

Increased Aerobic Capacity Reduces Susceptibility to Acute High-Fat Diet-Induced Weight Gain

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Objective: Aerobic capacity is the most powerful predictor of all-cause mortality in humans; however, its role in the development of obesity and susceptibility for high-fat diet (HFD)-induced weight gain is not completely understood.

Methods: Herein, a rodent model system of divergent intrinsic aerobic capacity [high capacity running (HCR) and low capacity running (LCR)] was utilized to evaluate the role of aerobic fitness on 1-week HFD-induced (45% and 60% kcal) weight gain. Food/energy intake, body composition analysis, and brown adipose tissue gene expression were assessed as important potential factors involved in modulating HFD-induced weight gain.

Results: HCR rats had reduced 1-week weight gain on both HFDs compared with LCR. Reduced HFD-induced weight gain was associated with greater adaptability to decrease food intake following initiation of the HFDs. Further, the HCR rats were observed to have reduced feeding efficiency and greater brown adipose mass and expression of genes involved in thermogenesis.

Conclusions: Rats with high intrinsic aerobic capacity have reduced susceptibility to 1-week HFD-induced weight gain, which is associated with greater food intake adaptability to control intake of energy-dense HFDs, reduced weight gain per kcal consumed, and greater brown adipose tissue mass and thermogenic gene expression.

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Introduction

Obesity is a global epidemic, impacting over 35% of the world population (1,2), and is fundamental to the development and progression of metabolic disease states. Therefore, understanding the factors influencing weight gain and obesity is critically important. A striking characteristic is the presence of individual phenotypes that are resistant to obesity despite exposure to the same obesogenic environment (3). This is undoubtedly driven by a complex interplay between polygenetic factors. The importance of these polygenetic characteristics is emphasized by the observation that up to two thirds of variability in body mass index is due to heritable factors (4). These putative genetic factors predispose individuals to susceptibility or protection against weight gain through metabolic, hedonic, hormonal, and satiety driven mechanisms (5-8).

Weight gain occurs following a shift to a positive energy balance through some combination of increased energy intake and/or reduced energy expenditure (9). However, the multisystem physiology and polygenetic factors that interact to ultimately control energy balance make it difficult to tease out individual components of weight gain that may be used as predictive tools or therapeutic targets. Observed changes in energy intake and body weight are not constant, with daily and weekly weight fluctuations observed. As such, long-term weight gain results as a series of small positive weight fluctuations due to changes in energy intake and expenditure in time scales such as weekends (10), holidays (11,12), and seasonally (13). Thus, any factors that could act to reduce the propensity to acutely gain weight in the face of the current energy-dense food landscape could be important tools in mitigating the increasing number of people with overweight and obesity.

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Additional Supporting Information may be found in the online version of this article.

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TABLE 1 Anthropometrics, food, and energy intake data

	HCR			LCR			Interaction
	LFD	45% HFD	60% HFD	LFD	45% HFD	60% HFD	
Anthropometrics							
Body weight (g)	361.1 ± 7.8	363.7 ± 11.8	382.0 ± 10.0 ^b	441.6 ± 8.4 ^a	446.8 ± 10.6 ^a	465.6 ± 11.6 ^{a,b}	
Fat-free mass (g)	320.7 ± 8.9	322.8 ± 13.6	325.2.7 ± 7.9	370.3 ± 9.5 ^a	374.8 ± 11.4 ^a	369.2 ± 8.8 ^a	
Fat mass (g)	50.0 ± 4.1	64.6 ± 7.8 ^b	56.6 ± 2.9 ^b	72.4 ± 4.3 ^a	89.2 ± 6.1 ^{a,b}	89.1 ± 4.8 ^{a,b}	
% Body fat	14.0 ± 1.0	16.4 ± 1.4 ^b	15.26 ± 0.8 ^b	16.20 ± 0.8 ^a	19.1 ± 0.9 ^{a,b}	19.33 ± 0.9 ^{a,b}	
Intake							
Food (g)	103.5 ± 3.6	98.8 ± 4.2	93.4 ± 3.3	111.6 ± 4.0 ^a	122.7 ± 5.6 ^a	122.5 ± 3.8 ^a	<i>P</i> < 0.05
Energy (kcal)	398.0 ± 13.9	467.3 ± 19.7 ^b	489.2 ± 17.1 ^b	429.5 ± 15.3 ^a	580.4 ± 26.7 ^{a,b}	641.8 ± 20.1 ^{a,b,e}	<i>P</i> < 0.05
Adjusted intake							
Food (g, covariate final BW)	116.3 ± 3.5	110.3 ± 3.8	101.3 ± 3.8 ^d	105.2 ± 3.2 ^c	113.2 ± 3.7	107.0 ± 4.2	<i>P</i> < 0.05
Covariate effect size		Covariate BW	<i>P</i> < 0.05	Partial η^2 0.361			
Energy (kcal, covariate final BW)	455.1 ± 15.4	519.1 ± 17.0 ^b	525.1 ± 16.8 ^b	399.9 ± 14.1 ^c	537.6 ± 16.6 ^b	572.3 ± 18.6 ^b	<i>P</i> < 0.05
Covariate effect size		Covariate BW	<i>P</i> < 0.05	Partial η^2 0.366			

Body weight ($n = 16$ – 24), fat-free mass ($n = 8$ – 16), fat mass ($n = 8$ – 16), and percent body fat after the 1-week HFD are expressed as means \pm SEM. Food and energy intake ($n = 16$ – 24) during the 1-week HFD is expressed as means \pm SEM. Food and energy intake following ANCOVA for body weight is expressed as the estimated means \pm SEM. Estimated effect size of the covariate for each ANCOVA analysis is presented as partial η^2 .

