

RESEARCH ARTICLE

Effects of Energy and Macronutrient Cycling on Weight Loss, Body Composition, and Markers of Health in Obese Women Participating in a Resistance-Based Exercise Program

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Abstract

Purpose: To determine whether adherence to a repeating 30-d non-linear diet intervention while participating in a supervised exercise program that includes resistance-exercise would promote weight and fat loss without weight loss plateau and whether alterations in carbohydrate and protein intake may influence results.

Methods: Fifty sedentary and obese pre-menopausal females (35.2 ± 7.6 years; 88.7 ± 18 kg, 32.6 ± 6 kg/m², $42.5 \pm 4.2\%$ fat) were randomly assigned to an exercise-only (EX) or EX plus diets containing higher proportions of carbohydrate or protein. Diets were hypoenergetic for 30-d (7-d at 1,200 kcal/d, 21-d at 1,500 kcal/d), more isoenergetic for 30-d (2,200 kcal/d), and repeated three times during a 24-wk intervention. Diets were either 45:30:24 (HCD) or 30:45:25 (HPD) carbohydrate:protein:fat. All participants performed 30-min resistance exercise (3 d/wk) and a brisk walking program (3 d/wk). Data were analyzed by general linear model (GLM) statistics with repeated measures and presented as mean changes from baseline (mean [UL, LL]).

Results: Participants experienced a significant reduction in body weight (EX -2.24 [-6.5, 2.0], EX+HCD -6.99 [-9.4, -4.5], EX+HPD -4.49 [-7.1, -1.8] %), fat mass (EX -3.45 [-10.9, 4.03], EX+HCD -12.15 [-16.4, -7.9], EX+HPD -8.54 [-13.2, -3.9] %), and percent body fat (EX -1.30 [-5.6, 3.0], EX+HCD -5.91 [-8.3, -3.5], EX+HPD -4.31 [-7.0, -1.6] %) with those in the EX+HCD experiencing a more linear response. Participants in the EX and EX+HCD groups maintained fat-free mass to a better degree (EX -0.89 [-4.0, 2.2], EX+HCD -3.21 [-5.0, -1.2], EX+HPD -1.92 [-3.8, 0.002] %). Participants dieting generally experiencing greater benefit with some evidence that those in the EX+HPD experienced greater changes in blood lipids.

Conclusion: A 30-day repeated non-linear diet can promote fat and weight loss without a plateau. Consuming a HPD may help maintain fat-free mass during weight loss and improve some markers of health compared to HCD interventions.

Trial registration

clinicaltrials.gov, # NCT04344821. Retrospectively registered 9, April 2020.

Keywords: diet; linear, non-linear, high protein, high carbohydrate, resistance-training

1. Introduction

According to the National Center for Health Statistics,¹ the age-adjusted prevalence of obesity among adults in the United States was 42.4% in 2017–2018. This includes a prevalence of 40.0% among younger adults aged 20–39, 44.8% among middle-aged adults aged 40–59, and 42.8% among older adults aged 60 and over.¹ Obesity is associated with several co-

morbidities, including diabetes, arthritis, pulmonary abnormalities, urinary incontinence, cataracts, and certain types of cancer.²⁻⁵ For this reason, strategies to help people effectively lose weight and/or maintain a healthy weight after weight loss has become a paramount public health issue.^{2,6} According to the American Society for Metabolic and Bariatric Surgery,⁷ preoperative weight loss and/or medical nutrition therapy should be encouraged before bariatric surgery to ensure the

candidate for surgery is not responsive to traditional and/or medical weight-loss interventions. There is also some evidence that preoperative weight loss (e.g., 10% body weight or 5% fat loss) may help reduce liver volume, reduce complications with bariatric surgery, and/or improve postoperative outcomes.^{8,9} Additionally, a goal for postoperative bariatric patients after reaching weight loss goals should be to transition toward consuming an energy and macronutrient sufficient diet in order to effectively maintain weight loss. However, one of the challenges with rapid weight loss as occurs with bariatric surgical interventions is a concomitant loss in fat-free mass and a reduction in resting energy expenditure that may increase the likelihood of weight regain.^{10,11} Consequently, bariatric surgeons must recommend effective preoperative and postoperative strategies to promote effective fat loss while maintaining muscle mass, strength, and functional capacity in bariatric patients.

We have previously reported that adherence to a circuit-style resistance exercise program and a higher protein hypoenergetic diet generally promotes greater weight and fat loss while maintaining muscle mass and preserving resting energy expenditure in pre-menopausal and post-menopausal women.¹²⁻²⁰ Moreover, this program promoted gains in aerobic capacity, muscular strength, endurance, and improved markers of health.¹²⁻²⁰ However, we have also noticed that most of the weight loss occurs during the first two to three months of weight loss, followed by a plateau.^{13,17} The plateau has been suggested to be due to a lack of adherence to diet and exercise interventions and/or metabolic adaptation.^{6,21-24} For this reason, there has been an interest in determining whether non-linear and/or intermittent diet approaches may prevent a plateau in weight loss thereby

promoting a gradual reduction in body weight and/or fat mass.⁶

The purpose of this study was to determine whether adherence to a repeated non-linear 30-d diet intervention while participating in an exercise program that includes resistance-exercise may promote gradual weight loss. Further, whether adherence to low-fat hypoenergetic diets with higher proportions of carbohydrate or protein would promote differential effects. We hypothesized that the use of a non-linear diet intervention approach while participating in a supervised exercise program would effectively promote weight loss in comparison to exercise alone without a plateau in and that consuming a diet with a higher proportion of protein would promote greater benefits. The primary outcomes were body weight, body composition, and resting energy expenditure. Secondary outcomes were resting hemodynamics, aerobic capacity, muscular strength, muscular endurance, and fasting glucose and blood lipids.

