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Differential weight loss with intermittent fasting or daily calorie restriction in low- and high-fitness phenotypes

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Abstract

Recent interest has focused on the benefits of time-restricted feeding strategies, including intermittent fasting, for weight loss. It is not yet known whether intermittent fasting is more effective than daily caloric restriction at stimulating weight loss and how each is subject to individual differences. Here, rat models of leanness and obesity, artificially selected for intrinsically high (HCR) and low (LCR) aerobic capacity, were subjected to intermittent fasting and 50% calorie restrictive diets in two separate experiments using male rats. The lean, high-fitness HCR and obesity-prone, low-fitness LCR rats underwent 50% caloric restriction while body weight and composition were monitored. The low-fitness LCR rats were better able to retain lean mass than the high-fitness HCR rats, without significantly different proportional loss of weight or fat. In a separate experiment using intermittent fasting in male HCR and LCR rats, alternate-day fasting induced significantly greater loss of weight and fat mass in LCR compared with HCR rats, although the HCR rats had a more marked reduction in *ad libitum* daily food intake. Altogether, this suggests that intermittent fasting is an effective weight-loss strategy for those with low intrinsic aerobic fitness; however, direct comparison of caloric restriction and intermittent fasting is warranted to determine any differential effects on energy expenditure in lean and obesity-prone phenotypes.

KEYWORDS
adiposity, alternate-day fasting, body composition, high- and low-capacity runners, intrinsic aerobic capacity, male, rat

1 | INTRODUCTION

With an increasing prevalence of obesity comes a heightened risk for the development of chronic health sequelae, including metabolic diseases, cardiovascular diseases, diabetes and cancer (Haslam & James, 2005; Withrow & Alter, 2011). Most weight-loss diet regimens, such as caloric restriction (CR), rely on limiting daily food intake, whereas time-restricted feeding and intermittent fasting (IF) limit the timing or duration of food availability (Hoddy et al., 2020). A common challenge with diet-induced weight loss is that the reduced weight is often difficult to maintain (Redman et al., 2009; Yamada et al., 2013). This is because reduced weight and negative energy balance stemming from food restriction suppress metabolic rate and physical activity (Martins et al., 2020; Redman et al., 2009; Yamada et al., 2013). For metabolic rate, energy expenditure is proportional to body mass; a decrease in weight is mirrored by a decrease in energy expenditure (Most & Redman, 2020). Metabolic adaptation occurs when energy expenditure is decreased below what is predicted for the reduced body mass, also known as adaptive thermogenesis (Dulloo et al., 2012; Müller & Bosy-Westphal, 2013; Rosenbaum & Leibel, 2010). This

adaptation might be determined by the degree of negative energy balance (Most & Redman, 2020).

Aerobic capacity is well recognized as a strong predictor of metabolic health (Goran et al., 2000; Ladenvall et al., 2016; Timmons et al., 2010). Yet the challenge remains of how to promote weight loss on a background of low aerobic fitness. To investigate this challenge, we studied the lean, high-capacity runner (HCR) and the obesity-prone, low-capacity runner (LCR) rats that contrast for the fitness phenotypes of high and low intrinsic aerobic exercise capacity (Koch & Britton, 2018). These rat models were developed by a two-way artificial selection on maximal treadmill running capacity within a large founder population of genetically heterogeneous rats (referred to using the nomenclature N:NIH) (Hansen & Spuhler, 1984; Koch & Britton, 2018). These contrasting rat models serve as a platform to investigate segregating differences in both behaviour and physiology that lead to a susceptibility or resistence to the development of obesity and a differential response to weight-loss strategies.

Here, we examine these rat models of leanness and obesity to observe intrinsic differences in response to IF and CR dietary regimens. Previously, Smyers et al. (2015) reported that HCR rats ate more, were more physically active and lost more weight relative to baseline body mass compared with LCR rats during 50% CR. This held true for both the males and the females, in which body weight was more comparable between the LCR and HCR. When examining body composition during daily 50% kcal restriction, female HCR rats lost more fat and lean mass than LCR rats during CR even with similar baseline body weights. In males, HCR rats also lost more weight as a proportion of their baseline body weight compared with LCR rats.

The HCR and LCR response to daily CR differed markedly from the response to IF (Smyers et al., 2020). Over 14 weeks of IF, LCR rats lost more weight than HCR, in contrast to the response to 50% daily CR (Smyers et al., 2015). The HCR rats were still more physically active than LCR on both IF and 50% CR (Smyers et al., 2020). Continued IF maintained changes in body weight, body composition and physical activity that persisted after 1 year (Smyers et al., 2020). Although intriguing, the ability of IF to induce marked weight loss in rats with low fitness compared with high fitness was investigated only in females. Given that male HCR and LCR rats are larger than females and that male HCR and LCR rats exhibit a larger divergence in body weight and composition than that seen in females, the response to IF needs to be investigated in male HCR and LCR rats to determine whether the magnitude of weight loss on IF is characteristic of the low-fitness phenotype in general. In the present study, the temporal pattern of weight and body composition changes was investigated in males using 50% CR, and using IF in a separate group of HCR and LCR rats.

New Findings

- **What is the central question of this study?**
  How does intrinsic aerobic capacity impact weight loss with 50% daily caloric restriction and alternate-day fasting?
- **What is the main finding and its importance?**
  Intermittent fasting is effective for weight loss in rats with low fitness, which highlights the importance of how intermittent fasting interacts with aerobic fitness.

2 | METHODS

2.1 | Ethical approval

All research adhered to the principles of the laboratory animal care guidelines and was approved by the Kent State University Institutional Animal Care and Use Committee and performed in accordance with the *Guide for the Care and Use and Laboratory Animals* (8th Ed, 2011, National Institutes of Health, Bethesda, MD, USA). All research complied with the ethical principles and standards for reporting animal experiments in *Experimental Physiology*.

2.2 | Diet

Water was available *ad libitum*, and food (5P00 MRH 3000; T R Last, Cabot, PA, USA) was available *ad libitum* except during caloric restriction and intermittent fasting. Prolab RMH 3000 chow pellets are composed of 26% protein, 14% porcine and plant oil fat and 60% carbohydrate, with a physiological fuel value of 3.46 kcal/g.

