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High-Intensity Interval Training with Energy Restriction Preserves Lean Tissue and Improves Glucose Tolerance in Obesity

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HIGH-INTENSITY INTERVAL TRAINING WITH ENERGY RESTRICTION PRESERVES LEAN TISSUE AND IMPROVES GLUCOSE TOLERANCE IN OBESITY

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ABSTRACT

Calorie restriction (CR) reduces body weight (BW) and adiposity, but is often less than expected due to hypometabolism produced by reductions in lean body mass (LBM). A comparable energy deficit created by combining CR and exercise energy expenditure (EE) has been shown to attenuate the reduction in EE produced by CR. Continuous moderate-intensity training (MIT) is commonly used to sustain energy balance or expedite weight loss, but high-intensity interval training (HIIT) may produce greater results. The purpose of this investigation was to determine if HIIT preserves LBM and improves energy metabolism to a greater extent than MIT in the presence of CR. Thirty-two 5-wk old male C57BL/6J mice were placed on a 45% kcal high-fat diet (HFD) for 11 weeks (*ad libitum*). Mice were then randomized to 4 groups for 15 weeks: 1) HFD (n = 8; remain on HFD); 2) HFD with 25% CR (n = 8); 3) HFD with 25% energy deficit induced by 12.5% CR and 12.5% EE mediated through HIIT (n = 8); and 4) HFD with 12.5% energy deficit induced by 12.5% CR and 12.5% EE mediated through MIT. HIIT consisted of 9-12 intervals of 2.5-minutes of treadmill running at 0.18-0.30 m/s with 1 minute of passive recovery. MIT consisted of 35-50 minutes of continuous treadmill running at 0.13-0.21 m/s. Body composition was assessed by Quantitative Magnetic Resonance (QMR) and resting energy expenditure (REE) by indirect calorimetry. Glucose tolerance tests were performed on all groups at 1.0 $g \cdot kg^{-1}$ BW (i.p.), while

insulin tolerance tests were performed at 0.75 $mU \cdot g^{-1}$ BW (i.p.). HFD produced a 92.3% increase in BW. REE was 15.6% lower in the CR group compared to Control (33.6 ± 1.2 kJ/day to 39.8 ± 1.4 kJ/day, $p < 0.05$). Twenty-five percent CR produced reductions in LBM and REE, whereas HIIT, but not MIT, preserved LBM and rescued CR-mediated reductions in REE, which was associated with improvements in glucose tolerance and insulin sensitivity. These results suggest that HIIT may produce a hypermetabolic state in the presence of CR, which could lead to long-term success in weight loss interventions.

Keywords: exercise, calorie restriction, metabolism, high-fat diet

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TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

Figure *Page*

LIST OF ABBREVIATIONS

ANCOVA	analysis of covariance
ANOVA	one-way analysis of variance
ARP	Animal Resource Program
AUC	area under the curve
BAT	brown adipose tissue
BW	body weight
CON	Control
CR	calorie restriction
CVD	cardiovascular disease
DIO	diet-induced obesity
EE	energy expenditure
FM	fat mass
HFD	high-fat diet
HIIT	high-intensity interval training
IACUC	Animal Care and Use Committee
IPGTT	intraperitoneal glucose tolerance test
ITT	insulin tolerance test
LBM	lean body mass
MIT	moderate intensity training
QMR	Quantitative Magnetic Resonance

REE	resting energy expenditure
RER	respiratory exchange ratio
SEM	standard error of the mean
SM	skeletal muscle
T2DM	type 2 diabetes mellitus
TDEE	total daily energy expenditure
VAT	visceral adipose tissue
WAT	white adipose tissue

CHAPTER 1

INTRODUCTION

Obesity and Obesity-Related Metabolic Disease

Over the past 35 years, the incidence of overweight and obesity in America has increased to epidemic proportions despite extensive lifestyle promoting strategies to reverse this trend (*1*). Obesity accounts for an alarming $147 billion dollars in healthcare costs annually due to related complications. More recent investigations indicate that obesity may contribute up to 20% of all-cause mortality primarily related to metabolic and cardiovascular complications(*2*). These findings highlight the need for more extensive investigation on the fundamental causes of obesity and the exploration of novel lifestyle and pharmacological strategies to reduce its economic and health-related burden.

Obesity is the product of chronic positive energy balance and manifests as the accretion of excess lipid storage in white adipose tissue (WAT). Ectopic lipid stored in skeletal muscle (SM), liver and more recently, brown adipose tissue (BAT) occurs in the presence of obesity and are well known to produce metabolic dysfunction resulting in a cluster of cardio-metabolic risk factors such as hypertension, dyslipidemia, systemic inflammation, and insulin resistance (*3-6*). While the extent of its contribution continues to be debated, the availability and consumption of energy-dense foods and reduced occupational/leisure-time physical activityare thought to be responsible for the rise in obesity(*7-10*). Since the 1950's, the typical American diet has transitioned toward excessive carbohydrate and fat consumption from highly processed and refined foods,

while physical activity has progressively declined (*11*, *12*). Indeed, over 50% of Americans do not meet physical activity recommendations for aerobic exercise; with lack of time being the greatest reported challenge (*13*). This steady increase in physical inactivity has begun to impact the body composition status of Americans (*14*, *15*). Long-term sedentary living in relation to low physical fitness has been named a risk factor for cardiovascular disease (CVD), type 2 diabetes mellitus (T2DM), and all-cause mortality (*16*). With the American lifestyle trending toward poor diet choices and sedentary living, it is widely assumed that the concurrent expansion in the obesity epidemic is in part occurring from preventable causes. In an effort to reverse the resulting health disparities, it is imperative to understand the various causes and explore feasible interventions that can contribute to novel treatment and prevention plans.