2.0 Methods

2.1 Experimental Design

This study was conducted at a university-based research setting as a randomized, parallel, prospective diet, and exercise intervention trial. This research protocol was reviewed and approved by the Baylor University Institutional Review Board prior to initiation of the study in accordance with the Declaration of Helsinki²⁵ and subsequently approved by the Texas A&M Human Participant Protection Board (IRB 2008-0643F) after the Principal Investigator moved to that institution. The study is also registered with clinicaltrials.org (#NCT04344821). Pre-menopausal females between the ages of 18 and 45 years with a Body Mass Index (BMI) between 25 and 40 kg/m² were randomly assigned to one of three

treatment groups: 1.) Exercise plus no diet intervention (EX); 2.) Exercise plus higher carbohydrate diet (EX+HCD); or, 3.) Exercise plus higher protein diet (EX+HPD). The exercise and diet interventions were modeled after the Curves[®] for Women (*Curves International, Waco, TX, USA*) fitness and weight management program.²⁶ This exercise and weight management program has been used by millions of women per year in over 70 countries worldwide since the early 1990's.²⁶ The specific focus of this investigation was to identify the impact of cycling different energy intake levels with diets containing higher proportions of carbohydrate or higher protein on weight loss, body composition, and markers of health and fitness to determine if this type of diet cycling may be an effective way of promoting continual weight loss without reductions in resting energy expenditure or fat-free mass. Primary outcome variables included body weight, body composition, and resting energy expenditure. Secondary outcome variables included resting hemodynamics, aerobic capacity, muscular strength, muscular endurance, and clinical blood panels.

2.2 *Participants*

Participants were recruited from local newspapers, radio advertisements, flyers, and the Internet. Participants meeting eligibility criteria attended a familiarization session. Entrance criteria stipulated the recruitment of sedentary pre-menopausal females between

the ages of 18-45 years with a BMI between 25 and 40 kg/m². Participants were not allowed to participate in the study if they: 1.) Had a recent history of weight change (± 3.5 kg within 3 months); 2.) Had a history of any metabolic disorders including known electrolyte abnormalities, heart disease, arrhythmias, diabetes, or thyroid disease; 3.) Had a history of hypertension, hepatorenal, musculoskeletal, autoimmune, or neurologic disease; 4.) Had been pregnant or lactating within the past 12 months or were planning to become pregnant during the next 12 months; 5.) Had participated in a planned exercise program or regularly exercised (≥ 30 min/d 3 days/wk) within the past three months; or, 6.) Had taken ergogenic levels of nutritional supplements that may affect muscle mass or weight loss within the three months prior to the start of the study. Figure 1 presents a consolidated standards of reporting trials (CONSORT) diagram. Approximately 324 women responded to research advertisements with 257 undergoing preliminary phone screening for eligibility. Of these, 142 were familiarized with the experimental protocol, consented to participate in the study, and underwent baseline testing. A total of 50 women who were 35.1 ± 7.6 years old, 88.7 ± 17.9 kg, 32.6 ± 5.8 kg/m², and 42.5 ± 4.2 % body fat) completed the study. Those who withdrew did so primarily due to time constraints and/or poor compliance to the exercise and/or diet protocol. Study recruitment spanned for approximately 24 months.

Recruitment	Responded to Research Advertisements (n=324)		
	Screened by Phone for Eligibility (n=257)		
	Consented, Randomized to Treatments, Agreed to Participate in Study (n=142)		
Allocation	EX (n=8)*	EX+HCD (n=69)	EX+HPD (n=65)
Intervention	Discontinued after baseline testing (n=1) due to: • Time Constraints (n=1)	Discontinued intervention (n=46) after 1 month (n=20), 2 months (n=10), 3 months (n=7), 4 months (n=9) due to: • Scheduling Conflicts • Non-adherence to exercise or diet program • Personal reasons	Discontinued intervention (n=45) after 1 month (n=22), 2 months (n=11), 3 months (n=10), 4 months (n=2) due to: • Scheduling Conflicts • Non-adherence to exercise or diet program • Personal reasons
Analyzed	EX (n=7)	EX+HCD (n=23)	EX+HPD (n=20)

* Participants who wanted to participate in study but did not want to be put on a diet.

Figure 1. Consolidated standards of reporting trials (CONSORT) flow chart.

2.3 Testing Sequence

Figure 2 provides an overview of the experimental design. Participants attended a detailed familiarization session before baseline testing. This included being familiarized about the study requirements, obtaining informed consent, obtaining health history information, and undergoing a general physical exam to assess study eligibility. Those meeting study eligibility criteria were then instructed about how to follow the diet plans and practiced using the exercise equipment. Participants were asked to record all food and energy-containing beverages for 4-days prior to each testing session (three weekdays and one weekend day). Participants were asked to refrain from vigorous physical activity, alcohol intake, and ingestion of over the counter medications for 48 h before testing. In addition, participants fasted for 12 h before reporting to the laboratory. All testing was performed at the same time of the month, during the 10

days following menstruation, and in the early morning hours at approximately the same time in order to control for diurnal variations in hormone levels and resting metabolism. The following measures were obtained at each testing session: body weight; body composition determined by dual-energy X-ray absorptiometry (DEXA); hip and waist measurements; resting energy expenditure (REE); resting heart rate and blood pressure measurement; fasting whole blood and serum samples; 1 repetition maximum (1RM) lifts on the bench press and leg press; upper and lower body muscular endurance (maximum repetitions performed at 80% of 1RM); and, a maximal cardiopulmonary exercise stress test. Participants also completed a medical safety and side effect report that was reviewed weekly. Follow-up testing included all baseline tests after 4, 8, 12, 16, 20, and 24 weeks with the exception that exercise capacity tests were only obtained after 12 and 24 weeks of intervention.