^a*P* < 0.05, main effect HCR vs. LCR.

^b*P* < 0.05, main effect LFD vs. HFD.

^c*P* < 0.05, HCR vs. LCR within diet.

^d*P* < 0.05, LFD vs. HFD within strain.

^e*P* < 0.05, 45% HFD vs. 60% HFD within strain.

Aerobic capacity has been described as the most powerful predictor of all-cause mortality in adults (14), and 40% to 70% of an individual's aerobic capacity is driven by complex genetic components (15). However, the mechanisms by which intrinsic aerobic capacity impacts diet-induced weight gain are not completely known. To assess how intrinsic aerobic capacities impact metabolic health, a rat model was produced based on selective breeding for high capacity running (HCR) or low capacity running (LCR) rats, resulting in divergent intrinsic aerobic capacities (16). Several studies have found that the HCR rats are resistant to high-fat diet (HFD)-induced metabolic diseases and increased adiposity (17,18), while in contrast, the LCR are extremely susceptible to dietary-induced obesity, insulin resistance, and fatty liver (19,20). Therefore, the HCR/LCR model presents a unique opportunity to study the role of aerobic capacity upon susceptibility or protection against weight gain. Here, we examine the potential role of intrinsic fitness to impact short-term, dose-dependent, HFD-induced weight gain through modulation of food/energy intake and excess energy storage. Our findings suggest that the high aerobic capacity HCR rat is protected from obesity due in part to a better acute regulation of energy intake and reduced feeding efficiency compared with LCR rats.

Methods

An overview of the methods utilized is provided below; detailed methods are provided in Supporting Information.

Animals

HCR/LCR rats' development and characterization have been previously described (16–22). Rats 25 to 30 weeks of age were singly housed (~75–77°F, 12-h light cycle) and acclimatized to the low-fat control diet (LFD) [D12110704 (10% kcal fat, 3.5% kcal sucrose, 3.85 kcal/g), Research Diets, Inc., New Brunswick, NJ] for 2 weeks before the initiation of the 1-week HFD [D12451 (45% kcal fat, 17% kcal sucrose, 4.73 kcal/g) or D12492 (60% kcal fat, 6.8% kcal sucrose, 5.24 kcal/g)]. Food intake was monitored for 1 week before HFD (week 0) and during the 1-week HFD (week 1). Change in food intake and change in energy intake was calculated as the difference between the week 0 and week 1 values. Feeding efficiency was calculated as the weight gained per kcal consumed during 1 week. The animal protocols were approved by the Institutional Animal Care and Use Committee at the University of Missouri and the Subcommittee for Animal Safety at the Harry S. Truman Memorial VA Hospital.

Body composition analysis

Fat mass was measured by magnetic resonance using the EchoMRI-900 analyzer (EchoMRI, Houston, TX) as previously described (18).

Adipose staining and morphometrics

Retroperitoneal and brown adipose tissue (BAT) was collected at sacrifice and formalin fixed. Hematoxylin/eosin (H&E) staining was

performed and retroperitoneal fat pad images from each animal were analyzed for cell volume and number as previously described (19).

mRNA expression

RNA and cDNA were prepared as previously described (23).

Western blot

Brown fat samples were prepared, separated by SDS-PAGE, and analyzed as previously described (23).

Citrate synthase activity

Citrate synthase activity was determined as previously described (23).

Statistical analysis

All data are presented as means \pm standard error of the mean (SEM). The main effects of phenotype and diet were tested by using two-way ANOVA utilizing SPSS (SPSS Inc., Armonk, NY). Two-way ANCOVA with body weight as the covariate was performed for several outcomes to statistically control for the consistent difference in body weight between the strains. Where significant main effects were observed, *post hoc* analysis was performed using least significant difference to test for any specific pairwise differences. Linear regression analysis was performed utilizing GraphPad Prism (GraphPad Software, Inc., La Jolla, CA). Data are represented as the regression lines within strains across diets, with dietary groups separately annotated. Goodness-of-fit as r^2 and the equation of the regression line for comparison of the associations are presented. For all analysis, statistical significance was set at $P < 0.05$.

Results

Animal characteristics

Body weight was $\sim 15\%$ less in the HCR compared with LCR before and after HFD, due to both lower initial fat-free and fat mass (data not shown). The 60% HFD increased body weight in both strains (5%, $P < 0.05$). One week of both the 45% and 60% HFD resulted in increased fat mass in both HCR and LCR rats (Table 1, $P < 0.05$). Final fat-free mass was lower in the HCR than LCR ($P < 0.05$), with no observed diet effect.