Positive Energy Balance with High-Fat Feeding

Positive energy balance achieved through excess energy consumption is highly associated with the development of obesity in both human and animal models (*17*). A positive correlation between dietary fat content and adiposity has been shown in a number of studies (*18-20*). Diet-induced obesity (DIO) in animal models is a common method used to mimic and explore the metabolic abnormalities that exist in human obesity. In fact, *ad libitum* high fat diet (HFD) quickly induces weight gain, liver steatosis, insulin resistance, hyperglycemia, and systemic inflammation in mice (*21-24*). Prolonged consumption of a HFD also impacts feeding behavior and satiety signaling, with the induction of hyperphagia(*25*). Chronic hyperphagia leads to further weight gain and disruptions to energy homeostasis (*26*, *27*). In order to correct the impairments

associated with HFD consumption, it is necessary to reduce energy density within the diet. Restricting overall energy intake has been shown to be effective in attenuating the negative metabolic effects of a HFD(*28, 29*).

Energy (Calorie) Restriction

Calorie restriction (CR) is utilized in many forms of dieting and is one of the most widely used methods of weight reduction(*30*). Reducing energy intake has been shown to reduce adiposity and prevent further weight gain occurring from poor diet composition, excess energy consumption, and aging (*29*). Particularly in those who are overweight, consistent negative energy balance and the resulting loss of fat mass (FM) is associated with improvements in multiplecardio-metabolic risk factors. These improvements include an overall decrease in body weight (BW), FM, visceral adipose tissue (VAT), fasting plasma insulin and glucose, blood lipids, blood pressure, and improved insulin sensitivity and glucose tolerance (*31, 32*). While CR may be effective in reducing body mass, a metabolic adaptation to negative energy balance is evident with a decline to total daily energy expenditure (TDEE) (*33*). TDEE is comprised of resting energy expenditure (REE), the thermic effect of feeding, and daily physical activity. As a result of the reduction in caloric intake, the thermic effect of digesting, absorbing, and metabolizing foodstuffs is reduced. The reduction in energy intake is also associated with a decrease in spontaneous physical activity (*33*). In addition to reducing WAT mass, a concurrent loss of lean body mass (LBM) occurs with CR and is associated with a decline in REE (*34, 35*). This corresponds to a reduction in non-activity and activity-relatedthermogenesis, which can be attenuated if a portion of the energydeficit is produced by exercise. While a

reduction in TDEE is evident with CR alone, the reduction is absent in the presence of exercise (*33*). Additionally, the long-term practice of reduced energy intake can be difficult to sustain, revealed by the frequent weight fluctuations that occur while dieting (*30*). Previous studies have speculated as to whether maximal metabolic improvements and subsequent prevention of weight re-gain occur when the energy deficit created to reduce adiposity is produced from a combination of CR and exercise training.

Aerobic Exercise Training

Aerobic exercise training is commonly recommended to improve health and maximize weight loss. Moderate intensity training (MIT), matching an intensity of 60-75% of maximal oxygen uptake, is typically the method of training chosen for weight reduction. This form of exercise has been shown to reduce overall WAT, preserve LBM, and improve insulin sensitivity and glucose tolerance (*36*).While generally promoted as a strategy for weight loss, beneficial changes to body composition occur even in the absence of reduced BW(*37, 38*). Results from clinical trials comparing exercise with and without weight loss suggest that exercise can still be advantageous in reducing VAT and in preventing further weight gain from excess energy intake(*36, 39, 40*). Regardless of whether a decline in body mass occurs, reductions to VAT, subcutaneous AT, and increases in SM mass have been seen in both lean and overweight individuals when performing regular aerobic exercise training (*37*). Insulin sensitivity is inversely correlated to VATindicating that the reduction of VAT seen with exercise training is valuable in reversing insulin resistance (*36, 41, 42*). Many of these improvements are

evident with CR, but CR does not produce exercise-mediated increases in SM mass, strength, andcardiorespiratory fitness (*43*).

While the benefits to energy balance and metabolism are evident with CR and exercise interventions alone, evidence suggests that a combination of CR and aerobic exercise provide greater benefits. CR produces a reduction in LBM, which is observed to a lesser extent with exercise, suggesting that a combination of each intervention is superior to either alone (*44, 45*). Exercise training attenuates the decline in REE that occurs from reduced caloric intake,which favors loss of WAT over LBM(*46, 47*). Since exercise of higher intensity has been shown to increase LBM, it is possible that this level of training may produce greater post-exercise and activity-related energy expenditure [EE] (*48*).

High-Intensity Interval Training

High-intensity interval training (HIIT), which consists of multiple bouts of near maximal exercise usually at 85-95% of VO_{2peak}, has been shown to produce equivalent, if not superior, advantages to MIT despite producing an energy deficit that is considerably lower(*49, 50*). Despite lower training volume, the reported benefits of HIIT includegreater improvements in VO_{2peak}, insulin action and sensitivity, and overall weight reduction(*51*). Existing studies comparing HIIT and MIT indicate that HIIT may also be superior in improving glucose disposal(*52*). In rodent models, to our knowledge, no one has compared HIIT and MIT in the presence of CR andDIO. What has not been explored, and the purpose of this study, was to determine if HIIT could attenuate the loss in LBMrelated to CR, and produce superior improvements in body composition than MIT in

the presence of CR. We hypothesized that HIIT would preserve LBM to a greater extent than MIT, and produce a greater decline in overall WAT.