2.3 | Animals

Male HCR and LCR rats were developed by and transferred from the University of Michigan. Two experiments were performed on individually housed rats. The first study included 48 rats that were aged 361 ± 39 days [mean ± SD; generation 27; HCR (n = 24); LCR (n = 24)]. These rats were subjected to either 2 or 21 days of 50% CR or were part of the control group fed *ad libitum*. At phenotyping (treadmill running endurance tests at 3–4 months of age), HCR rats had significantly longer maximal running time (HCR, 77.7 ± 7.7 min; LCR, 15.3 ± 1.9 min), maximal running distance (HCR, 2263.6 ± 373.4 m; LCR, 205.1 ± 30.6 m), top speed attained (HCR, 48.5 ± 3.8 m/min; LCR, 17.1 ± 0.9 m/min) and work performed (HCR, 1377.3 ± 153.6 J; LCR, 177.0 ± 30.9 J). Within phenotype, there were no group differences except that the LCR rats later subjected to 2 days of 50% CR showed marginally lower work than the other groups of LCR (156 compared with 183–192 J; Student's two-tailed t-tests). At the end of 50% CR, animals were killed in the middle of the light phase by rapid decapitation; rats were briefly restrained and held by wrapping them in a clean medical towel and then quickly decapitated using a clean, sharpened, large decapitator (Harvard Apparatus, Holliston, MA, USA).The investigator carrying out

this procedure was experienced in rapid decapitation of rats; prior anaesthesia was not used as this would interfere with brain gene expression.

The second study examined weight loss on IF in 16 male rats aged 384 ± 25 days [generation 37; HCR (n = 8); LCR (n = 8)]. For this study, these rats remained on IF for 7 weeks and served as their own control to measure changes in body weight and body composition between HCR and LCR. At phenotyping, HCR rats had significantly longer maximal running time (HCR, 71.7 ± 2.0 min; LCR, 11.7 ± 2.7 min), maximal running distance (HCR, 1968.2 ± 88.4 m; LCR, 147.2 ± 41.9 m), top speed attained (HCR, 45.4 ± 1.5 m/min; LCR, 15.3 ± 1.5 m/min) and work performed (HCR, 1295.6 ± 101.2 J; LCR, 127.0 ± 38.1 J). Rats subjected to IF were later euthanized with CO_2.

A 12 h–12 h light–dark cycle was maintained during both studies, with the light phase starting at 07.00 EST, at 21.7 ± 0.5° (SD) for 50% CR and 23.1 ± 0.9 °C for IF, and the humidity was between 30 and 55% for IF and CR. Water was provided to each rat *ad libitum* throughout the duration of both studies. Daily food intake and body weight were measured for 1 week before the onset of CR or IF. After a week of baseline measurement, the rats on 21 and 2 days of 50% CR were fed *ad libitum* until they began CR. During baseline measurements and during CR, food intake and body weights were measured between 10.00 and 11.00 h (i.e., 4–5 h after lights-on). For IF, after 1 week of baseline measurement, all the rats on IF were subjected to alternate-day fasting and were fed *ad libitum* every other day, with no food given on the alternating fasting days for 7 weeks. During this study, each rat had two separate cages, one for fasting days and one for feeding days; SSP Alpha-dri bedding (T R Last) was used to facilitate identification of any leftover food to be measured. As with the baseline measurements, during alternate-day fasting the food intake and body weight were measured at 18.30 h (i.e., 30 min before the onset of the active phase). After the end of the experiments, the IF rats were killed by exposure to a rising concentration of carbon dioxide.

2.4 | Body composition analysis using EchoMRI

To measure body composition, rats underwent magnetic resonance spectroscopy (EchoMRI-700; EchoMRI, Houston, TX) at baseline and after CR (Nixon et al., 2010). For rats on IF, body composition was measured weekly for 8 weeks to quantify lean and fat mass (each in grams). This was done at the same time and day each week, resulting in alternating fasting and feeding days.

2.5 | Cytokines and qPCR analysis

After the conclusion of the study, during in the light phase, rats subjected to 50% CR and their control counterparts were killed by rapid decapitation and trunk blood was collected. Serum levels of cytokines interleukin (IL)-1β, IL-1α, tumor necrosis factor-α (TNF-α), IL-6, IL-10 and leptin were determined by the CTSC Bioanalyte Core Center at Case Western Reserve University using the Luminex xMAP multiplexing ELISA system, as described previously (Almundarij et al., 2016).

2.6 | Statistical analysis

RStudio (RStudio IDE Desktop Open Source Edition) software was used to analyse the data collected for both the CR study and the IF study, and SPSS (IBM SPSS Statistics) was used to analyse the data from multiplex analysis. ANOVA was used to compare body weight and body composition within and between lines. For cytokine analysis, outliers were identified using the outlier labelling rule (calculated as 2.2 × the upper and lower quartiles). Repeated-measures ANOVAs were used to compare food intake, body weight, fat mass and lean mass between HCR and LCR rats over 7 weeks of IF. A two-way repeated-measures ANOVA was used to compare baseline and final values for body weight on fasted and non-fasted days and to compare food intake between HCR and LCR rats over time. Upon analysis of food intake and body weight using two-way repeated-measures ANOVAs, sphericity was violated and corrected for the main effect of time using the Huynh–Feldt correction. Two-way ANOVAs were used to compare fed versus fasted values between HCR and LCR rats for weight loss, fat loss and lean mass loss. Tukey's HSD *post hoc* test was used when making comparisons between groups, and Student's *t*-tests were used after repeated-measures analysis. Student's unpaired two-tailed *t*-tests were used compare weekly body weight, fat mass and lean mass between HCR and LCR rats. Analysis of covariance (ANCOVA) was used to determine whether baseline fat mass (covariate) affected weight and fat loss and to determine whether the overall change in food intake (covariate) affected weight loss. Effect sizes (Cohen's *d*) were calculated for the CR- and IF-fed rats. Data are represented as the mean ± SD.

3 | RESULTS

3.1 | Body composition and circulating cytokines

The ability of CR to induce weight loss and alter body composition was compared between male HCR and LCR rats. The HCR and LCR animals differed significantly in baseline body weight, fat mass and lean mass before and after the onset of CR, such that LCR rats had higher baseline body weight, fat mass and lean mass, consistent with prior studies (Smyers et al., 2015; Table 1). When analysing the body composition from 2 days of 50% CR, there were no significant difference in the loss of body weight or fat mass (in grams) from baseline between HCR and LCR animals (Table 1). The HCR rats, however, lost significantly more lean mass from baseline than LCR rats on 2 days of CR (P = 0.003; Table 1). Two days of continuous energy restriction did not allow enough time for noticeable changes between the two phenotypes in body weight or fat mass, in contrast to lean mass.