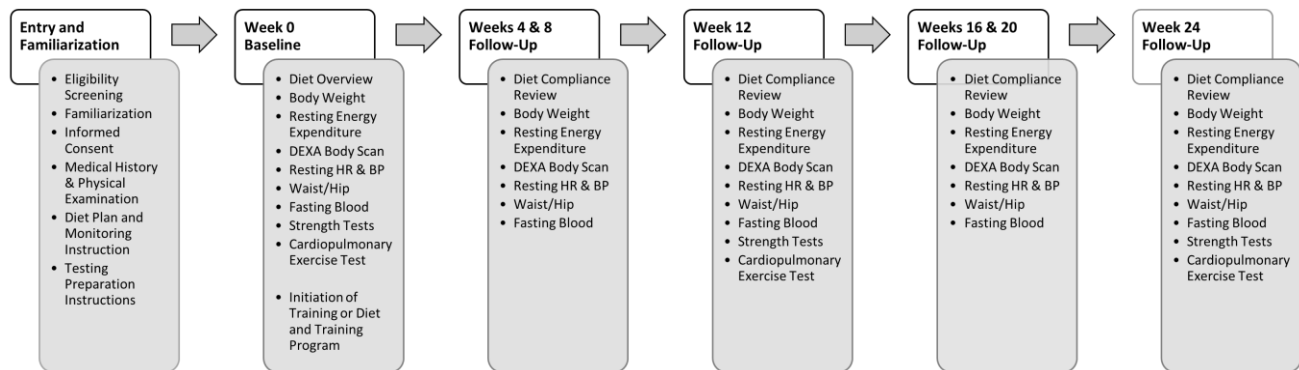


Figure 2. Overview of study testing and timeline.

2.4 Diet Intervention

As consistent with several previously published studies from our research group,^{12,16-20,27} participants were randomly assigned at baseline in a block fashion according to age and body mass to one of the following groups: 1.) Exercise-only (EX); 2.) Exercise plus a higher carbohydrate diet (EX+HCD); or, 3.) Exercise plus a higher protein diet (EX+HPD). Given that the primary reason individuals were interested in participating in the study was to experience weight loss, a number of women initially assigned to the EX group declined to participate unless they were assigned to one of the diet treatments. In these cases, the participants were randomly re-assigned based on age and weight to one of the exercise and diet interventions. Thus, the EX group has fewer participants than the diet intervention groups. Participants in the EX group participated in exercise training

sessions while maintaining their normal dietary habits. Participants assigned to the diet interventions followed the Curves 30/30 weight management program²⁶ as summarized in Table 1. Meal plans were prepared by a registered dietitian and provided interchangeable meals providing 1,200 kcal/d for 1 week followed by 1,500 kcal/d for 3-weeks during each diet phase. This was followed by meal plans providing 2,200 kcals/d for 4-weeks during a metabolic recovery diet phase. Participants repeated this 8-week diet cycle three times during the study. Thus, diets were considered low energy, low-fat diets with the primary difference being the amount of carbohydrate and protein ingested. Previous research in our lab has demonstrated that this diet intervention generally increases protein intake to 24% to 35% while maintaining fat intake between 25% and 30% based on analysis of food logs.^{12,16-20,27}

Table 1. Diet Cycling Energy Intake and Macronutrient Plan.

Weeks	0-4		4-8	8-12		12-16	16-20		20-24
Days	7	21	28	7	21	28	7	21	28
Energy Intake (kcal/d)	1,200	1,500	2,200	1,200	1,500	2,200	1,200	1,500	2,200
Higher Carbohydrate Diet									
Carbohydrate (% g/d)	45 135	45 169	45 248	45 135	45 169	45 248	45 135	45 169	45 248
Protein (% g/d)	30 90	30 113	30 165	30 90	30 113	30 165	30 90	30 113	30 165
Fat (% g/d)	25 33	25 42	25 61	25 33	25 42	25 61	25 33	25 42	25 61
Higher Protein Diet									
Carbohydrate (% g/d)	30 90	30 113	30 165	30 90	30 113	30 165	30 90	30 113	30 165
Protein (% g/d)	45 135	45 169	45 248	45 135	45 169	45 248	45 135	45 169	45 248
Fat (g/d)	25 33	25 42	25 61	25 33	25 42	25 61	25 33	25 42	25 61

2.5 Training Protocol

Participants performed the 30-min Curves resistance-based circuit exercise program 3 d/wk as well as walked for 30-min/d at a brisk pace on non-circuit training days throughout of the 24-week study. The circuit included 13 bi-directional hydraulic concentric-only resistance exercise machines which involved all major muscle groups (i.e., elbow flexion/extension, knee flexion/extension, shoulder press/latissimus dorsi pull, hip abductor/adductor, chest press/seated row, horizontal leg press, squat, abdominal crunch/back extension, chest flies, oblique, shoulder shrug/dip, hip extension, and side bends). During each training session, participants were coached to perform as many repetitions as possible within a 30-s period on each resistance machine. Between machines, participants performed floor-based aerobic exercises or stepping exercise for 30-s to maintain an elevated heart rate. Participants completed the entire circuit twice during each workout and then performed stretching exercises. All workouts were supervised by trained fitness instructors who monitored proper exercise technique and maintenance of adequate exercise intensity. Previous work from our lab indicated this workout regimen yields an average exercise heart rate of 126 ± 15 bpm (80% of maximal heart rate), exercise intensity of $65 \pm 10\%$ of peak oxygen uptake, resistance exercise intensities ranging between 61% and 82% of 1RM, and expends approximately 314 ± 102

kcal per workout.^{26,28-30} Compliance with the exercise program was set a priori at a minimum of 70% compliance (50/72 exercise sessions).

3.0 Procedures

3.1 Dietary Assessment

Participants were given dietary plans and meal menus to follow at the start of the study and instructed by a registered dietitian about how to follow the diet and select meal and/or food substitutions to provide variety to the diets. Diet compliance was checked by research assistants and a registered dietitian following exercise sessions as well as by reviewing food logs before each testing session. Food logs were analyzed using the ESHA Food Processor v8.6 Nutritional Analysis software (ESHA Research Inc., Salem, OR).