CHAPTER 2

METHODS

Animals and Diets

Male C57BL/6J mice (n = 32) were purchased from Jackson Laboratories (Bar Harbor, ME) at 5 weeks of age. Upon arrival to the UAB animal facility, mice were group housed (3-5 per cage) and allowed to acclimate for 7 days, consuming a standard chow diet and water *ad libitum*. At 6 weeks of age, the micewere individually housed and maintained on a 12:12 reverse light-dark cycle in a temperature-controlled room at 22-23°C. The shelf placements of the shoebox cages were rotated weekly to ensure that all animals were exposed to an equal amount of light. Each of the mice were then placed on an *ad libitum* HFD containing 45% kilocalories from fat (Table 1; Research Diets, #D12451) for 11 weeks. Following the HFD phase, micewere randomly assigned to one of four groups for an additional 15 weeks (n = 8 per group): 1) Control (CON), remain on HFD 2) HFD with 25% CR 3) HFD with a 25% energy deficit created by 12.5% CR and 12.5% EEfromHIIT and 4) HFD with a 25% energy deficit created by 12.5% CR and 12.5% EE mediated through MIT. CR for all groups was calculated based on the average weekly intake of the CON group. The amount of CR for each group was recalculated at the beginning of each week and pellets were cut to the exact weight needed to achieve the imposed restriction. For the duration of the study, food intake was measured every 24 hours withBWmeasurements every 2-3 days. Shoebox cages, bedding, and hoppers were replaced once per month. The UAB Institutional Animal Care and Use Committee

(IACUC) approved the investigation (Appendix), and all conditions conformed to the care procedures employed by the UAB Animal Resource Program (ARP).

TABLE 1. Diet Composition – Research Diets, #D12451.

Macronutrient	kcal %	Source
Protein	20	Casein, L-Cystine
Carbohydrate	35	Sucrose, Maltodextrin, Cornstarch
Fat	45	Lard, Soybean Oil

*Total energy = 4.73kcal/g

Body Composition and Indirect Calorimetry

Body composition wasmeasured in the UAB Small Animal PhenotypingSubcore by Quantitative Magnetic Resonance (QMR) following 11 weeks of HFD, and at 4-week intervals during the treatment phase. Energy intake and expenditure, locomotor activity (horizontal and vertical), and substrate utilization were evaluated following the HFD (start of 15-week treatment) and during the final week of the study using a TSE PhenoMaster indirect calorimetry system (TSE Instruments, Chesterfield, MO) in the Small Animal PhenotypingSubcore.

Exercise Interventions

Treadmill running was performed on a modified dog treadmill (JOG A DOG, Ottawa Lake, MI) 5 days per week at a fixed incline of 25° for 15 weeks. All exercise was performed within the first two hours of the dark cycle under dim red lighting. The treadmill included a dividing overlay with enclosed compartments containing safety padding for each mouse.HIIT consisted of 9-12 intervals of 2.5 minutes of running at 0.18-0.30 m/s with 1 minute of passive recovery between intervals. MIT consisted of 35-50 minutes of continuous running at 0.13-0.21 m/s. Time and speed were increased every two weeks throughout the exercise-training phase of the study. Both non-exercise groups (CON and CR) observed exercise sessions from enclosed control chambers on the outside of the treadmill to provide similar handling and treadmill exposures. If animals resisted treadmill running, they were encouraged with continuous nudging to their hind legs.

Aerobic Capacity

$VO_{2peak}$was measured in all groups using a mouse modular treadmill (TSE Instruments, Chesterfield, MO) following 11 weeks of HFD, and at 4 and 12 weeks of the exercise intervention. Following a 10-minute warm up, speed was gradually increasedby 0.03 m/s every 2 minutes with a fixed grade (25° inclination)until oxygen consumption leveled off despite increased running speed and a respiratory exchange ratio (RER) of 1.0 was achieved. The running speed at which VO_{2peak} was obtained was defined as $speed_{max}$.VO_{2peak} was used to calculate the intended duration of each exercise intervention. Energy expenditure per session was estimated from VO_{2peak} values per

group and the assumption that HIIT and MIT were performed at 85% and 65% of maximal oxygen consumption, respectively.

Glucose and Insulin Tolerance Testing

At weeks 10 and 11 of exercise, an intraperitoneal glucose tolerance test (IPGTT)and insulin tolerance test (ITT) wasperformed on all mice. Glucose tolerance tests were performed following a 6-hour fast at 1.0 $g \cdot kg^{-1}$BW(i.p.). Following a baseline blood glucose measure (0 time point), glucose was administered, followed by additional blood glucose measurements at 15, 30, 60, 90, and 120 minutes. Insulin tolerance tests were performed one week laterfollowing a 2-hour fast at 0.75 $mU \cdot g^{-1}$ BW (i.p.). Both tests were performed within the first two hours of the dark cycle. Following a baseline blood glucose measure (0 time point), insulin was administered, followed by additional blood glucose measurements at 0, 15, 30, 45, and 60 minutes. A glucometer was used for all blood glucose measurements (OneTouch Ultra, Lifespan).

Tissue Collection and Handling

Animals were sacrificed 24 hours after the final exercise session whichbegan early in the dark cycle. Following a 6-hour fast, whole blood and tissues (interscapular BAT, epididymal WAT, retroperitoneal WAT, inguinal WAT, liver, kidneys, heart, brain, and SM) were obtained immediately following decapitation and snap frozen in liquid N_2. Portionsof each adipose tissue depot were placed in cassettes and stored in formalin for future analyses.

Statistical Analysis

Power analyses suggested that a minimum of 6 mice would be required to detect differences between groups. This was determined using the variance in our primary variables of interest at an effect size of 0.8 and an alpha level of 0.05, which coincides with our experience for the detection of differences in the majority of variables of interest and the amount of specific tissue available from each animal. Power calculations were made using SAS for Window software (version 9.1; SAS Institute, Cary, NC). Differences between groups were analyzed by one-way analysis of variance (ANOVA) using GraphPad Prism (La Jolla, CA). Tukey-Kramer post-hoc testing was used to explore significant differences determined by ANOVA. Group differences in EE (kJ/mouse/day) were compared by analysis of covariance (ANCOVA)using IBM SPSS Statistics (version 23.0; IBM Corp., Armonk, NY), calculating least squares means that account for variation in EE attributable to differences in LBM, FM, physical activity, and energy intake among groups. Relationships between variables were determined using Pearson Product-Moment Correlations. Significance was set *a priori* at $P < 0.05$.Data are expressed as mean ±standard error of the mean (SEM).