Although the absolute weight loss (in grams) approached significance, the LCR showed only a trend toward more body weight

TABLE 1 Body weight and composition before and after 2 or 22 days of 50% caloric restriction (CR) or *ad libitum* (control) intake in male high- and low-capacity runner (HCR and LCR) rats

	Baseline			Calorie restriction			Change from baseline		
Group	Body weight (g)	Fat mass (g)	Lean mass (g)	Body weight (g)	Fat mass (g)	Lean mass (g)	Body weight (g)	Fat mass (g)	Lean mass (g)
Control intake									
HCR	395.88 ± 30.09	54.13 ± 17.03	286.90 ± 14.18	401.88 ± 32.47	55.23 ± 16.03	289.36 ± 16.17	6.00 ± 9.65	1.11 ± 5.71	2.49 ± 6.85
LCR	563.25 ± 46.62	143.26 ± 35.98	345.30 ± 10.66	570.38 ± 50.76	144.39 ± 39.74	350.63 ± 10.77	7.13 ± 16.02	1.13 ± 11.54	5.36 ± 3.99
HCR ≠ LCR (*P*-value)	< **0.001**	< **0.001**	< **0.001**	< **0.001**	< **0.001**	< **0.001**	0.532	0.996	0.323
2 days at 50% CR									
HCR	386.94 ± 34.12	49.95 ± 14.42	281.70 ± 29.24	373.50 ± 31.38	52.33 ± 16.33	272.70 ± 28.58	−13.44 ± 5.68	2.38 ± 4.52	−8.95 ± 4.16
LCR	538.94 ± 40.16	127.32 ± 18.86	340.80 ± 30.0	527.06 ± 36.70	122.65 ± 21.47	338.97 ± 27.92	−11.88 ± 10.86	−4.67 ± 9.18	−1.83 ± 3.65
HCR ≠ LCR (*P*-value)	< **0.001**	< **0.001**	**0.001**	< **0.001**	< **0.001**	< **0.001**	0.480	0.072	**0.003**
21 days at 50% CR									
HCR	411.13 ± 45.53	55.47 ± 21.91	298.60 ± 27.0	321.31 ± 38.93	24.87 ± 15.48	243.50 ± 23.60	−89.81 ± 22.67	−30.60 ± 15.53	−55.12 ± 14.59
LCR	604.31 ± 55.75	160.82 ± 35.82	368.00 ± 33.86	493.38 ± 45.49	96.85 ± 31.80	325.75 ± 27.33	−110.94 ± 17.82	−63.96 ± 12.05	−42.30 ± 12.01
HCR ≠ LCR (*P*-value)	< **0.001**	< **0.001**	< **0.001**	< **0.001**	< **0.001**	< **0.001**	0.051	< **0.001**	0.076

Values are the mean ± SD. Body weight was assessed at the time of measurement of body composition. Control intake represents rats that ate *ad libitum* for 21 days. Effect size (Cohen's *d*) between lean mass loss in HCR and LCR rats for 2 days at 50% CR was $d = 2.55$, and fat loss for 21 days at 50% CR was $d = 1.82$. Significant differences (*P*-values shown in bold) were found between HCR and LCR rats at baseline, after CR, and in the number of grams lost or gained (Student's two-tailed *t*-test).

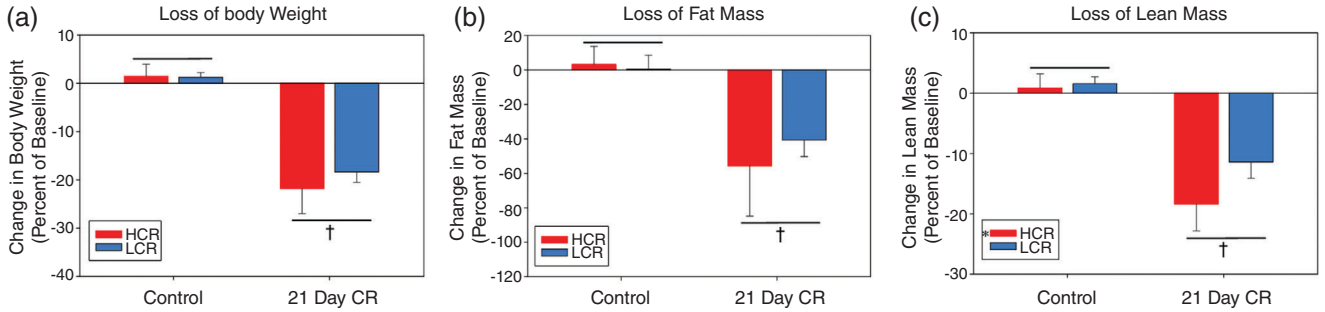

FIGURE 1 Body weight and composition in male rat high- (HCR) and low-capacity runners (LCR) subjected to 21 days of 50% caloric restriction (CR), which induced a significant loss of body weight (a), fat mass (b) and lean mass (c). There was significantly greater loss of lean mass in HCR than LCR rats subjected to 50% CR and a non-significant trend for greater weight loss in HCR than in LCR rats ($P = 0.051$). Values are the mean ± SD. †Significant ($P < 0.05$) loss in rats subjected to 21 days of 50% CR compared with control rats fed *ad libitum*; *(in key) HCR rats significantly different ($P < 0.05$) from LCR rats during 21 days of CR

TABLE 2 Circulating cytokine levels in high-capacity runner (HCR) and low-capacity runner (LCR) rats on *ad libitum* (control) food intake or subjected to 50% caloric restriction (CR) for 2 or 21 days