3.2 Resting Energy Expenditure and Metabolism

Resting energy expenditure (REE) was assessed using a Parvo Medics TrueMax 2400 Metabolic Measurement System (ParvoMedics, Inc., Sandy, UT, USA). This test was performed in a fasted state with the participants lying supine on an exam table. A clear, hard plastic hood and a soft, clear plastic drape were placed over the participants' neck and head in order to determine resting oxygen uptake and energy expenditure. A dilution pump controlled the

flow of air to ensure a consistent percentage of carbon dioxide (0.8 – 1.2%) flowing through the hood. Participants remained motionless without falling asleep for approximately 20 min. Data were recorded after the first ten minutes of testing during five minutes in which criterion variables (e.g., VO_2 L/min) changed less than 5%.³¹ Test–retest measurements from a study previously reported²⁰ revealed that test–retest correlations (r) of collected VO_2 in L/min ranged from 0.315 to 0.901 (mean 0.638) and the coefficient of variation ranged from 8.2 to 12.0% (mean: 9.9%) with a mean intra-class coefficient of 0.942, $p < 0.001$.

3.3 Body Composition and Anthropometric Measures

Height and body weight were measured according to standard procedures using a calibrated electronic scale (*Cardinal Detecto Scale Model 8430, Webb City, MO, USA*), with a precision of ± 0.02 kg. Waist and hip circumference was measured using a Gulick tensiometer tape measure using standard criteria.³² Body composition and bone density (excluding the cranium) were evaluated using calibrated Hologic Discovery W (*Hologic Inc., Waltham, MA, USA*) dual-energy X-ray absorptiometry (DEXA) equipped with APEX Software (*APEX Corporation Software, Pittsburg, PA, USA*). Test–retest reliability studies performed with this DEXA machine have previously yielded mean coefficients of variation for total bone mineral content and total fat-free/soft tissue mass of 0.31–0.45% with a mean intra-class correlation of 0.985.³³

3.4 Exercise Capacity

Resting heart rate was determined by palpation of the radial artery using standard procedures.³² Blood pressure was assessed by auscultation of the brachial artery using an aneroid sphygmomanometer using standard

clinical procedures.³² Resting heart rate and blood pressure measurements were taken in the supine position after resting for 5-min. Participants were attached to a Quinton 710 ECG (*Quinton Instruments, Bothell, WA, USA*) and walked on a Trackmaster TMX425C treadmill (*JAS Fitness Systems, Newton, KS, USA*). Expired gases were collected using a Parvo Medics 2400 TrueMax Metabolic Measurement System (*ParvoMedics, Inc., Sandy, UT, USA*). Participants then performed a standard symptom-limited maximal Bruce treadmill exercise test according to standard procedures.³² Calibration of gas and flow sensors was completed prior to testing and was found to be within 3% of the previous calibration point.

A standard isotonic Olympic bench press (*Nebula Fitness, Versailles, OH, USA*) was used for the isotonic bench press testing. A 1RM testing procedure was performed using standard procedures with 2-min recovery between attempts.³² Following 1RM testing, participants rested for 5-min and then performed a maximum number of repetitions at 80% of 1RM on the bench press to determine upper body muscular endurance. Participants were then given 5-min of rest and then had their lower body 1RM maximal strength determined using a hip sled/leg press (*Nebula Fitness, Versailles, OH, USA*) using standard testing procedures with 2-min rest recovery between attempts.³² Participants then rested for 5-min and performed a maximum number of repetitions at 80% of hip sled/leg press 1RM to assess lower body muscular endurance. Total lifting volume was calculated by multiplying the number of repetitions performed during the endurance test times the amount of weight lifted expressed in kg. Test to test reliability of performing these strength tests in our lab has yielded low mean coefficients of variation (C_v) and high reliability for the bench press

(Cv: 1.9%, intra-class $r = 0.94$) and hip sled/leg press (Cv: 0.7%, intra-class $r = 0.91$).

3.5 *Blood Collection and Analysis*

Fasted serum samples were collected in BD Vacutainer® SST™ Serum Separation Tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) using standard phlebotomy techniques from an antecubital vein in the forearm. Blood samples were then centrifuged at 1,200 rpm for 15-min. Serum samples were analyzed for glucose and lipid levels by Quest Diagnostics (Quest Diagnostics, 5850 Rogerdale Road, Houston, TX, USA 77072) using an Olympus AAU 5400 Chemistry Immuno Analyzer (Olympus America Inc., Center Valley, PA, USA). Test-retest reliability of performing assays using this system range from 2% to 6%.

3.6 *Statistical Analysis*

A priori power calculation was set at >0.80 and was based on the observed change in fat mass between diet groups from previous research in our lab utilizing similar diet and exercise interventions.^{14-20,34-36} This analysis revealed that a sample size of 15-20 participants per group was sufficient to detect meaningful changes (~2 kg) in fat mass among exercise-only and hypoenergetic diet groups. Participants were included in the analysis if they completed at least 5-months of intervention. Missing data were extrapolated, carrying forward the last observed value. This was found to be more representative of individual responses than conducting data imputation analysis. Data were analyzed using IBM® SPSS® version 26 Statistics for Windows (IBM Corp., Armonk, NY, USA). General linear modal (GLM) multivariate analysis was used to determine

differences between groups at baseline. Since a significant difference was observed among groups in body weight, baseline body weight was used as a covariate in subsequent analyses. Related variables were analyzed using univariate, multivariate and repeated measures GLM statistics. The overall multivariate Wilks' Lambda and Greenhouse-Geisser univariate p-levels are reported. Data were considered significant when the probability of type I error was 0.05 or less, and statistical trends toward significance were noted if the p-level ranged between 0.05 and 0.10. Partial eta squared effect sizes (η_p^2) are reported as an indicator of effect size³⁷. An eta squared around 0.02 was considered small, 0.13 medium, and 0.26 large.³⁷ Tukey's least significant differences (LSD) post-hoc analyses were performed to determine differences among groups. Mean changes from baseline as well as percent changes from baseline were calculated and analyzed using one-way ANOVA to determine mean changes with 95% confidence intervals (CI). Mean changes with 95% CI's completely above or below baseline were considered significantly different.³⁷ Data are presented as means \pm standard deviations, mean [95% CI] change from baseline, or mean [95% CI] percent change from baseline.