CHAPTER 3

RESULTS

High-Fat Diet-Induced Obesity

Five week old male C57BL/6J mice (n = 32) were maintained on a 45% kcal fat diet for 11 weeks to induce obesity. HFD increased BW by 92.3% from 20.2 ± 0.2 g to 38.8 ± 0.8 g. Mean energy intake over the 11-week period was 55.2 ± 0.8 kJ/day, while mean TEE was 48.7 ± 1.3 kJ/day. Following the DIO phase of the study, mice were randomized to one of four weight-matched groups (CON, CR, HIIT, MIT). As a result, there were no significant differences in baseline BW between groups (Fig. 1).

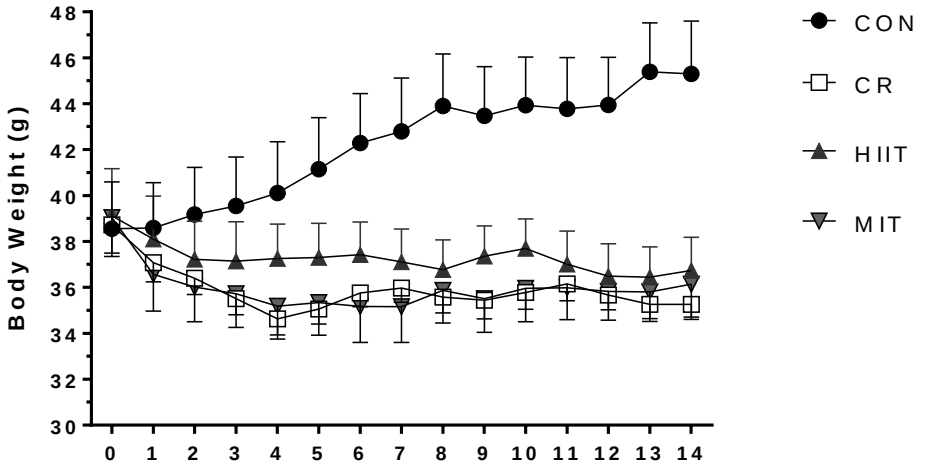

FIGURE 1. Body weight changes per week in the Control and treatment groups. Values are weekly means ± SEM. Values with the same letter are not significantly different.

High-Intensity Interval Training Preserves Lean Body Mass and Resting Energy Expenditure

Control animals received the HFD*ad libitum* for a total of 26 weeks and experienced a progressive increase in body mass throughout the study period (Fig. 1). Each treatment (CR, HIIT, MIT) produced a similar reduction in BW (Fig. 1) and adiposity (Fig. 2A) compared to CON. As expected, CR produced a significant reduction in LBM, and this was attenuated by the HIIT group only (Fig. 2B). These findings indicate that HIIT, but not MIT, preserved LBM in the presence of CR.

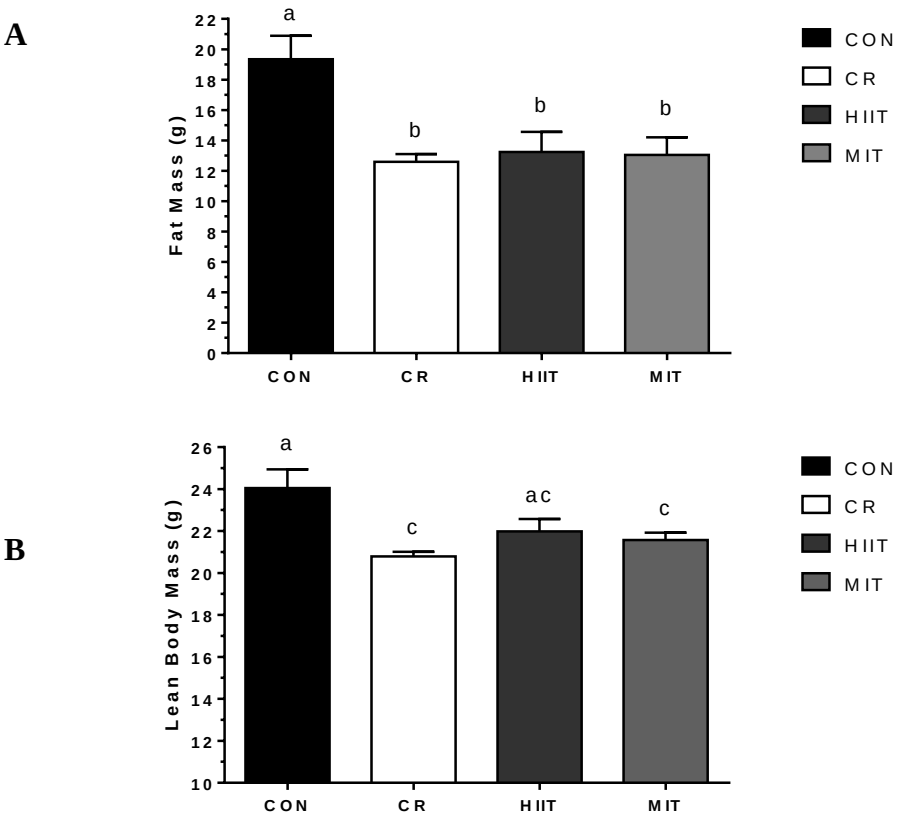

FIGURE 2. Differences in fat mass (A) and lean body mass (B) per group following the 26-week study period. Values are means ± SEM. Values with the same letter are not significantly different.

Average energy intake (kJ/day) between groups was: 57.6±0.6 (CON), 41.8 ± 0.0 (CR), 47.1 ± 0.3(HIIT), and 45.9 ± 0.3 (MIT) with CR and exercise groups fed 25% and 12.5% below CON intake, respectively. ANCOVA was used to assess the impact of the treatments on REE and TDEE after adjusting for variation in TDEE associated with LBM and FM for each group. REE was 15.6% lower in the CR group compared to CON (39.8±1.4 to 33.6±1.2 kJ/day, $p<0.05$).While 24-hour REE for CR and MIT were significantly lower than CON, HIIT rescued this reduction in REE to levels that were not significantly different from CON, despite significantly lower LBM and FM (Table 2). TDEE was not significantly different between any of the treatment groups compared to CON, but a trend was observed for higher TDEE with HIIT (Table 2).