		Cytokine (pg/ml)					
Group		Leptin	Interleukin-1α	Interleukin-1β	Interleukin-6	Interleukin-10	Tumor necrosis factor-α
Control intake	HCR	9,851 ± 3,136	35.9 ± 14.95	55.4 ± 25.04	31.9 ± 8.51	54.7 ± 23.16	20.5 ± 5.91
	LCR	13,003 ± 2,147	40.7 ± 11.35	60.0 ± 19.68	32.5 ± 7.61	64.4 ± 11.58	20.9 ± 3.19
2 days of CR	HCR	8,075 ± 4,417	28.6 ± 2.57	32.6 ± 9.99	28.5 ± 5.01	36.8 ± 7.79	17.5 ± 2.19
	LCR	11,679 ± 3,970	33.2 ± 12.91	89.5 ± 71.96	29.6 ± 9.07	75.2 ± 41.89	18.5 ± 4.79
21 days of CR	HCR	3,556 ± 2,696	30.8 ± 5.63	45.6 ± 25.73	25.6 ± 3.45	42.6 ± 20.08	18.6 ± 2.20
	LCR	7,988 ± 3,553	36.9 ± 14.21	52.3 ± 27.87	26.8 ± 4.36	50.4 ± 15.41	19.7 ± 3.08
Main effect	diet	$P < 0.01$	n.s.	n.s.	n.s. ($P = 0.051$)	n.s.	n.s.
	line	$P < 0.01$ (LCR > HCR)	n.s.	$P = 0.043$ (LCR > HCR)	n.s.	$P = 0.011$ (LCR > HCR)	n.s.
Interaction		n.s	n.s.	n.s.	n.s.	n.s.	n.s.

Values are the mean ± SD. n.s., not significant.

loss ($p = 0.051$; Table 1), and the HCR showed a trend toward more lean mass loss ($p = 0.076$; Table 1) after 3 weeks of 50% CR, despite the LCRs much higher baseline body weight and lean mass ($P = 0.051$; Table 1). Consistent with prior experiments (Smyers et al., 2015, 2020), HCR rats were less able to maintain lean mass when subjected to food restriction (Figure 1c). After 21 days of 50% CR, LCR rats lost significantly more fat mass from baseline than HCR rats ($P < 0.001$; Table 1). Compared with control rats, rats subjected to 50% CR for 21 days lost a greater proportion of baseline body weight (Figure 1a), fat mass (Figure 1b), and lean mass (Figure 1c). A total of 21 days of 50% CR induced a significantly greater proportional loss of baseline lean mass in HCR animals ($P = 0.001$); the proportion of weight loss and fat loss, however, did not reach significance between the two phenotypes (Figure 1). Compared with HCR animals, LCR rats were more resistant to the loss of lean mass and lost significantly more grams of fat mass from baseline than HCR rats after 3 weeks of 50% CR.

At baseline, HCR rats later subjected to 3 weeks of 50% CR ate 22.63 ± 1.65 g of chow and LCR rats 21.20 ± 2.06 g; HCR rats selected for 2 days of 50% CR consumed 21.47 ± 1.85 g and LCR rats ate 20.27 ± 1.28 g. Upon examination of daily food intake on days 2 and 21 of 50% CR, analysis revealed no significant difference in food intake between HCR and LCR animals. Over 21 days of 50% CR, HCR rats 10.04 ± 0.83 g and LCR rats ate 10.01 ± 0.83 g of chow per day. Likewise, during 2 days of 50% CR, HCR rats ate 10.07 ± 0.95 g and LCR rats 9.87 ± 0.75 g of chow per day. Before and after 50% CR in rats subjected to either 2 or 21 days of CR, there was no significant difference in food intake between HCR and LCR animals.

Plasma leptin was significantly different between HCR and LCR rats and between rats on control (*ad libitum*) feeding, 2 and 21 days of CR. The 50% CR significantly decreased leptin levels, and LCR rats had significantly higher leptin levels overall (Table 2). Analysis of serum levels of the circulating cytokines IL-1β and IL-10 showed a significant main effect of phenotype, with no main effect of food restriction and

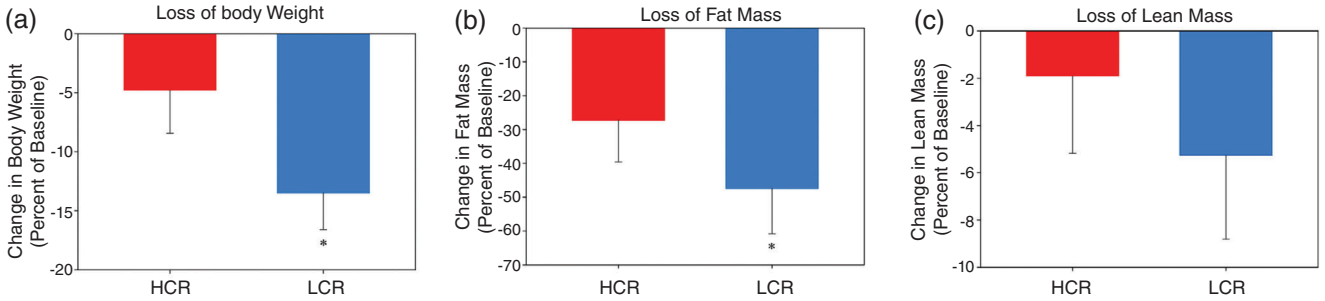

FIGURE 2 Body weight and composition in male high- (HCR) and low-capacity runner (LCR) rats subjected to 7 weeks of intermittent fasting (IF). (a,b) The LCR rats lost significantly more of their baseline body weight (a) and fat mass (b) than HCR rats on IF. (c) There was no significant difference in the percentage of lean mass lost from baseline between HCR and LCR rats. Values are the mean ± SD. *Significantly ($P < 0.05$) more loss in LCR than in HCR rats

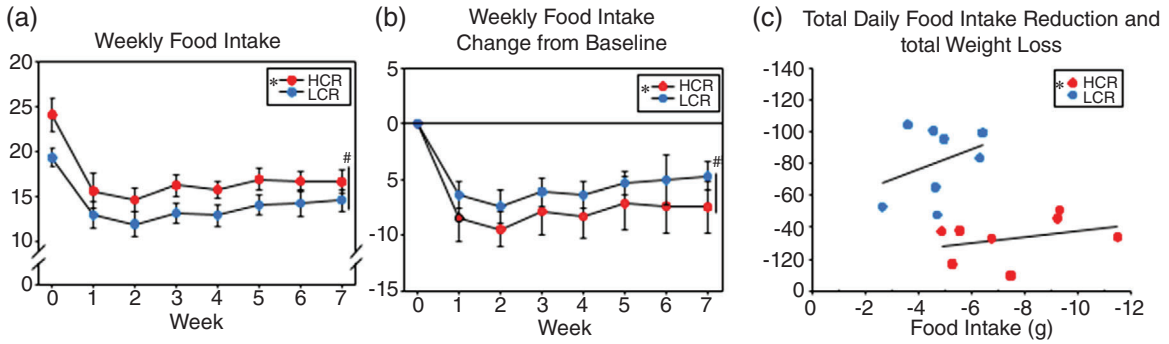

FIGURE 3 Weekly food intake across 7 weeks of intermittent fasting (IF) in high- (HCR) and low-capacity runner (LCR) rats. (a) The HCR and LCR rats reduced their food intake significantly from baseline. The HCR rats ate more than LCR rats at baseline and during each week of IF. (b) The HCR rats showed a greater reduction from baseline food intake at weeks 1, 2, 4 and 7. (c) Compared with LCR rats (ANCOVA), HCR rats showed a significantly ($P < 0.05$) greater reduction in food intake for a given weight loss. Values are the mean ± SD. #Significant ($P < 0.05$) interaction between time and phenotype; *(in key) HCR rats significantly ($P < 0.05$) different from LCR rats