4.0 *Results*

4.1 *Participant Demographics*

Table 2 presents the baseline participant demographics. One-way ANOVA revealed that there were significant differences among treatment groups in body weight and BMI, with no other baseline differences observed. Therefore, body weight was used as a covariate in subsequent GLM analyses.

Table 2. Baseline Characteristics.

	EX	EX+HCD	EX+HPD	Mean	p-level
Age (yrs)	35.0 ± 7.2	34.3 ± 6.4	36.0 ± 8.7	35.2 ± 7.6	0.779
Weight (kg)	85.0 ± 16.2	82.5 ± 14.4 ^a	97.0 ± 19.6 ^b	88.7 ± 17.9	0.022
Height (cm)	164.9 ± 3.7	164.2 ± 6.4	165.2 ± 7.5	164.7 ± 6.5	0.880
BMI (kg/m ²)	31.2 ± 5.4	30.47 ± 4.1	35.5 ± 6.6	32.6 ± 5.8	0.011
Body fat (%)	40.8 ± 3.1	41.92 ± 3.4 ^a	43.8 ± 5.0 ^b	42.5 ± 4.2	0.170

Data are expressed as means ± standard deviations for the exercise only group (EX), exercise plus higher carbohydrate hypo-energetic diet (EX+HCD), and exercise plus higher protein hypo-energetic diet (EX+HPD). a = p<0.05 difference from EX, b = p<0.05 difference from EX+HC, c = p<0.05 difference from EX+HP.

4.2 Energy and Macronutrient Intake

Analysis of initial dietary records revealed that participants assigned to diet interventions reported consuming 1,940 [1,745, 2,133] kcals/d, 239 [210, 268] g/d of carbohydrate, 76 [68, 93] g/d (0.91 [0.83, 1.00] g/kg/d) of protein, and 45 [39, 52] g/d of fat at baseline. Self-reported energy intake in the diet groups decreased to 1,321 [1,226, 1,415] kcals/d after first phase of the diet with significant differences observed between diet groups in protein intake (EX+HCD 65 [58, 73], EX+HCD 96 [73, 120] g/d, $p=0.02$; EX+HCD 0.88 [0.69, 1.07], EX+HCD 1.20 [0.92, 1.46] g/kg/d, $p=0.07$; EX+HCD 20.6 [17.6, 23.4], EX+HCD 28.4 [22.4, 34.4] %, $p=0.03$). Self-reported energy intake increased to 1,524 [1,173, 1,875] kcals/d during the more isoenergetic metabolic recovery period with similar differences observed in protein intake. These findings agree with previous reports from our lab, indicating that this diet protocol results in a decrease in caloric intake with differential protein intake during the assigned diet phases, and that participants tend to maintain lower than assigned energy and macronutrient intake during the recovery / weight maintenance phase of the diet.^{12,16-20,27}

4.3 Body Weight and Composition

Supplemental Table 1 presents body composition data observed among groups during the study, while Figure 3 shows mean changes from baseline with 95% CI's. GLM analysis revealed an overall Wilks' Lambda Time ($p=0.001$) and Group x Time ($p=0.735$) effects for body composition variables. Univariate analysis revealed no significant Group x Time interactions. However, pairwise-comparison revealed significant reductions in body mass, fat mass, and percent body fat over time in diet groups. Additionally, analysis of mean changes from baseline with 95% CI's found that dieting promoted significantly greater reductions in body weight, fat mass, and percent body fat in comparison to the EX intervention. No significant differences were observed between the types of diet interventions. In percentage terms, participants in the diet and exercise interventions experienced an overall significant and gradual reduction in body weight (EX -2.24 [-6.5, 2.0], EX+HCD -6.99 [-9.4, -4.5], EX+HCD -4.49 [-7.1, -1.8] %), fat mass (EX -3.45 [-10.9, 4.03], EX+HCD -12.15 [-16.4, -7.9], EX+HCD -8.54 [-13.2, -3.9] %), and percent body fat (EX -1.30 [-5.6, 3.0], EX+HCD -5.91 [-8.3, -3.5], EX+HCD -4.31 [-7.0, -1.6] %) while participants in the EX and EX+HCD groups maintained fat-free

mass to a better degree (EX -0.89 [-4.0, 2.2], EX+HCD -3.21 [-5.0, -1.2], EX+HPD -1.92 [-3.8, 0.002] %) as evidenced by the 95% CI's not crossing baseline values. However, pairwise comparison revealed no significant

differences between diet groups. Progressive fat loss was observed, particularly in the higher carbohydrate group, although changes from month to month were less pronounced during the last two months.