TABLE 2. Resting and Total Daily Energy Expenditure.

GROUP	BW (g)	FM (g)	LBM (g)	REE (kJ/day)	TDEE (kJ/day)
CON (n=8)	45.0±1.5	19.3±1.7	24.0±0.6	42.6±1.2 †39.8±1.4	57.0±1.6 †51.9±1.4
CR (n=8)	34.8±1.5	12.6±1.2	20.8±0.6	32.2±1.2 †33.6±1.2	46.9±1.6 †49.6±1.2
HIIT (n=7)	36.6±1.6	13.2±1.3	22.0±0.6	36.0±1.3 †36.6±1.2	51.2±1.7 †52.2±1.2
MIT (n=8)	36.0±1.5	13.0±1.2	21.6±0.6	33.8±1.2 †34.7±1.1	48.9±1.6 †50.5±1.1

†Adjusted for FM and LBM.

Changes in Cardiorespiratory Fitness and Physical Activity

HIIT and MIT increased VO_{2peak} similarly (12 and 9.6%, respectively), and final VO_{2peak} was significantly different when compared to CON. We found no differences in physical activity between all treatments compared to CON during the dark cycle (Fig. 3A), when feeding typically occurs, or the light cycle during "fasting" (Fig. 3B).

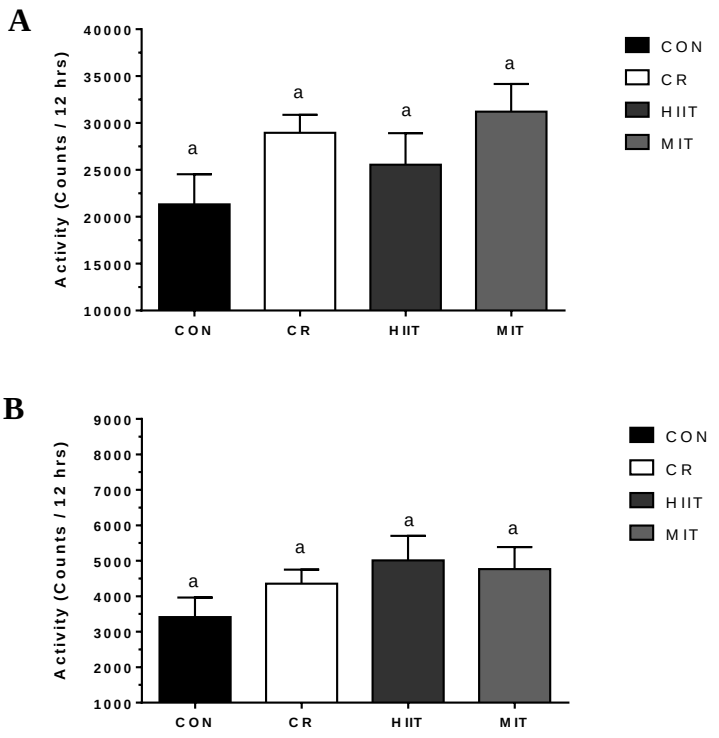

FIGURE 3. Physical activity counts obtained by the TSE phenotyping system. A) Dark cycle activity. B) Light cycle activity. Results are means ± SEM.Values with the same letter are not significantly different.

Metabolic Flexibility, Glucose Tolerance and Insulin Tolerance Testing

Respiratory exchange ratio values from indirect calorimetry were used to explore metabolic flexibility between each of the four groups. Impairments in metabolic flexibility are recognized by a diminution of the normal increase in RER that occurs

during the feeding period, and this has been attributed to compromised insulin-dependent glucose uptake in peripheral tissues(*53, 54*). In the current study, mice from the CON group had an attenuated increase in the normal excursion of RER during feeding such that dark and light cycle RER were nearly identical (Fig. 4). In contrast, CR and MIT produced similar shifts in carbohydrate utilization and a greater shift in fat utilization during the light cycle compared to CON. While not significantly different from CR or MIT, HIIT produced the numerically greatest flexibility in night to day RER excursion.

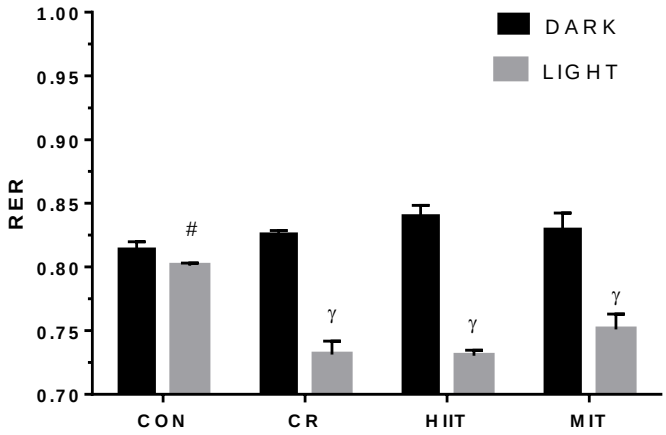

FIGURE 4. Respiratory Exchange Ratio (RER) for each group during dark and light cycles. Values are means ± SEM. Values with different symbols indicate significant between group differences for the dark and light cycle excursion.

Glucose tolerance was similar between CR and CON. MIT did not result in significant reductions in glucose tolerance when compared as the absolute response (Fig. 5A), total area under the curve [AUC] (Fig. 5B) or incremental AUC (Fig. 5C). In agreement with our findings related to metabolic flexibility, glucose tolerance was significantly greater with HIIT compared to each of the other groups.