The 45% HFD resulted in increased 1-week weight gain in both HCR and LCR rats (2.3 \times and 3.3 \times , respectively, $P < 0.05$) compared with LFD (Figure 1A). The 60% HFD produced greater weight gain in HCR and LCR rats than both the LFD (3.7 \times and 4.6 \times , respectively, $P < 0.05$) and the 45% HFD (58% and 40%, respectively, $P < 0.05$). Importantly, HCR rats demonstrated less weight gain ($\sim 65\%$) compared with LCR regardless of diet ($P < 0.05$). Together these findings resulted in a significant interaction of strain and diet on 1-week weight gain ($P < 0.05$). ANCOVA for body weight was utilized to determine if the difference in body weight influenced HFD-induced weight gain. Using final body weight as a covariate did not change the outcomes (data not shown). Further, the partial η^2 for the analysis was relatively low (0.117) suggesting that only a small percentage of the observed variance was due to body weight differences. Importantly, differences in weight gain between the HCR and LCR strains was driven by

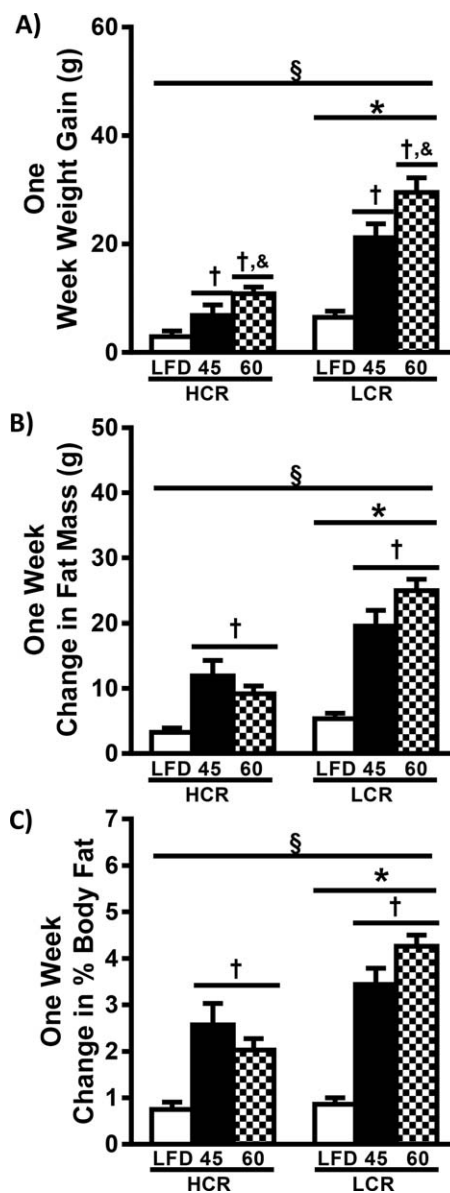


Figure 1 High intrinsic aerobic capacity reduces HFD-induced weight gain. Body weight and composition were determined before and following 1 week of HFD. One-week (A) weight gain, (B) change in fat mass ($n = 8-16$), and (C) change in percent body fat ($n = 8-16$) presented as means \pm SEM. § $P < 0.05$, interaction; * $P < 0.05$, main effect HCR vs. LCR; † $P < 0.05$, main effect LFD vs. HFD; & $P < 0.05$, main effect 45% HFD vs. 60% HFD.

increases in fat mass (Figure 1B). HCR rats gained less fat mass than LCR within all diets ($P < 0.05$), while both the 45% and 60% HFD resulted in greater fat mass gain in both strains compared with LFD ($P < 0.05$). The observed differences in fat mass resulted in similar alterations in change in percent body fat (Figure 1C). One-week change in fat-free mass was minimal, highly variable, and not different between strains or diets (data not shown). ANCOVA analysis of the change in fat and fat-free mass was not possible as both data sets failed the homogeneity of regression test. Together these data add to our previous data (18) suggesting higher intrinsic aerobic