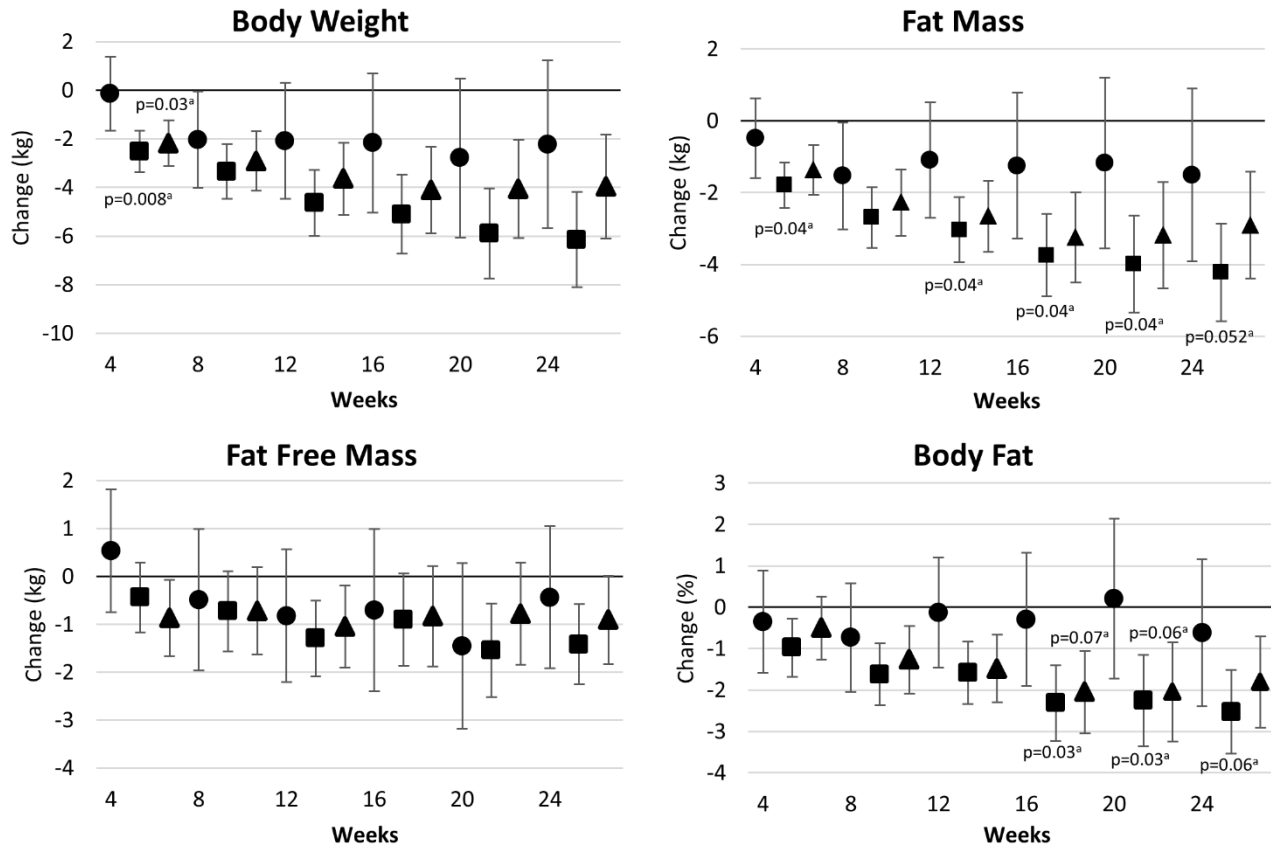


Figure 3. Body composition changes from baseline for the exercise (EX = ●), exercise plus higher carbohydrate diet (EX+HCD = ■), and exercise plus higher protein diet (EX+HPD = ▲) groups. Data are means and 95% confidence intervals (CI). Mean changes and 95% CI's completely above or below baseline represent a significant change from baseline values. Superscripts a = $p < 0.05$ difference from EX.

4.4 Resting Energy Expenditure

Supplemental Table 2 presents resting energy expenditure and respiratory exchange ratio data, while Figure 4 shows mean changes from baseline with 95% CI results. General Linear Model analysis revealed overall Wilks' Lambda Time ($p = 0.185$) and Group x Time ($p = 0.896$) effects for resting energy expenditure variables. Univariate

analysis revealed no significant Time or Group x Time effects. Analysis of mean changes from baseline with 95% CI's found that those in the exercise-only group experienced a significant reduction in resting energy expenditure after 20 weeks of intervention while those in the diet intervention groups were able to maintain resting energy expenditure to a greater degree. This finding was also seen when

evaluating changes in REE expressed in kcals/kg/d. RER values decreased from baseline in the EC-HCD group after 4-weeks of intervention suggesting a greater fat

oxidation. However, no significant differences were observed among treatments thereafter.

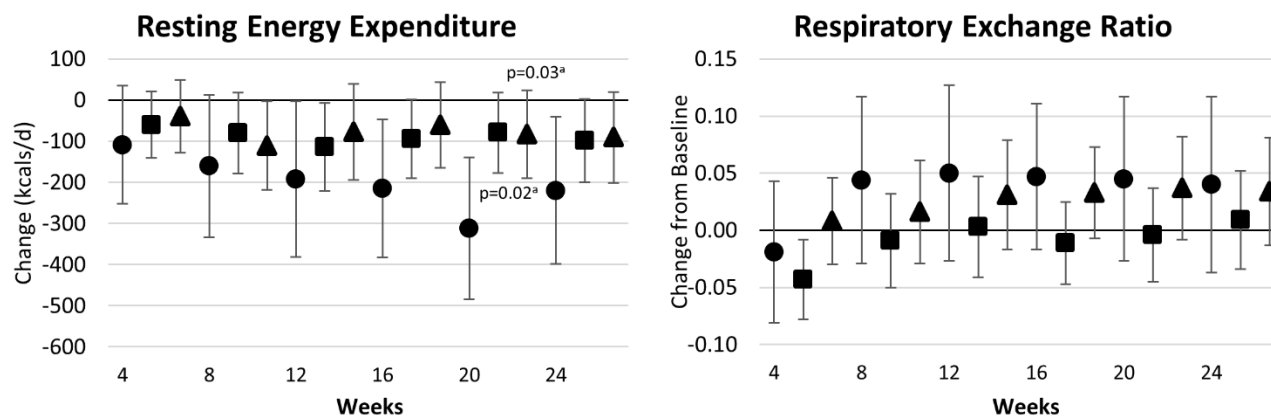


Figure 4. Resting energy expenditure changes from baseline for the exercise-only (EX = ●), exercise plus higher carbohydrate diet (EX+HCD = ■), and exercise plus higher protein diet (EX+HPD = ▲) groups. Data are means and 95% confidence intervals (CI). Mean changes and 95% CI's completely above or below baseline represent a significant change from baseline values. Superscript a = $p < 0.05$ difference from EX.