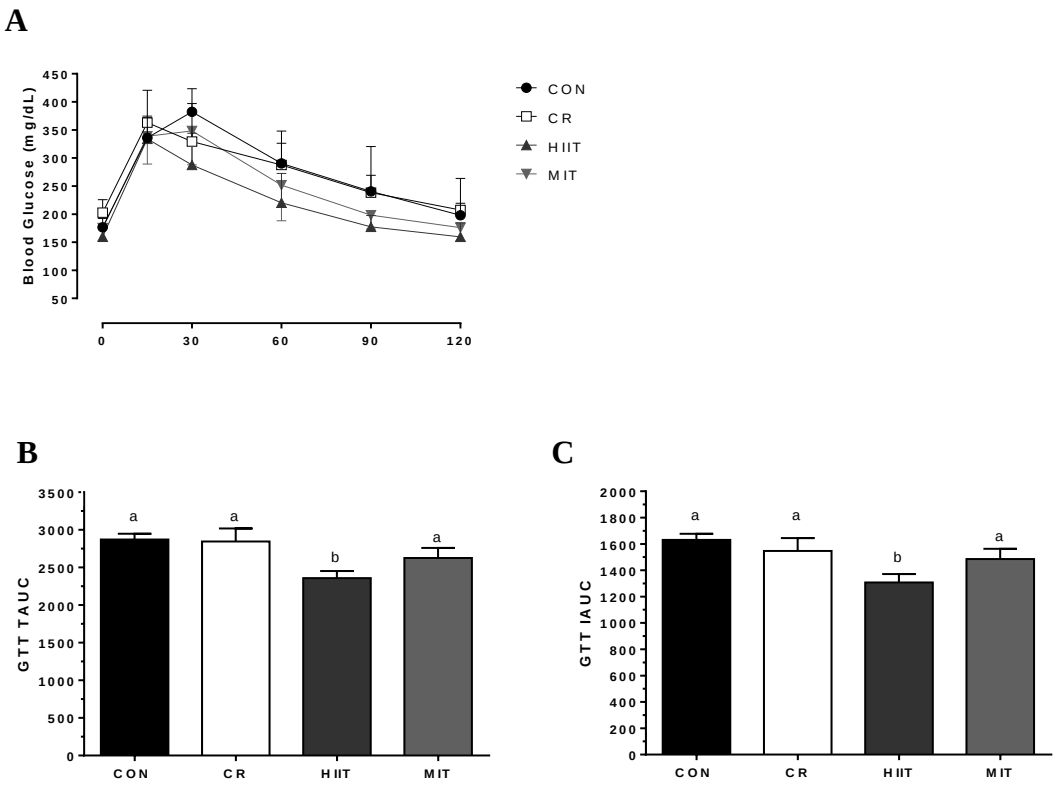

FIGURE 5. A) Absolute blood glucose responses following GTT (1 mg·kg^{-1}i.p.). B) Total area under the curve. C) Incremental area under the curve. Results are means ± SEM. Values with the same letter are not statistically significant.

Serum glucose concentrations were significantly lower 15 minutes following the ITT in the CR group compared to CON but were not statistically significant beyond this time point despite numerically lower values throughout (Fig. 6A). HIIT produced a similar improvement in insulin tolerance at 30 minutes that remained similar to CR throughout the ITT (all other time points were no different from control despite similar trends). In support of these findings, we found practically relevant and consistent data that did not achieve statistical significance when calculated as total (Fig. 6B) and incremental (Fig. 6C) AUC.

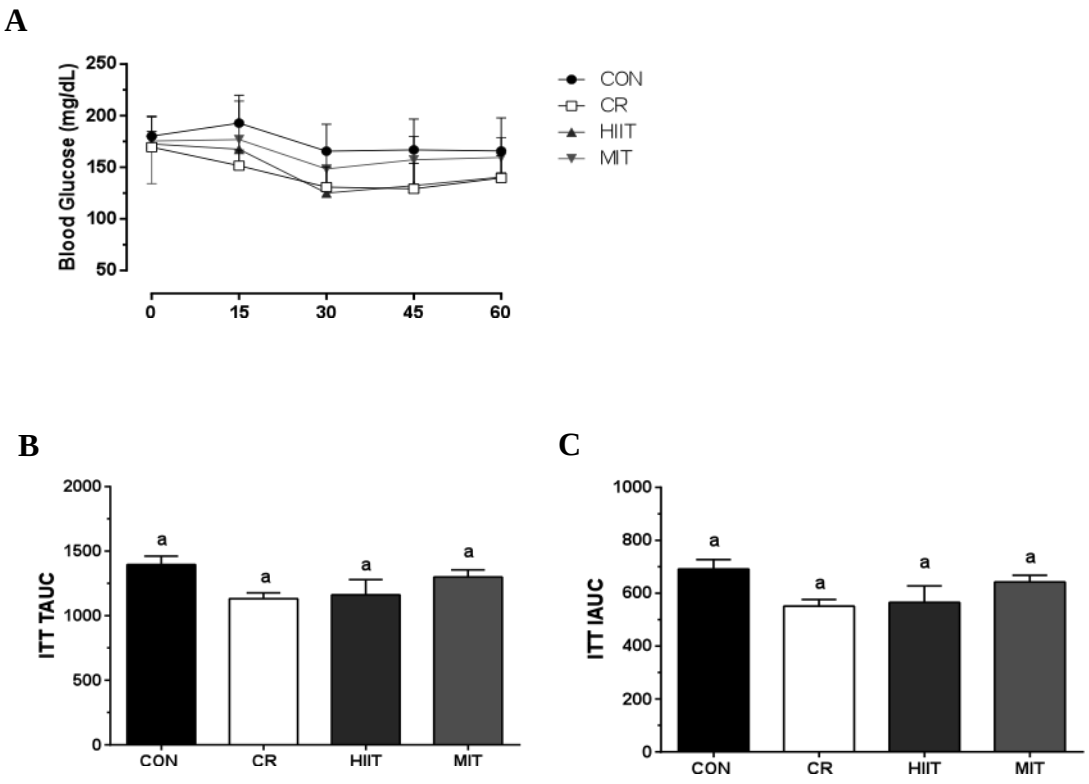

FIGURE 6. A) Absolute blood glucose responses following ITT (0.75 mU·kg^{-1}i.p.). B) Total area under the curve. C) Incremental area under the curve. Results are means ± SEM. Values with the same letter are not statistically significant.