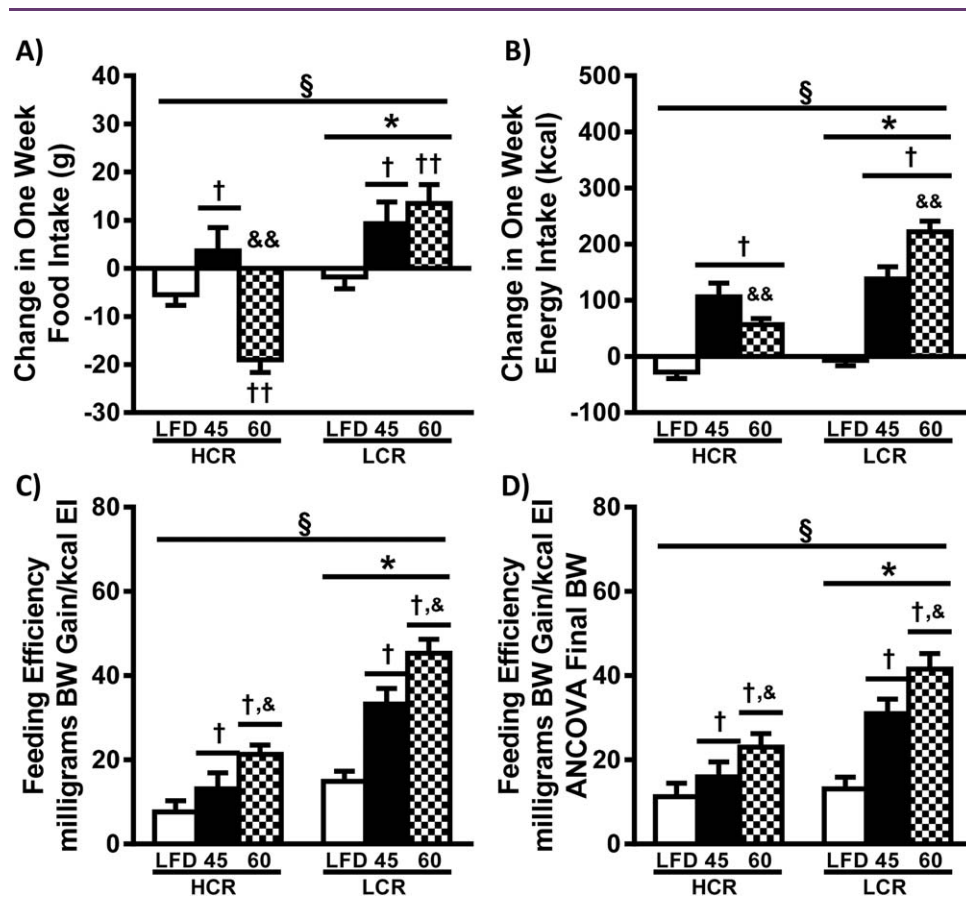


Figure 2 High intrinsic aerobic capacity is associated with food intake adaptability and reduced feeding efficiency following HFD. Change in food and energy intake was calculated as the difference between the LFD lead-in week and 1 week of HFD, and feeding efficiency is the weight gain in milligrams for the week divided by the total weekly energy intake. (A) Change in food intake, (B) change in energy intake, (C) feeding efficiency, and (D) estimated feeding efficiency adjusted for final body weight (ANCOVA) ($n = 16-24$) presented as means \pm SEM. § $P < 0.05$, interaction; * $P < 0.05$, main effect HCR vs. LCR; † $P < 0.05$, main effect LFD vs. HFD; & $P < 0.05$, main effect 45% HFD vs. 60% HFD; †† $P < 0.05$, LFD vs. HFD within strain; && $P < 0.05$, 45% HFD vs. 60% HFD within strain.

capacity is associated with reduced acute HFD-induced weight gain that is primarily driven by increased adiposity in the LCR rats.

Food and energy intake

Data for food and energy intake (FI and EI, respectively) are presented in Table 1 and Figure 2. FI during the 1-week 45% and 60% HFDs was lower in HCR compared with LCR (Table 1, $P < 0.05$). Compared with LFD, HFDs tended to increase FI in the LCR ($P = 0.060$). Following adjustment of FI by ANCOVA, the main effect of strain disappears (Table 1). The partial η^2 (0.361) highlights that the body weight difference between the strains is a moderate determinant of FI. We also examined how FI was altered within each animal during the transition from LFD to HFD (Figure 2A). FI after 1 week of HFD was subtracted by the LFD FI that was measured 1 week prior. Introduction of the 45% HFD increased FI in both strains ($P < 0.05$). Interestingly, introduction of the 60% HFD resulted in a similar increase in FI in the LCR, while the HCR displayed a dramatic reduction in FI following transition to the 60% HFD ($P < 0.05$).

Because HFDs have different energy density than LFD, it is important to additionally examine EI. Both HFDs resulted in increased EI compared with the LFD condition within both strains (Table 1, $P < 0.05$). While no difference was observed between HFD in HCR, the LCR rats on the 60% HFD had a 10% higher EI compared with the 45% HFD ($P < 0.05$). HCR rats had lower EI compared with LCR, effects primarily driven by the 24% and 31% lower EI in the HCR on the 45% and 60% HFD, respectively. There was a significant interaction for EI as a result of the LCR significantly increasing EI to a greater degree on both of the HFDs compared with the HCR ($P < 0.05$). ANCOVA analysis of EI once again diminished differences between the strains (Table 1, $\eta^2 \sim 0.366$). However, the weight-adjusted mean EI of LFD for the HCR rats was higher compared with LCR ($P < 0.05$). Both HFDs resulted in greater weight-adjusted EI compared with LFD in HCR and LCR rats ($P < 0.05$). Change in EI (Figure 2B) was lower in HCR rats compared with LCR ($P < 0.05$), with both HFDs being higher than LFD across both strains ($P < 0.05$). The divergent change in FI observed on transition to the 60% HFD resulted in a smaller increase in EI in the HCR compared with the 45% HFD, while the LCR demonstrated a significantly larger increase in EI compared with the 45% HFD.

Together these data suggest that the HCR rats have greater adaptability than the LCR for lowering FI when provided with an energy-dense HFD, a trait that significantly influences HFD-induced weight gain.

Relationship between FI/EI and weight gain

Regression analysis of the body weight gain versus change in FI for the HCR and LCR rats is displayed in Figure 3A. The dynamic differences in FI and lower weight gain changes result in no observed correlation within the HCR, while the LCR rats demonstrate a significant association ($P < 0.0001$, $r^2 = 0.545$). As previously observed (24), change in EI was highly associated with change in body weight across strains and diets ($P < 0.0001$, $r^2 = 0.751$, $y = 0.094x + 6.134$). Figure 3B represents the within-strain association of body weight gain versus change in EI across all three diets, with both HCR and LCR rats having significant associations ($P < 0.0001$, $r^2 = 0.403$, $y = 0.049x + 6.203$; $P < 0.0001$, $r^2 = 0.822$, $y = 0.101x + 6.822$, respectively). Together these data suggest a stronger association between FI/EI and weight gain in the LCR than in the HCR rats.