no interaction. Interleukin-1α and IL-16 did not differ significantly between HCR and LCR rats or with food restriction, although for IL-6 the main effect of diet approached significance ($P = 0.051$), and there was also no significant difference in circulating TNF-α levels between HCR and LCR rats on CR or control diets. For the cytokines assessed here, 2 and 21 days of 50% CR reduced leptin levels compared with 21 days of a diet fed *ad libitum*, with LCR rats having overall lower levels of leptin; the cytokines IL-1β and IL-10 were significantly different between phenotypes, with LCR animals having higher levels of both cytokines than HCR animals.

3.2 | Male rats with low fitness lost more body weight, fat mass and lean mass on intermittent fasting

Similar to the HCR and LCR rats before 50% CR (Table 1), before initiation of 7 weeks of IF, LCR rats weighed significantly more than HCR rats at baseline, and LCR rats also had significantly more baseline fat and lean mass (Table 3). After 7 weeks of IF, LCR rats lost 3.6 times more absolute body weight than HCR rats, with a large effect size of body weight ($d = 2.55$; Table 3). Not only did LCR rats lose a greater proportion of their baseline body weight, they also lost a greater proportion of their baseline fat mass than HCR rats. Indeed, LCR animals lost 13.5% of their baseline body weight and nearly half (47.4%) of their baseline fat mass, whereas HCR animals lost only 4.8% of their baseline body weight and 27.1% of their baseline fat mass (Figure 2a,b). Therefore, the majority of the weight lost on IF and the more marked loss by LCR rats were attributable to loss of fat mass rather than lean mass (Figure 2). In fact, LCR rats lost more than three times more fat mass than HCR rats, an effect size of $d = 1.79$ (Table 3). For lean mass, in contrast, there was much less overall loss, and the proportional loss of 5.30% in LCR and 1.88% in HCR animals ($d = 1.08$; $P = 0.048$; Table 3; Figure 2c). In summary, on IF, LCR rats lost significantly more fat mass, which accounted for the majority of their weight loss.

Food intake was examined to determine the extent to which a decrease in food intake contributed to the enhanced weight loss in LCR relative to HCR rats while on IF. Although HCR weighed less than LCR animals, HCR rats ate more at baseline ($P < 0.001$) and during each

TABLE 3 Body weight and composition before and after 7 weeks of intermittent fasting (IF) in male high- and low-capacity runner (HCR and LCR) rats

Group	Baseline			Intermittent fasting			Change from baseline		
	Body weight (g)	Fat mass (g)	Lean mass (g)	Body weight (g)	Fat mass (g)	Lean mass (g)	Body weight (g)	Fat mass (g)	Lean mass (g)
HCR	408.38 ± 26.09	67.50 ± 12.31	281.11 ± 16.80	388.52 ± 23.02	48.34 ± 7.43	275.83 ± 20.00	−19.85 ± 15.61	−19.16 ± 9.28	−5.29 ± 9.67
LCR	530.80 ± 47.93	130.79 ± 56.60	326.23 ± 19.80	458.93 ± 40.92	68.53 ± 29.43	308.94 ± 19.31	−71.88 ± 18.80	−62.26 ± 32.75	−17.29 ± 12.36
HCR ≠ LCR (*P*-value)	**< 0.001**	**0.008**	**< 0.001**	**< 0.001**	0.081	**0.004**	**< 0.001**	**0.003**	**0.048**

Values are the mean ± SD. Body weight was assessed at the time of measurement of body composition on a fed day at the end of 7 weeks. Effect size (Cohen's *d*) between HCR and LCR body weight loss was $d = 2.55$, fat mass loss ($d = 1.29$) and lean mass loss ($d = 1.08$). There were significant differences (*P*-values are in bold) between HCR and LCR rats (Student's two-tailed *t*-test).

week of IF ($P < 0.05$), as shown in Figure 3a. A repeated-measures ANOVA of food intake across 7 weeks revealed a significant main effect of time ($P < 0.001$) whereby both HCR and LCR rats reduced their food intake with IF, a significant main effect of phenotype ($P = 0.001$) whereby HCR and LCR rats had differential changes in food intake over time, and a significant interaction between time and phenotype ($P < 0.001$) whereby HCR and LCR rats showed differential decreases in food intake over IF. A two-way ANOVA comparing food intake before and at the end of IF showed a significant main effect of time ($P < 0.001$) whereby HCR and LCR rats ate less over IF, and a significant main effect of phenotype ($P < 0.001$) whereby HCR rats ate more than LCR animals; there was no interaction between time and phenotype. Student's unpaired (two-tailed) *t*-tests showed that the change from baseline weekly food intake was significantly different between HCR and LCR rats at weeks 1, 2, 4 and 7 ($P < 0.05$), when HCR animals had the larger decrease in food intake from baseline (Figure 3b). Interestingly, HCR rats ate more than LCR rats, and although both LCR and HCR animals decreased their food intake, the larger decrease was seen in the HCR phenotype.

When examining the effect that reduced food intake had on weight loss during week 7 of IF, an ANCOVA revealed that total weight loss was significantly related to the decrease seen in total daily food intake ($P < 0.037$). This differed between HCR and LCR rats such that LCR rats had a greater loss of body weight for a given decrease in food intake ($P < 0.001$; Figure 3c). In summary, LCR rats decreased their average food intake from 19.36 to 14.66 g, a decrease of 4.7 g intake, with a weight loss of 71.88 g, whereas HCR rats decreased their food intake from 24.11 to 16.64 g, a decrease of 7.47 g intake, with a weight loss of 19.85 g. On IF, HCR rats ate 69.0% of their *ad libitum* intake, whereas LCR ate 75.7% of their *ad libitum* intake. Although HCR rats had a greater reduction in food intake than LCR, the HCR rats were still eating more than LCR, and the LCR rats lost more weight.