4.5 Exercise Capacity

Supplemental Table 3 depicts health and fitness-related variables. General Linear Model analysis revealed overall Wilks' Lambda Time ($p=0.019$) and Group x Time ($p=0.429$) effects for fitness-related variables. Univariate analysis demonstrated that time to peak aerobic capacity (VO_2) significantly increased over time ($p=0.003$). Pairwise comparisons revealed significant increases over time in peak VO_2 , 1 RM bench press, and lower extremity lifting volume. Those in the diet groups experienced significantly greater increases in time to peak VO_2 .

4.6 Health-Related Variables

Supplemental Table 4 presents health-related variables analyzed during the study. GLM analysis revealed an overall Wilks' Lambda Time ($p=0.738$) and Group x Time ($p=0.014$) effects for health-related variables. Univariate analysis revealed a significant

interaction effect in resting heart rate with those in the diet groups experience a greater reduction in resting heart rate after 4 & 8-weeks of intervention. There was some evidence that dieting promoted greater reductions in systolic and diastolic blood pressure as well as waist and hip circumference compared to the exercise-only group.

4.7 Blood Glucose and Lipids

Supplemental Table 5 shows fasting glucose and lipid-related data. General Linear Model analysis revealed overall Wilks' Lambda Time ($p=0.559$) and Group x Time ($p=0.196$) effects for glucose and blood lipid variables. Univariate analysis revealed no significant Time or Group x Time effects. However, pairwise comparison analysis revealed some evidence that those in the diet interventions maintained lower blood glucose levels and experienced greater reductions from baseline in total cholesterol,

LDL cholesterol, the ratio of total cholesterol to HDL cholesterol, and triglycerides.

5.0 Discussion and Conclusions

Given that preoperative and postoperative bariatric patients are often prescribed diet interventions, bariatric surgeons need to recommend effective weight loss and maintenance strategies. Rapid weight loss is associated with reductions in fat and muscle mass and concomitant decreases in resting energy expenditure. The loss of fat-free mass and REE may make it more likely for individuals to regain lost weight over time. Since it's more challenging to regain fat-free mass, the weight gain is often fat mass resulting in increases in percent body fat despite maintaining lower body weight after an intervention². Resistance training and adherence to a higher protein hypoenergetic diets have been suggested as a means of preserving fat-free mass and REE during weight loss.^{12,38} Additionally, adherence to non-linear diet approaches has been suggested to lessen metabolic adaptation and thereby promote gradual weight and fat loss without plateaus.^{6,21} The purpose of this study was to determine whether adherence to a repeated non-linear 30-d diet intervention while participating in an exercise program that includes resistance-exercise may promote gradual weight loss. Further, whether adherence to low-fat hypoenergetic diets with higher proportions of carbohydrate or protein would promote differential effects. We hypothesized that the use of a non-linear diet intervention while participating in a supervised exercise program that includes resistance-exercise would effectively promote weight loss without weight loss plateau in comparison to exercise alone and that consuming a diet with a higher proportion of protein would promote greater benefits. If effective, this type of diet cycling approach might serve as a more feasible diet

and exercise intervention for preoperative and/or postoperative bariatric patients.

5.1 Primary Outcomes

Results from the present study indicated that adherence to a resistance-based circuit training exercise program with walking on non-circuit training days promoted modest but non-significant changes in weight (EX -2.22 [-5.7, 1.23] kg), fat mass (EX -1.5 [-3.9, 0.9] kg), fat-free mass (EX -0.4 [-1.9, 1.0] kg), and percent body fat (EX -0.6 [-2.4, 1.2] %). These findings are consistent with other studies indicating that exercise alone may only promote small changes in body weight and composition without diet intervention³⁹. As expected, adhering to a 30-d non-linear weight loss program promoted significantly greater weight loss (EX+HCD -6.1 [-8.1, -4.2], EX+HPD -4.0 [-1.1, -1.8] kg) and fat loss (-4.2 [-5.6, -2.9], EX+HPD -2.9 [-4.3, -1.4] kg) than those exercising alone. These changes were more clinically significant in the EX+HCD groups when expressed in percentage terms (weight: EX+HCD -7.0 [-9.4, 4.6], EX+HPD -4.6 [-7.1, -1.8] %; fat mass (EX+HCD -12.1 [-16.4, -7.9], EX+HCD -8.5 [-13.2, -3.9] %). Additionally, those exercising and dieting were able to effectively maintain resting energy expenditure (EX -2.1 [-4.2, 0.1], EX+HCD -0.03 [-4.2, 0.1], EX+HPD -0.2 [-1.6, 1.1] kcal/kg/d) while not experiencing significant loss in fat-free mass (EX -0.4 [-1.9, 1.0], EX+HCD -1.4 [-2.3, -0.6], EX+HCD -0.9 [-1.8, 0.1] kg; EX -0.9 [-4.0, 2.2], EX+HCD -3.2 [-5.0, -1.5], EX+HCD -1.9 [-3.8, 0.002] %). The amount of weight and fat loss was consistent with our previous reports.^{12-14,16,17,19,27} The magnitude of weight loss observed also meets recommendations that bariatric surgery candidates should try to lose 5% of fat mass prior to surgical intervention, if possible, to improve surgical outcomes.^{8,9} These findings indicate that diet

cycling during exercise training can serve as an effective means to promote weight loss without significant reductions in resting energy expenditure or fat-free mass. These findings are consistent with studies showing that non-linear diet interventions can lead to similar weight loss.^{21,40-45} However, it should be noted that weight and fat loss plateaued toward then end of the intervention in the higher protein group while continuing to progressively decrease in the higher carbohydrate group. Therefore, we accept our first hypothesis that adherence to repeated 30-d cycles of hypoenergetic diets can serve as an effective means of promoting fat loss and that progressive weight loss without an observable plateau is possible when following the higher carbohydrate version of this diet.