Feeding efficiency

Feeding efficiency (FE) is displayed in Figure 2C and represents a comparison of weight gained per kcal consumed during the 1-week HFDs. HCR rats displayed ~50% lower FE than LCR across all diets ($P < 0.05$). The 45% HFD increased FE in both HCR and LCR rats (70% and 2.2 \times , respectively, $P < 0.05$) compared with LFD. The 60% HFD produced greater FE in HCR and LCR rats than LFD (2.8 \times and 3.0, respectively, $P < 0.05$) and 45% HFD conditions (62% and 36%, respectively, $P < 0.05$). While the adjustment of FE for final body weight by ANCOVA was significant (Figure 2D, $P < 0.05$), the relationships are similar to the absolute data and the very small partial η^2 (~0.043) suggests that body weight differences were not responsible for the observed differences in FE. The large differences in FE between HCR and LCR rats across diets suggest that additional factors such as previously described differences in weight-adjusted resting energy expenditure (REE) (18) and metabolic efficiency (21), impact the observed association between intrinsic aerobic capacity and HFD-induced weight gain.

Adipose mass, morphometrics, and gene expression

Adipose depot weights and retroperitoneal adipose tissue morphometric analysis are shown in Figure 4. As expected, epididymal and retroperitoneal adipose mass was less in HCR rats compared with LCR (Figure 4A, B, $P < 0.05$). The LCR rats increased epididymal and retroperitoneal adipose mass following HFD, while HCR on HFDs was not different from LFD. Adipocyte cell size in the retroperitoneal fat pad was smaller in HCR compared with LCR. (Figure 4C, E, $P < 0.05$). Only the LCR demonstrated an increase following HFD, with only the 45% HFD reaching significance. Further, HCR had more adipocytes per frame compared with LCR (Figure 4D, E, $P < 0.05$), but this was not altered by HFD in either strain.

The previously described weight-adjusted differences in REE (18) and the observed differences in FE and weight gain could be due in part to differences in BAT mass-dependent thermogenesis. We observed no differences in BAT mass between strains, and only the

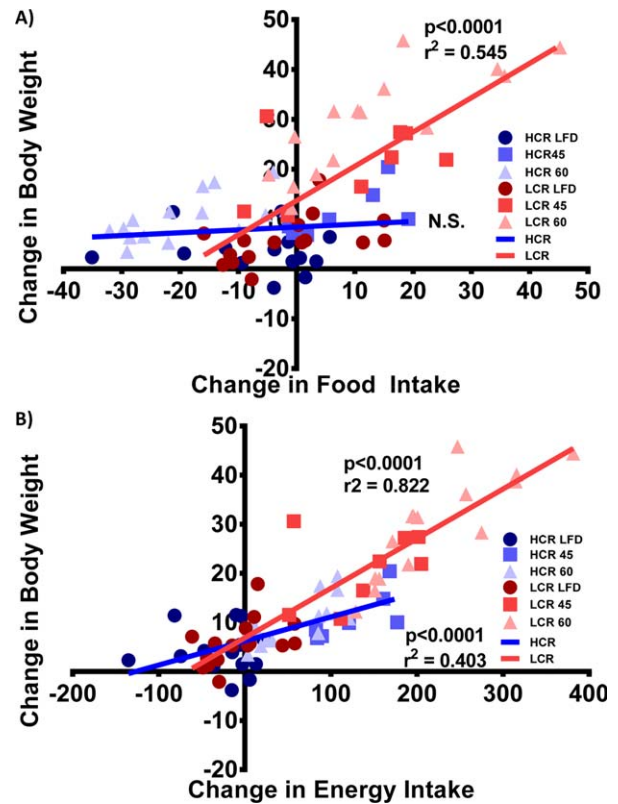
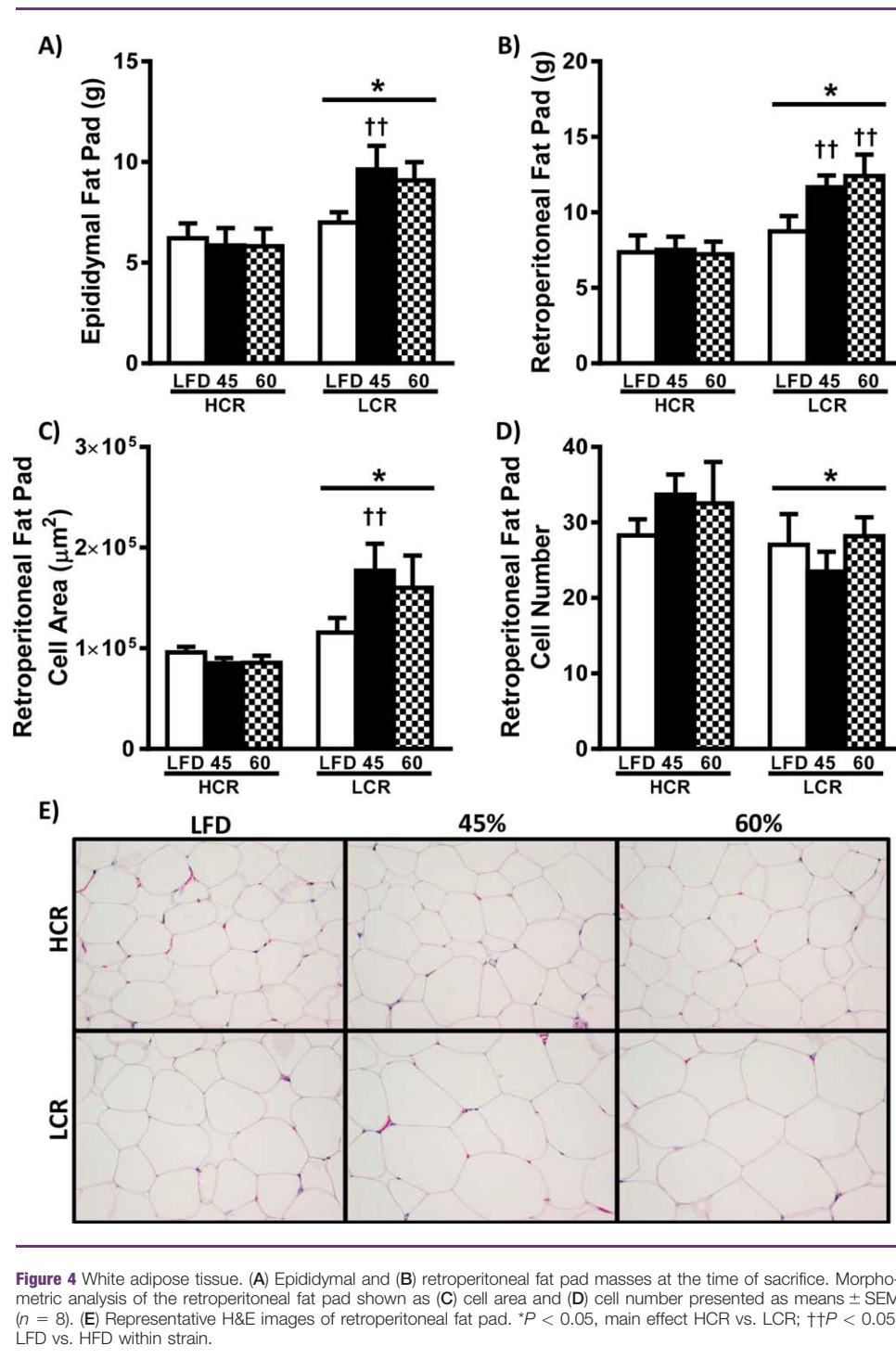