When comparing the method of food restriction between daily 50% CR and IF, male LCR rats subjected to IF (arguably, a modest restriction of food intake of < 25%; Figure 2b) lost a similar amount and proportion body fat (62 g, 47.4%) to the male LCR rats on much more severe daily food restriction (64 g, 39.8%), with relative preservation of lean mass on IF (Figures 1b and 2b). The ability of a relatively benign total energy deficit with IF to decrease adiposity in LCR stands in contrast to the vulnerability of HCR rats to a more severe restriction, during which the HCR rats lost > 10 g more lean mass (Table 1; Figure 1c).

Some variables changed between fed and fasted days, whereas others showed less day-to-day volatility in response to acute food availability. Repeated-measures 2 × 7 ANOVA for the average weekly (7 weeks) fed minus fasted days for body weight showed a significant main effect of time ($P < 0.001$) such that HCR and LCR rats had a change in body weight between fed and fasted days over time (higher body weight after a fed day), and a significant main effect of phenotype ($P = 0.009$) such that LCR rats had a greater change between body weights on fed and fasted days; there was no significant interaction between time and phenotype ($P = 0.160$). Student's *t*-tests (two-tailed) showed that the LCR rats weighed more than HCR rats on both fed and fasted days each week ($P < 0.05$; Figure 4a). To compare changes

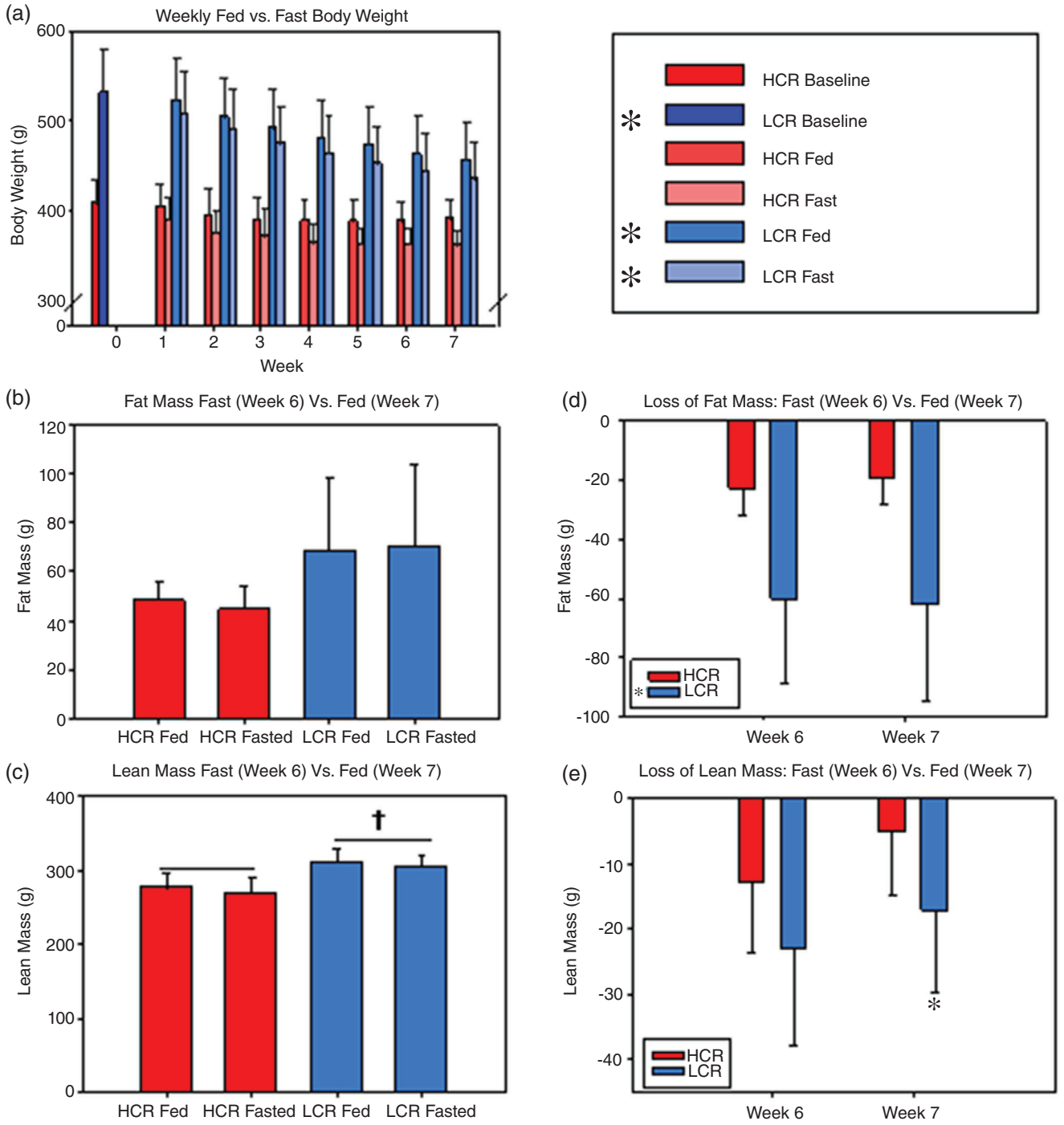

FIGURE 4 Rat body weight and composition during fed and fasted days of intermittent fasting (IF) in high- (HCR) and low-capacity runners (LCR). (a) The LCR rats had higher fed- and fasted-day body weights than HCR rats in each week of IF. (b) The HCR and LCR fat mass did not differ between phenotypes during weeks 6 (on a fasted day; $P = 0.06$) or 7 (on a fed day; $P = 0.08$). (c) The LCR rats had more lean mass than HCR rats at both weeks 6 (fasted) and 7 (fed). (d) The LCR rats lost more fat mass than HCR rats during both weeks 6 (fast) and 7 (fed). (e) The LCR rats lost more lean mass than HCR rats during week 7 (fed), but not week 6 (fast) of IF. Values are the mean ± SD. †Significantly ($P < 0.05$) more fasted and fed lean mass in LCR than in HCR rats; *(in key or under error bar) significantly ($P < 0.05$) more in LCR than HCR rats

in body composition between fed and fasted days between HCR and LCR rats subjected to IF, the last fasted and fed days when body composition was measured (i.e., weeks 6 and 7) were analysed using Student's *t*-tests (two-tailed). As shown in (Figure 4d,e), comparing the change in fat mass and lean mass from the final fasted and final fed measurements between HCR and LCR rats, analysis revealed a greater change in fat mass between fed and fasted days in HCR than in LCR rats ($P = 0.02$; fasted fat mass was 3.6 ± 1.09 g lower than fed fat mass in HCR rats, and 1.69 ± 1.64 g higher in LCR rats), but there was no difference in the change of lean mass between HCR and LCR rats