Correlations

Lean body masswas positively correlated with REE (r= 0.67, $p < 0.05$) and TDEE ($r = 0.77$, $p < 0.05$). Resting energy expenditure was positively correlated with GTT AUC ($r = 0.52$, $p < 0.05$) and ITT AUC ($r = 0.50$, $p < 0.05$). Total daily energy expenditure was also positively correlated with GTT AUC ($r = 0.44$, $p < 0.05$) and ITT AUC ($r = 0.42$, $p < 0.05$). Theestablished relationships between LBM and REE/TDEE suggest that the increase in LBM of the HIIT group was significantly associated with increased EE. The relationships reported between REE/TDEE and GTT AUC and ITT AUC imply that

increased REE contributes at least in part to the improvement in glucose tolerance and insulin sensitivity.

CHAPTER 4

DISCUSSION

Reducing dietary energy intake (dieting) is one of the leading and most effective strategies in reducingBW and adiposity. However, reductions in caloric intake often produce results that are less than expected in the short-term and difficult to sustain with time. While it is not entirely clear, it is generally well accepted that reductions in the size of the metabolizable mass, energy cost of physical activity and the thermic effect of food produced by the caloric deficit produce metabolic and neurophysiological responses that limit weight loss and increase risk of weight regain (*31, 33*). When at least a portion of an energy deficit is achieved with aerobic exercise, CR-mediated reductions in activity-related EE are not only reversed but also increased compared to control(*33*). These findings suggest that aerobic exercise may be an effective strategy to maximize weight/fat loss and prevent long-term weight regain. Nevertheless, one of the greatest challenges for the field is the common report from participants about the lack of time available to engage in physical activity/exercise.

High-intensity interval training has been used for many years as a training strategy to improve exercise and sports performance(*55*). More recent studies show that low-volume HIIT performed as multiple bouts of 30-120s each at >85% of VO_{2peak} produces results which are just as effective or superior to traditional continuous aerobic exercise performed at moderate intensity(*56*). Indeed, HIIT has been shown to increase non-activity related energy expenditure following a single bout of exercise(*57*). The

increase in EE appears to be at least partially related preservation ofLBM produced by HIIT. We are unaware of any studies that have simultaneously compared the effects ofHIIT and MIT in the presence of CR following DIO. Therefore, the purpose of this study was to determine if HIIT could attenuate the loss in LBMresulting from CR, and produce superior improvements to body composition than MIT.

In the current investigation, a 45% kcal HFD for 11 weeks produced a 92.3% increase in BW of male C57BL/6J mice when started at 6 weeks of age. A 25% deficit in energy intake produced by CR or a combination of CR with HIIT or MIT produced rapid and similar between group reductions in BW compared to CON. Although not statistically significant, HIIT resulted in average BWs that were numerically higher than CR alone or MIT + CR (HIIT: 36.5 ± 1.6g, CR: 34.8 ± 1.5g, MIT: 36.0 ±1.5g). An expanding body of literature has established that CR-induced decreases in BW do not occur without a loss of LBM, which is associated with lower REE (*35*). In fact, Ravussin et al(1985) reported that 10-16 weeks of CR resulted in significant declines in 24-hour EE, REE, and LBM (*34*). Our results also indicate that CR produced the expected reduction in LBM compared to CON. When uncorrected for BW, LBM of the HIIT group matched that of CON despite the fact that the mice were 18.7% smaller. These findings indicate that LBM was preserved in response to HIIT, which supports our hypothesis that HIIT would preserve LBM to a greater extent than MIT in the presence of CR.We observed the expected reduction in REE with CR, and HIIT was the only exercise condition thatwas able to rescue this effect. Our findings support the literature in that exercise of greater intensity results in prolonged elevated post-exercise EE, which could explain the augmented REE seen with HIIT(*58-60*). This is promising for future weight

loss and exercise considerations, where HIIT could bethe best-utilized method in attenuating the negative outcomes of CR while taking less overall time than typical aerobic exercise.

Metabolic flexibility is defined as the ability to effectively switch substrate utilization from primarily fat to carbohydrate between fasted and fed states (*61*). Lower RER during a fasted state is indicative of fat metabolism, while refeeding coincides with a greater utilization of carbohydrate apparent by a higher RER. Insulin resistance and glucose intolerance have been linked to impairment of the required switch from fat to carbohydrate with refeeding, which is evident in obesity(*62*). Our data support these previous findings in that our obese CON animals had almost identical RER values within the fasting (light) and feeding (dark) cycles. Each of the dietary/exercise treatments produced significant improvements in the dynamic range of RER excursions in the transition from the light to dark cycle. However, HIIT again produced numerically higher, although statistically similar, increases in metabolic flexibility than CR alone or MIT + CR. While it is suggested that both CR and exercise can induce improvements to metabolic flexibility while in the presence of a HFD, it is not known if there is a relationship between exercise intensity and metabolic flexibility(*28, 63*). Because of the existing relationship between impaired metabolic flexibilityand insulin resistance/glucose intolerance, and the fact that HIIT may be superior in improving these metabolic impairments, it is reasonable to suggest that HIIT may be optimal in enhancing metabolic flexibility(*50, 54*). This could be a valuable focus in future studies researching the benefits of higher intensity aerobic exercise.