Figure 3 Change in food/energy intake vs. weight gain. Linear regression of 1-week body weight gain vs. (A) change in food intake and (B) change in energy intake (blue = HCR, red = LCR, LFD = •, 45% = ■, 60% = ▲) presented with P value and r^2 when appropriate.

LCR increased BAT mass in response to both HFDs (Figure 5B, $P < 0.05$). However, body weight-adjusted BAT mass was greater in HCR compared with LCR regardless of diet (Figure 5E, $P < 0.05$). Previously, our group has observed greater BAT mass as a percentage of body weight and reduced weight gain in female HCR rats compared with LCR following ovariectomy (20).

To evaluate whether these weight-adjusted differences in BAT were associated with potential functional changes, we assessed tissue H&E and the expression of genes relevant to nonshivering thermogenesis and mitochondrial content. In Figure 5A, representative H&E images suggest that BAT of HCR rats have increased locularity and mitochondrial content compared with LCR. The mRNA expression of mitochondrial biogenesis transcriptional activator, peroxisome proliferator-activated receptor γ coactivator-1 α (PGC-1 α), was higher in the HCR compared with LCR rats (Figure 5C, $P < 0.05$), with significant changes occurring due to both HFDs compared with LFD ($P < 0.05$). Citrate synthase activity, a marker of mitochondrial content, was increased in BAT homogenate from HCR rats compared with LCR (Figure 5F, $P < 0.05$), with reductions due to HFD in both strains. Uncoupling protein-1 (UCP1) mRNA expression was greater in HCR rats compared with LCR ($P < 0.05$), with no diet effects (Figure 5B). Importantly, the reduced UCP1 mRNA expression was associated with increased UCP1 protein expression in HCR BAT compared with LCR rats (Figure 5G,



$P < 0.05$). Interestingly, the observed differences in PGC-1 α mRNA did not produce similar differences in protein expression (Supporting Information Figure 1). Additionally, expression of certain genes involved in FAO, BAT activation, and lipid storage were differentially expressed across the strains (Supporting Information Figure 1). Collectively these data suggest that the observed increase in weight-adjusted REE in the HCR may due to greater nonshivering thermogenesis (18), which may help protect against the more dramatic acute HFD-induced weight gain observed in the LCR.