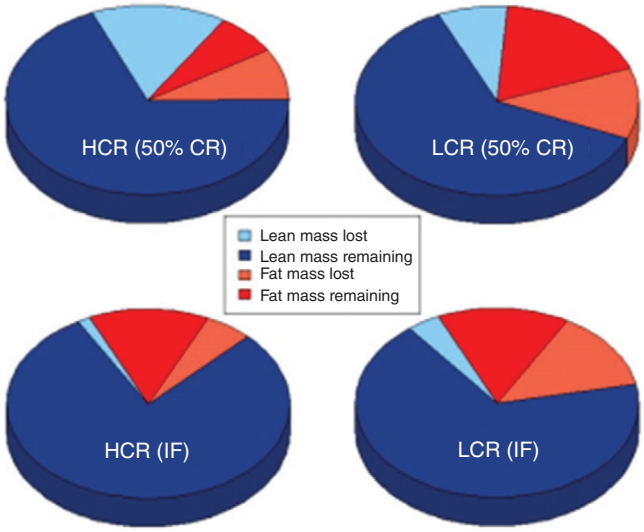

FIGURE 5 Relative change in rat body composition with 50% caloric restriction (50% CR) and intermittent fasting (IF) in male high- and low-capacity runners (HCR and LCR)

($P = 0.51$; fasted lean mass was 7.5 g lower than fed lean mass in HCR rats, and 5.85 g lower in LCR rats). The fat mass in HCR and LCR rats did not differ at week 6 (fasted day) or week 7 (fed day; $P > 0.05$; Figure 4b), but LCR rats had more lean mass than HCR rats at both weeks 6 and 7 of IF ($P < 0.05$; Figure 4c). In both HCR and LCR rats, the day-to-day changes of body weight while on IF were primarily attributable to daily fluctuations of lean mass rather than fat mass.

4 | DISCUSSION

Previously, we reported that female LCR rats lost more weight than HCR rats during IF, which contrasts with 50% CR, during which HCR rats lost a greater proportion of their baseline body weight than LCR rats (Smyers et al., 2015). Here, we confirm that IF induces greater weight loss in male LCR rats (Figure 5). This greater loss of body weight and fat mass in the male rats with low intrinsic aerobic fitness is consistent with the greater loss of body weight and fat mass previously identified in female LCR rats (Smyers et al., 2020), and underlines the potential for IF to be a beneficial weight-loss regimen for obesity-prone rats.

The underlying phenotypic differences in intrinsic aerobic capacity in HCR and LCR rat models produce a differential response to both methods of energy restriction discussed here. By artificial selective breeding, these contrasting models exemplify the differences in aerobic fitness capacity (Koch & Britton, 2008; Wisløff et al., 2005). As such, LCR rats have a much higher risk for the development of cardiovascular disease and metabolic syndrome (Koch & Britton, 2008). Previously, these rats showed differential weight gain on a high-fat diet and insulin sensitivity in male rats (Bikman et al., 2009; Morris et al., 2014, 2016, 2017, 2019; Naples et al., 2010; Noland et al., 2007; Novak et al., 2010; Park et al., 2016), and some deleterious health effects of a high-fat diet in LCR rats were not seen in HCR rats (Novak et al., 2010). Despite the general vulnerability to weight gain and the corresponding health sequelae in low-fitness rats, IF is a promising dietary regimen that results in a marked weight and fat loss in this rat model of intrinsic aerobic capacity (Smyers et al., 2020).

According to human clinical trials, low aerobic fitness is one of the strongest predictors of cardiovascular disease and all-cause mortality in healthy adults (Kodama et al., 2009; Kokkinos et al., 2008; Ladenvall et al., 2016). Kodama et al. (2009) compiled data from 33 eligible studies to reveal that lower maximal aerobic capacity is associated with higher cardiovascular disease and all-cause mortality (Kodama et al., 2009). High levels of fitness in children also reduce obesity-related co-morbidities (DuBose et al., 2007). Keeping this in mind, humans with low aerobic capacity or even those who do not exercise might benefit from IF for weight loss and the treatment of obesity. Indeed, in overweight and obese individuals, IF has resulted in significant weight loss, improvements of inflammatory markers and improvements of high-density lipoprotein cholesterol and triglyceride concentrations (Patterson et al., 2015). Aerobic capacity is therefore a major driver for the development of obesity and thus serves as a mediator of the response to calorie restriction and intermittent fasting for weight and fat loss.

Our previous experiment showed that 50% CR induced greater proportional weight loss in HCR than in LCR rats (Smyers et al., 2015). In the present study, however, the difference in weight loss between the two phenotypes did not reach significance. This inconsistency here and elsewhere (Mukherjee et al., 2020) could be attributable to the relatively low effect size, reflected in the marginal difference in weight loss seen here (Figure 1a). This stands in contrast to the robust ability of IF to reduce body weight and adiposity in the LCR rats. Comparing daily food restriction with IF, a more consistent and notable phenotypic difference is apparent when examining body composition, whereby LCR rats lost similar amounts and proportions of body fat on 50% CR and IF, whereas HCR rats lost 55.12 ± 14.54 g of lean mass on 50% CR compared with 5.29 ± 9.67 g on IF. This raises the question as to why LCR rats lost more fat and body weight than HCR rats on IF, which is a less restrictive diet, albeit for a longer duration of food restriction. Possible contributing factors include food intake, energy expenditure, or both. When examining food intake, HCR rats ate more than LCR rats both before and during IF (Figure 3a). The HCR rats, however, decreased their food intake more than LCR rats, although LCR rats lost more weight (Figures 3b,c). This implies that there is an additional energetic contribution to this phenotypic difference in the amount of weight lost, such as changes in energy expenditure, whereby LCR rats might experience less severe suppression of energy expenditure than HCR rats. It is known that HCR rats have higher energy expenditure (EE), owing to their heightened aerobic capacity and activity-related EE (Gavini et al., 2014; Mukherjee et al., 2020), and might therefore be affected in a different way from LCR rats in response to energy restriction. A direct comparison of the effects that IF and CR have on EE is warranted, because the differential change in body composition between HCR and LCR rats could stem from differential suppression of EE when subjected to different modes of

food restriction. The difference in size between male HCR and LCR rats complicates expenditure analysis, whereas female HCR and LCR rats have less size variation and are more easily weight matched to control for the effect of weight when measuring EE. Likewise, the ability of different modes of food restriction to alter body composition could stem from the intermittent nature of food restriction, the overall lower severity of energetic restriction, or both.