The observed increase in metabolic flexibility was associated with improvements in insulin tolerance during the early (≤ 30 minutes) timepoints for CR and HIIT, while no differences were observed for MIT. Emerging evidence suggests that HIIT may be superior to MIT, which agrees withour findings. Infact, studieshave reported improved insulin action in SM, adipose tissue and liver in response to HIIT, but not with MIT (*64*). Additional studies in humans have observed similar findings suggesting that HIIT may be preferential with regards to improving glucose tolerance and insulin sensitivity(*65, 66*).

The HIIT group was the only treatment group that produced improved glucose tolerance following the HFD-induced obesity. Other studies have shown similarities between HIIT and MIT in reducing obesity, but with improved glucose tolerance exclusive to training at higher intensities(*50, 52*). It has been suggested that these improvements are directly related to exercise-induced adaptations within SM, which we speculate to be heightened when the intensity of the exercise is greater(*32, 56*). The adaptations occurring from exercise include increases in SM mass, glycogen storage, and concentration of the GLUT-4 transporter in SM (*67*). Further evidence supporting a higher level of exercise intensity with regards to glucose handling comes from a study comparing HIIT and MIT, where glucose tolerance was greater with HIIT alone (*68*). Although we did not investigate the mechanisms by which HIIT improves metabolic flexibility and insulin/glucose tolerance, it is possible that the preservation of LBM and improvements in function may contribute to the superior outcomes of HIIT. What is particularly interesting is that while CR produced significant improvements in insulin sensitivity, there were no improvements in glucose tolerance. In contrast, HIIT improved both insulin sensitivity and glucose tolerance in the presence of CR, suggesting that

combining HIIT with CR is a superior approach to not only improve fasting, but also dynamic capacity to metabolize glucose following a meal. This is important as it has been suggested that glucose tolerance may represent a more valid assessment of glucose disposal(*69*).

Although our study employed an excellent model of treadmill running in obese mice, we encountered several limitations through out the process. Our original plan to prescribe exercise based off of EE calculations from VO_{2peak} was not possible because of problems that arose during testing. Despite acclimation, the stress induced by the treadmill chamber and exercise test produced skewed values early on, which may have been higher than anticipated due to a combination of hyperventilation and low aerobic capacity. We were unable to properly calculate the given time and intensity necessary for the desired energy deficit induced by each exercise method. Alternatively, we chose to base our exercise protocol off of previously published work on optimal methods of treadmill running and exercise testing in mice in order to ensure the accuracy of our testing, as well as a true difference in the intensity and duration of each method (*70, 71*). Unfortunately, we cannot report that each exercise method produced a 12.5% energy deficit. However, we are able to confirm that the EE achieved through MIT and HIIT were equal. Making the assumption that HIIT and MIT groups exercised at 85% and 65% of maximal oxygen uptake, respectively, we were able to calculate the total exercise-induced EE from our VO_{2peak} values. We did so by averaging oxygen consumption per group (L/min) and multiplying by exercise duration over the course of the 15 weeks. Conversion to energy allowed us to estimate the expended energy over the entire training period.

It should also be noted that one of the mice in our HIIT group appeared to be hypophagic compared to other animals and had considerable difficulty performing the prescribed intensity of exercise. The mouse continued to exercise for the first 5 weeks of the study, but eventually experienced a spinal cord injury and paralysis during treadmill exercise. The animal was immediately removed from the study and monitored by a UAB Veterinarian. By recommendation of the Veterinarian, the animal was euthanized within 2 days of the accident due to lack of improvement in condition. Necropsy was not performed on the mouse so we are unable to speculate as to the reason for the reduced energy intake and injury on the treadmill. We did not experience any other problems with mice in the HIIT or other treatment groups.

Conclusions

A 25% CR produced reductions in LBM and REE, whereas HIIT preserved LBM, increased REE and improved glucose tolerance and insulin sensitivity. These results suggest that HIIT may produce a hypermetabolic state in the presence of CR, which could lead to long-term success in weight loss interventions.

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APPENDIX

Institutional Animal Care and Use Committee Approval

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Institutional Animal Care and Use Committee (IACUC)

NOTICE OF APPROVAL

DATE: September 9, 2014

TO: ERIC P. PLAISANCE, Ph.D.
EB -207
(205) 996-7909

FROM:

Robert A. Kesterson, Ph.D., Chair
Institutional Animal Care and Use Committee (IACUC)

SUBJECT: Title: Role of Energy Restriction and High Intensity Interval Exercise Training on Adaptive Thermogenesis and Metabolism
Sponsor: Internal
Animal Project_Number: 140910194

As of September 9, 2014 the animal use proposed in the above referenced application is approved. The University of Alabama at Birmingham Institutional Animal Care and Use Committee (IACUC) approves the use of the following species and number of animals:

Species	Use Category	Number In Category
Mice	A	38

Animal use must be renewed by September 8, 2015. Approval from the IACUC must be obtained before implementing any changes or modifications in the approved animal use.

Please keep this record for your files, and forward the attached letter to the appropriate granting agency.

Refer to Animal Protocol Number (APN) 140910194 when ordering animals or in any correspondence with the IACUC or Animal Resources Program (ARP) offices regarding this study. If you have concerns or questions regarding this notice, please call the IACUC office at (205) 934-7692.

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM
Institutional Animal Care and Use Committee (IACUC)

MEMORANDUM

DATE: 13-Aug-2015

TO: Plaisance, Eric P.

FROM: Robert A. Kesterson, Ph.D., Chair

Institutional Animal Care and Use Committee (IACUC)

SUBJECT: NOTICE OF APPROVAL

The following application was approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee (IACUC) on 13-Aug-2015.

Protocol PI: Plaisance, Eric P.

Title: Role of Energy Restriction and High Intensity Interval Exercise Training on Adaptive Thermogenesis and Metabolism

Sponsor: UAB DEPARTMENT

Animal Project Number (APN): IACUC-10194

This institution has an Animal Welfare Assurance on file with the Office of Laboratory Animal Welfare (OLAW), is registered as a Research Facility with the USDA, and is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

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