Discussion

Aerobic capacity is arguably the most powerful predictor of all-cause mortality (14), but its impact on susceptibility for weight gain and obesity is relatively unknown. Herein we report that HCR rats bred for high intrinsic aerobic capacity display reduced susceptibility for HFD-induced acute weight gain compared with LCR rats bred for low intrinsic aerobic capacity. The protective phenotype in the HCR rats is marked by reduced FI/EI and FE. Moreover, the

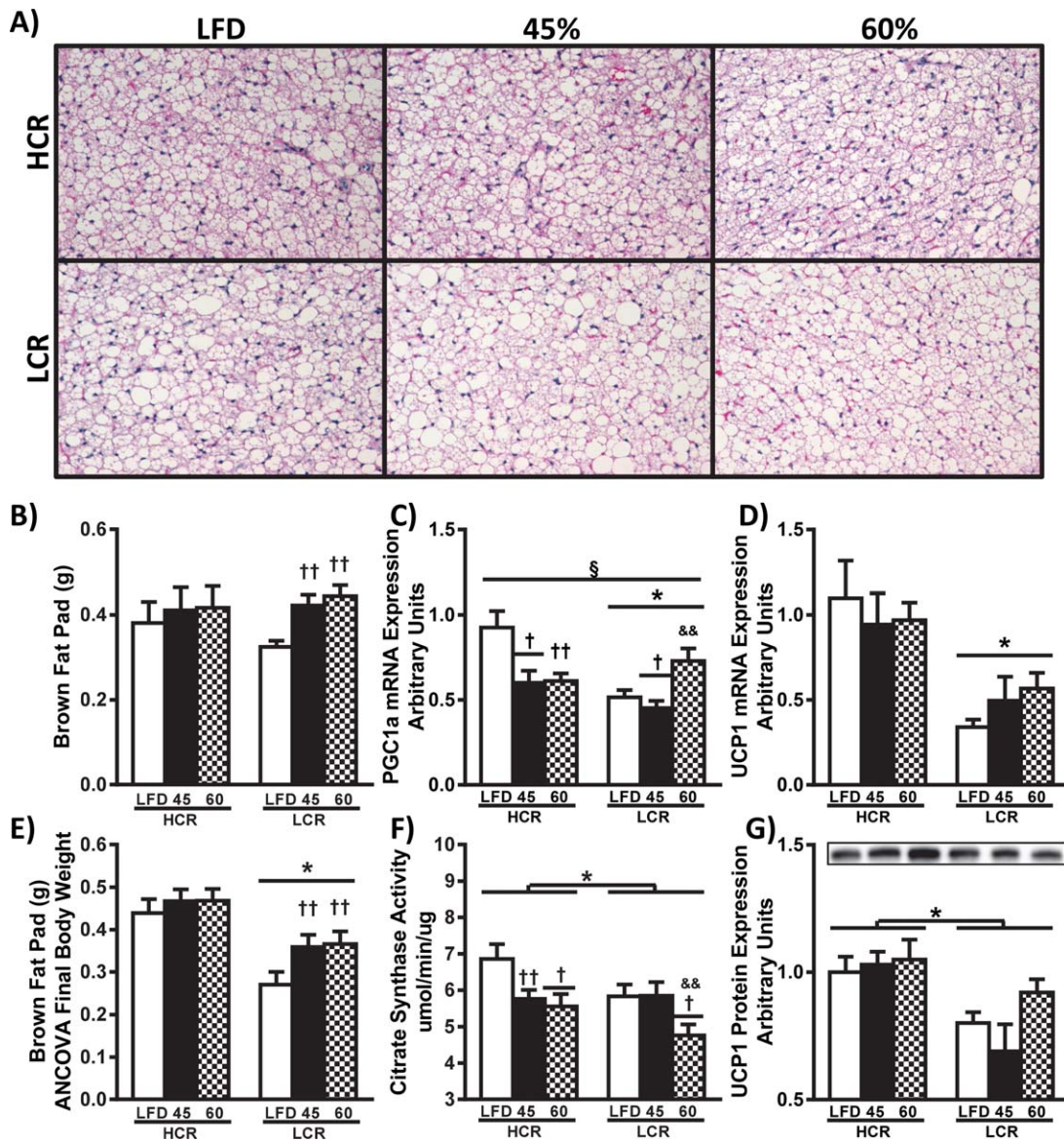


Figure 5 (A) Representative H&E images of brown adipose tissue. (B) Fat pad mass was determined at sacrifice and estimated mass of brown fat pad adjusted for final body weight (ANCOVA) is shown in (E). Gene expression in brown adipose was determined by RT-PCR and Western blot analysis: relative mRNA expression of (C) UCP1 and (D) PGC1α were normalized to *CycA*, and Western blot analysis of brown adipose homogenate for (F) UCP1. Brown adipose mitochondrial content was assessed by (G) citrate synthase activity. All data presented as means ± SEM ($n = 8$). $§P < 0.05$, interaction; $*P < 0.05$, main effect HCR vs. LCR; $†P < 0.05$, main effect LFD vs. HFD; $††P < 0.05$, LFD vs. HFD within strain; $\&\&P < 0.05$, 45% HFD vs. 60% HFD within strain.

reduced FE in the HCR rats may be due to a greater weight-adjusted brown adipose mass that also display higher mitochondrial content and higher expression of thermogenic genes and proteins (18).

In sedentary mature *ad libitum* fed rodents, acute HFD-induced weight gain is driven by increased FI/EI. Increased voluntary FI/EI of HFDs in both rodents and humans (25,26) is influenced by a complex interplay of metabolic and hedonic factors (5-8). In agreement with previous studies using rats that are obesity prone/resistant (24,27), the HCR/LCR HFD-induced change in EI strongly associated with subsequent weight gain. EI is a function of both the

energy density and quantity of the food consumed, with greater energy density of HFDs resulting in increased weight gain in both humans (28) and rodents (29). In this study, the HCR maintained or reduced FI following transition to the HFDs, while the LCR consumed more food despite the higher energy density of the HFDs. All told, the smaller weight gain in the HCR rats following transition to the HFDs is partially due to avoiding overconsumption.