As shown in Table 2, CR-induced weight loss was accompanied by a significant decrease in leptin in both HCR and LCR animals, as predicted with fat loss. Also, consistent with Novak et al. (2010), the greater body size and adiposity of the LCR rats coincided with significantly higher leptin levels, with no differential decrease in leptin with weight loss (Novak et al., 2010). There were also higher levels of the pro-inflammatory cytokine IL-1β in LCR rats. Counterintuitively, there were also higher levels of the anti-inflammatory cytokine IL-10 in the low-fitness LCR rats. Although obesity is associated with inflammation (Engin, 2017), the association between adiposity and individual cytokines is not entirely predictable; for example, elevated levels of IL-10 are reported in obese women with metabolic syndrome, whereas low levels of IL-10 are present in obese women without metabolic syndrome (Esposito et al., 2003). Some evidence supports the idea that IF decreases pro-inflammatory cytokines, such as TNFα, IL-1β and IL-6 (Arumugam et al., 2010; Liu et al., 2018; Patterson et al., 2015). Indeed, Liu et al. (2018) subjected high-fat diet-fed mice to IF and found that 8 weeks of IF improved adipose tissue markers of inflammation. Arumugam et al. (2010) suggested that age might modulate the effect of diet on inflammation; young mice on an IF-fed diet exhibited a decrease in the inflammatory cytokines TNFα and IL-6, whereas inflammatory cytokines were increased in older mice.

The absence of change in inflammation-related cytokines in HCR and LCR rats subjected to CR (Table 2) stands in contrast to a report that time-restricted feeding, which restricts the duration of food availability, reduced IL-1β in mice (Chung et al., 2002; Sherman et al., 2011). Interestingly, higher levels of IL-10 after 30% CR were observed in aged rhesus monkeys (Willette et al., 2013). Despite the relevance of inflammation to the health impact of obesity (Engin, 2017), there was no significant effect of food restriction and weight loss on the cytokines identified here apart from leptin. Similar to our findings in male HCR and LCR rats on IF and 50% CR, Trepanowski et al. (2017) found that overweight or obese individuals who were either on an alternate-day fasting or CR regimen had similarly reduced leptin levels, but neither diet affected other measured adipokines, including TNF-α and IL-6. Cytokine levels are under circadian control, and perhaps the timing of serum collection influenced cytokine levels somewhat (Keller et al., 2009; Liu et al., 2018). The effect of IF on circulating cytokines is of interest, although unfortunately, the experimental design and sample size in the present study precluded the collection of serum samples before and after IF.

With alternating days of fasting, changes in body weight corresponded to acute food availability, such that there was an increase in body weight after feeding and a decrease after fasting (Figure 4a). This weight fluctuation is primarily attributable to daily changes in lean mass, rather than fat mass, consistent with prior evidence (Figure 4c,e; Smyers et al., 2020). Previously, Smyers et al. (2020) measured water intake in HCR and LCR rats during IF and found that the fluctuations seen in lean mass, and therefore in body weight, might be attributable, in part, to the change in water intake with food availability on fed days; although water was available *ad libitum*, there was less water intake on fasted days compared with fed days. Limited evidence from human clinical trials indicates similar day-to-day responses and, not surprisingly, fasting days are associated with increased hunger and lower physical activity compared with fed days (Beaulieu et al., 2020). The prevalence of day-to-day changes in energy expenditure and physical activity with food availability is an important under-addressed issue, because it would be expected to impact diet adherence.

Consistent with our recent report that female LCR rats were more responsive to IF, in the present study we have shown that male LCR rats lost significantly more weight with alternate-day fasting, and this weight loss was primarily from the loss of fat mass (Table 3). Conversely, 3 weeks of CR induced a greater loss of lean mass in HCR rats (Table 1), consistent with our previous experiments, in which HCR rats appeared to have a greater vulnerability to the loss of lean mass with food restriction than LCR rats (Smyers et al., 2015). Even so, our findings suggest that IF is an effective weight-loss regimen for the low-fitness LCR phenotype; however, direct comparisons are needed to determine whether this greater weight loss in LCR rats is secondary to timing or the severity of food restriction, in addition to the potential for IF to lessen the adaptation in energy expenditure seen with weight loss. Altogether, these datasets highlight not only the ability of IF to promote weight and fat loss, but also the interaction with aerobic fitness.

AUTHOR CONTRIBUTIONS

All experiments were performed in Colleen M. Novak's laboratory at Kent State University. Conceptualization: A.E.D., M.E.S., L.B., S.L.B., L.G.K. and C.M.N. Acquisition of funding: S.L.B., L.G.K. and C.M.N. Resources: S.L.B., L.G.K. and C.M.N. Project administration: A.E.D., M.E.S. and C.M.N. Supervision: A.E.D. and C.M.N. Investigation: A.E.D., M.E.S., L.B. and D.M.M. Methodology: A.E.D., M.E.S., L.B. and C.M.N. Data curation: A.E.D., M.E.S. and C.M.N. Formal analysis: A.E.D. and M.E.S. Visualization and writing of original draft: A.E.D. Review and editing of the manuscript: A.E.D., M.E.S., L.B., D.M.M., S.L.B., L.G.K. and C.M.N. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

COMPETING INTERESTS

None declared.

DATA AVAILABILITY STATEMENT

All data supporting the results of this paper are located within this paper. The LCR and HCR rat models are maintained as an exercise

rat model resource for researchers at The University of Toledo, Toledo, OH, USA; contact L.G.K. (lauren.koch2@utoledo.edu) or S.L.B. (brittons@umich.edu) for information.

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