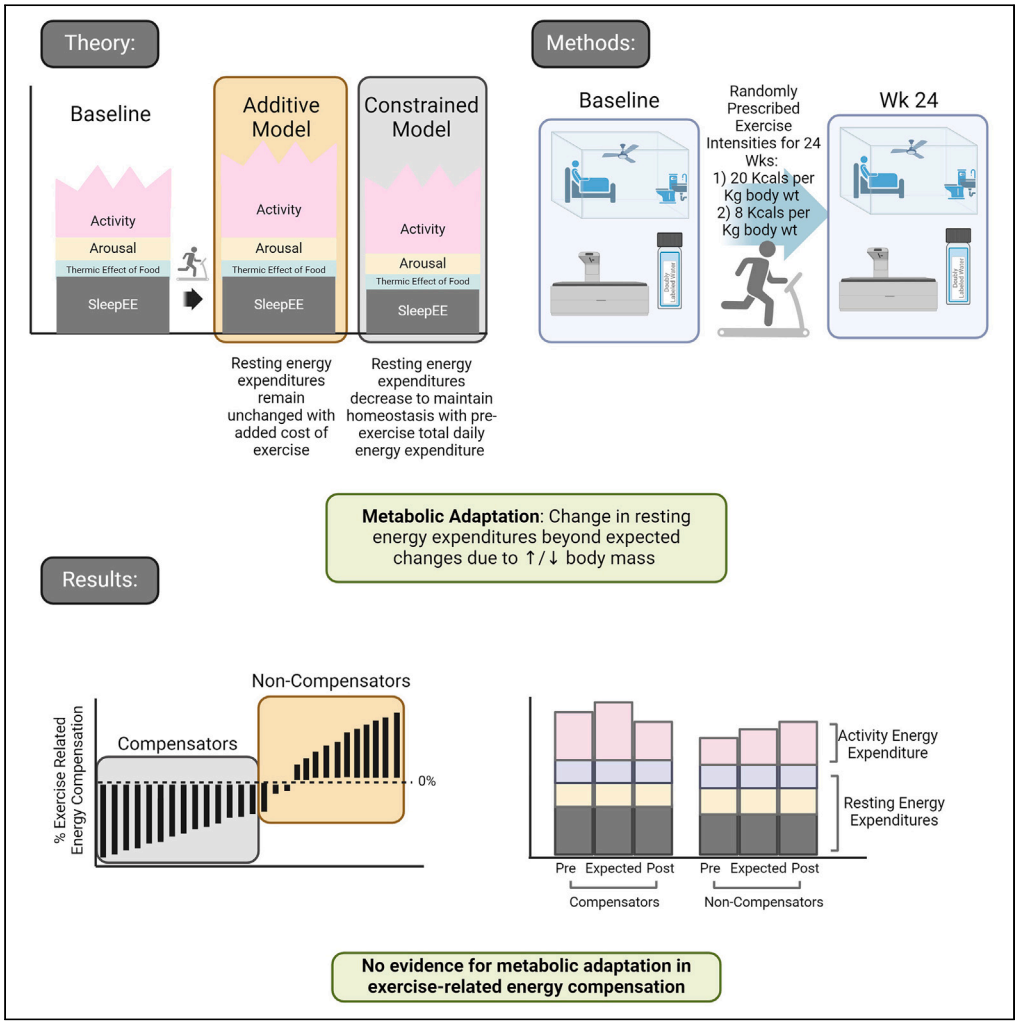
iScience

CellPress
OPEN ACCESS

Article

No evidence for metabolic adaptation during exercise-related energy compensation



E.W. Flanagan, G. Sanchez-Delgado, C.K. Martin, E. Ravussin, H. Pontzer, L.M. Redman

leanne.redman@pbrc.edu

Highlights

Half of participants exhibited ExEC following a 24-week exercise intervention

The degree of ExEC was related to baseline total daily energy expenditure

Metabolic adaptation was not evident in those with ExEC

Flanagan et al., iScience 27, 109842
June 21, 2024
https://doi.org/10.1016/j.isci.2024.109842

iScience

CellPress
OPEN ACCESS

Article

No evidence for metabolic adaptation during exercise-related energy compensation

E.W. Flanagan,[1] G. Sanchez-Delgado,[2,3,4] C.K. Martin,[1] E. Ravussin,[1] H. Pontzer,[5] and L.M. Redman[1,6,*]

