glycogen (µg/mg)**
- **SHM OVX SHM OVX**
- **HCR LCR**
- **SOLEUS**
- **GASTROCNEMIUS**

B. 

- **BAT (g)**
- **SHM**
- **OVX**
- **HCR**
- **LCR**

C. 

- **BAT/WAT (µg/g)**
- **SHM**
- **OVX**
- **HCR**
- **LCR**

D. 

- **Total BAT glucose uptake (µg/min)**
- **SHM**
- **OVX**
- **HCR**
- **LCR**
Figure 5

A. Solas glucose uptake (μg/min/mg)

- HCR SHM
- HCR OVX
- LCR SHM
- LCR OVX

Glucose clearance (mg/kg/min)

\[ r = 0.441, p = 0.02 \]

B. Gastroc glucose uptake (μg/min/mg)

Glucose clearance (mg/kg/min)

\[ r = 0.680, p < 0.001 \]

C. BAT glucose uptake (μg/min/mg)

Glucose clearance (mg/kg/min)

\[ r = 0.490, p = 0.014 \]

D. Subcutaneous glucose uptake (μg/min/mg)

Glucose clearance (mg/kg/min)

\[ r = 0.569, p = 0.005 \]
Figure 5 Cont.

Visceral glucose uptake (μg/min/mg) vs Glucose clearance (mg/kg/min)

E

\( r = 0.278, p = 0.111 \)