To date, we are aware of no detailed studies that have solely focused on hedonic pathways driving FI/EI in the HCR/LCR. However, a recent study examining the interaction between hunger and

environmental novelty on FI found that the LCR consumed food (normal chow) more quickly after a 24-h fast in a novel environment than the HCR (30). This suggests that the drive for the LCR to re-acquire dietary energy is more powerful than the need to explore or be vigilant to potential threats in a new environment. These results in the LCR were associated with differential expression of CART in the nucleus accumbens and higher circulating levels of leptin and ghrelin. In this study, we explored a different paradigm, changes in FI/EI after the transition from a low fat to a more palatable and energy-dense HFD. The differences in FI/EI following a transition to HFD in the HCR/LCR model could be due to hepatic energy sensing and vagal afferent pathways mediating control in the CNS (18), a hypothesis originally tested separately by Friedman (31) and Langhans (32). These groups showed that reductions in hepatic fat oxidation lead to greater FI. We have shown repeatedly that the LCR have reduced hepatic mitochondrial content and fat oxidation compared with the HCR. Additional potential metabolic predictors of weight gain in human subjects have included low REE, low whole-body fatty acid oxidation capacity (FAO), and metabolic inflexibility (3,5), all characteristics that we show to occur in the LCR rats compared with HCR (18). The numerous combinations of potential metabolic modulators of differences in EI and weight gain between the HCR and LCR rats underscores the difficulty of dissecting out any individual causal factors; however, it emphasizes that the polygenetic nature of selection for divergent aerobic capacity has resulted in multifactorial components that underlie their susceptibility for HFD-induced weight gain. Importantly, other factors that control EI, such as hedonic control, as well as, hormonal and satiety feedback signaling cannot be ignored (5,7), but are beyond the scope of this article.

As previously stated, susceptibility for HFD-induced weight gain has been ascribed to low metabolic rate (8), decreased fat oxidation (33), reduced spontaneous activity (8), low sympathetic nervous system activity, and increased storage of dietary fat (34). In this study, we observed the HCR rats had lower FE compared with LCR within all diets. The decreased FE of the HCR rats could be due to greater metabolic flexibility as suggested by the previously reported lower RQ on HFD compared with LCR (18,22). Importantly, in the same study HCR rats have higher whole-body FAO on LFD compared with LCR (18). The higher whole-body FAO in the HCR over the LCR is likely due to the greater skeletal muscle and hepatic mitochondrial content and FAO capacity witnessed in the HCR (17,19,35). In humans, subjects classified as high fat oxidizers had lower weight gain compared with low fat oxidizers, which was independent of 24 h metabolic rate (36). Further, maintenance of weight loss in subjects with previous obesity is associated with higher FAO rates compared with those who regained weight (37). These data suggest that the observed reduction in FE of HCR rats on HFD is due in part to increased adaptability to utilize fat for energy production. However, other factors that could impact FE such as increased tone of sympathetic nervous system (21), higher REE, and cage activity (18-20,22) have also been observed in HCR compared with LCR rats.

REE represents ~75% of total energy expenditure in sedentary, caged rodents, and thus significantly impacts energy balance following a transition to a HFD. The HCR display higher weight-adjusted REE on LFD and following 3 days of HFD (18,20). Elevated BAT thermogenesis produces an increase in REE, and results in improvements in numerous metabolic disease states (38). Recent studies suggest that increased BAT thermogenesis attenuates HFD-induced weight gain

(39,40). Following body weight adjustment, the HCR display greater BAT regardless of diet. This data are in agreement with our previous finding that female HCR rats display greater BAT (per body weight) which correlated with higher REE, and protection from weight gain following ovariectomy (20). In this study, we observed HCR BAT was more multilocular, had greater mitochondrial content, and altered expression of several genes and proteins putatively involved in BAT thermogenesis between HCR and LCR rats. Classic markers of increased BAT thermogenesis, PGC-1 α and UCP-1, were higher in HCR rats compared with LCR. These data suggest that high intrinsic aerobic capacity results in a phenotype of greater weight-adjusted BAT mass and possibly enhanced BAT thermogenesis, as evidenced by greater mitochondrial content and UCP-1 protein, which could potentially explain the observed higher REE reported in our previous studies (18,20). More studies are needed to validate the role of BAT in the HCR phenotype.

In summary, we demonstrate that selection for high intrinsic aerobic capacity results in a phenotype of reduced 1-week weight gain following initiation of both a 45% and a 60% HFD. The protection against weight gain in the HCR is linked to a greater adaptability to control FI upon transition to the HFDs, reduced FE, and possibly increased BAT thermogenesis. In contrast, the LCR display greater acute HFD-induced weight gain, putatively due to an inability to adapt FI/EI after transition to HFDs, augmented FE, and reduced BAT size and thermogenic gene expression. These data provide insight into potential mechanisms by which intrinsic aerobic capacity impacts weight gain, factors which may have particular importance in our obesogenic environment in which energy is available in overabundance. **O**

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