In terms of diet types, we have generally reported that both higher carbohydrate and higher protein hypoenergetic diets are effective in promoting weight and fat loss while participating in this exercise program with those consuming higher protein diets generally observing slightly greater benefits^{12-20,27} consistent with previous reports.^{6,46} However, not all studies have reported that higher protein hypoenergetic diet interventions promote greater fat loss.²⁰ In the present study, no statistically significant differences were observed in changes in weight or fat loss between diets although participants following the higher carbohydrate diet generally experienced greater weight and fat loss success, particularly toward the end of the diet intervention and when expressed as a percent change from baseline. However, there was some evidence that those in the higher protein diet group were able to maintain fat-free mass to a better degree toward the end of the 24-week diet. Thus, we are unable to accept our second hypothesis that adherence to a non-linear higher protein diet during a supervised exercise program that includes resistance

training would promote greater weight and/or fat loss.

5.2 Secondary Outcomes

The results of the present study indicated that participation in this exercise program promoted general improvement in markers of fitness and health. This includes significant improvements in peak aerobic capacity (8.1 [1.0, 15.1] %), time to reach peak aerobic capacity (+7.9 [2.8, 13.1] %), bench press 1RM (+9.1 [4.6, 14.2] %), leg press lifting volume endurance (+21.9 [1.2, 43.6] %), waist circumference (-3.7 [-5.9, -1.5] %), total cholesterol (-4.3 [-7.9, -1.88] %), LDL cholesterol (-8.0 [-13.9, -2.7] %), and HDL cholesterol (+4.5 [0.2, 8.8] %). Analysis of mean changes from baseline with 95% CI's revealed evidence that those in the diet groups experienced greater reductions in total cholesterol, LDL cholesterol, and triglycerides in comparison to the exercise-only group and that those following the higher protein diet experienced some additional benefit. These findings are consistent with our prior reports indicating that following this exercise and/or diet intervention promotes improvements in strength, endurance, aerobic capacity, blood lipid profiles, and insulin sensitivity.¹⁴⁻²⁰ Additionally, they are consistent with reports indicating that exercise and/or diet-induced weight-loss improves markers of health.^{14-20,47-49} These findings generally support our second hypothesis, but more research is needed to confirm these findings. Nevertheless, use of these types of exercise and diet interventions could be used to in preoperative bariatric patients to promote weight loss and improve risk to commodities and/or after surgery to help them preserve muscle mass during weight loss.

5.3 *Strengths and Limitations*

The major strength of this study was that it examined the effects of following a repeated non-linear 30-d hypoenergetic diet intervention on weight loss efficacy and markers of health in obese women participating in an exercise program that incorporated resistance-training. Additionally, higher carbohydrate and protein diets were compared to determine whether variation in macronutrient intake would influence outcomes. The major limitations included that the population studied was limited to studying volunteers responding to study advertisements who desired to lose weight from a small geographical area; that some women did not want to participate in the exercise-only arm of the study and thereby were re-randomized for assignment to one of the diet interventions groups creating an unequal *n*-size in treatment groups; and, the fact it is difficult to prevent attrition when conducting exercise and diet interventions lasting six months in duration. Thus, these limitations need to be considered when interpreting results.

5.4 *Conclusion and Future Directions*

Results from this study indicate that use of repeated 30-day diet interventions (1,200 kcals/d for 7-d and 1,500 kcals/d for 21-d) followed by allowing participants to increase energy intake up to 2,200 kcals/d for 30-d was effective in promoting loss in body and fat mass. Changes were more linear in nature in those following the higher carbohydrate hypoenergetic diet. Additionally, higher carbohydrate and higher protein diets effectively promoted weight and fat loss without marked loss in fat-free mass or resting energy expenditure. While differences in weight and fat loss were not significantly different between groups, those following the higher carbohydrate diet generally lost a greater percentage of weight

and fat as well as observed a more linear loss over time. In contrast, those consuming the higher protein diet generally maintained fat-free mass to a greater degree. Both diet interventions promoted clinically significant fat mass loss and improvements in markers of health. These findings suggest that a non-linear diet approach may be an effective type of diet for clinicians to consider recommending for patients who may benefit from preoperative and postoperative exercise and nutrition intervention. Additional research should compare the effects of linear and non-linear low-fat diet approaches with different levels of carbohydrate and protein while participating in a resistance-based exercise program on weight loss, body composition, resting energy expenditure, and markers of health and fitness.

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Declarations***Ethics approval***

This study was reviewed and approved by Baylor University's and Texas A&M University's Institutional Review Board (IRB 2008-0643F) in compliance in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable.

Availability of data and materials

Data and/or statistical analyses are available upon request on a case by case basis for non-commercial scientific inquiry and/or educational use as long as IRB restrictions and research agreement terms are not violated.

Author Contributions

R.B.K. served as Principal Investigator, designed the study, obtained external funding, provided project oversight, directed the research lab, assisted in data analysis, and finalized the manuscript. J.K. served as the study coordinator, oversaw all testing and data entry, and contributed to statistical analyses. M.B., J.M.O., C.C.B., M.M., S.S., Y.P.J., M.K., D.K., B.L., and R.D. assisted with data collection, entry, and analyses. C.R. served as lab coordinator and provided

project oversight. All authors reviewed and approved this submission.

Conflicts of Interest

Curves[®] International (Waco, TX, USA) provided funding for this project through an unrestricted research grant to Baylor University when the Principal Investigator and the Exercise & Sport Nutrition Lab were affiliated with that institution and to Texas A&M University after R.B.K. and his research team moved to that institution. R.B.K. currently serves as a non-paid member of the Curves Scientific Advisory Board. All researchers involved independently collected, analyzed, and interpreted the results of this study and have no financial interests concerning the outcome of this investigation. Publication of these findings does not constitute an endorsement by the investigators or their institutions of the programs or materials investigated.

Supplementary Materials

The following tables are provided in a supplemental file: Supplemental Table 1. Body Composition Data; Supplemental Table 2. Resting Energy Expenditure (RER) Data; Supplemental Table 3. Fitness-related Variables; Supplemental Table 4. Health-related Variables; Supplemental Table 5. Fasting Blood Glucose and Lipid Data.

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