SUMMARY

The constrained energy model posits that the increased total daily energy expenditure (TDEE) in response to exercise is often less than the energy cost of the exercise prescribed. The mechanisms behind this phenomenon, coined "exercise-related energy compensation" (ExEC), are poorly understood, and it is unknown if ExEC is coupled with metabolic adaptation. Using a randomized controlled 24-week exercise intervention, individuals who demonstrated ExEC were identified. Changes to all components of TDEE and metabolic adaptation were assessed using doubly labeled water over 14 days and room calorimetry over 24-h 48% of individuals exhibited ExEC (-308 ± 158 kcals/day). There were no statistically significant differences in sex, age, or BMI between ExEC and non-ExEC. ExEC was associated with baseline TDEE ($r = -0.50$, $p = 0.006$). There were no statistically significant differences in metabolic adaptations for 24 h, sleep, or resting expenditures. These findings reveal that ExEC occurs independent of metabolic adaptation in sedentary components of EE.

INTRODUCTION

The classic and simple model of non-surgical weight loss posits that the relationship between energy intake and energy expenditure (EE) must be perturbed to invoke a sustained negative energy balance. Hence, caloric restriction and increased energy expenditure through exercise are first-line therapeutics for weight loss. On average, exercise alone has a limited effect on weight loss with trials citing poor adherence and compensatory changes in eating behaviors which increase energy intake.[1,2] Yet, exercise interventions lead to highly variable changes in weight, with some individuals losing weight, and others ultimately experiencing weight gain.

Physiological adaptations and compensatory behaviors that contribute to less weight loss than expected in response to an exercise intervention have been coined "weight compensation."[3] While weight compensation has been primarily attributed to opposing and concomitant increases in energy intake,[4] less is known about the potential contributions from physiological adaptations which may promote changes in energy expenditure. One such contributor in the context of weight loss is that metabolic rate decreases more than expected based on tissue loss, termed metabolic adaptation. Metabolic adaptation occurs in interventions combining diet and exercise, although to a lesser extent than weight loss induced by diet alone.[5,6] Metabolic adaptation, while opposing further weight loss, is generally accepted to be a physiological benefit to weight loss, as it is associated with reductions in oxidative damage, core temperature (indicative of a reduced metabolic rate and a biomarker of longevity),[7] and thyroid hormones.[8,9]

A less understood hypothesis of energy homeostasis in response to intervention is the energy compensation hypothesis, also termed "constrained energy expenditure." Total energy expenditure has been shown to not simply be the product of basal energy expenditure and the added cost of exercise. As such, the constrained energy theory proposes that increased total daily energy expenditure (TDEE) in response to exercise is less than the actual energy cost of the prescribed exercise and is due to declines in resting energy expenditures (REEs) including basal metabolic rate and non-exercise activity thermogenesis (NEAT).[10,11] Indeed, several studies have shown a decline to NEAT in response to adoption of new exercise regimen,[12] but no studies have yet clearly identified declines in components of sedentary energy expenditures. In opposition to the "additive model" of energy expenditure, the constrained model highlights that TDEE often remains constant or "constrained," even when activity is excessively high, due to an upper limit in the amount of energy that humans can expend (Figure 1).[13–16] The energy which is "lost" or unaccounted for is referred to as exercise-related energy compensation (ExEC). Similar to observations seen with metabolic adaptations, it is hypothesized that ExEC is, to an extent, a beneficial response and related to downregulated

[1]Pennington Biomedical Research Center, 6400 Perkins Road, Baton Rouge, LA 70808, USA
[2]Department of Medicine, Division of Endocrinology, Centre de Recherche du Centre Hospitalier Universitaire de Sherbrooke, Université de Sherbrooke, 12e Avenue N Porte 6, Sherbrooke, QC J1H 5N4, Canada
[3]University of Granada, Institute of Nutrition and Food Technology "José Mataix" and Sport and Health University Research Institute, Cuesta del Hospicio Viejo s/n, 18012 Granada, Spain
[4]Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y Nutrición (CIBERobn), Instituto de Salud Carlos III, Av. de Monforte de Lemos, 5, 28029 Madrid, Spain
[5]Department of Evolutionary Anthropology, Duke University, 211 Biological Sciences, Durham, NC 27708, USA
[6]Lead contact
*Correspondence: leanne.redman@pbrc.edu
https://doi.org/10.1016/j.isci.2024.109842

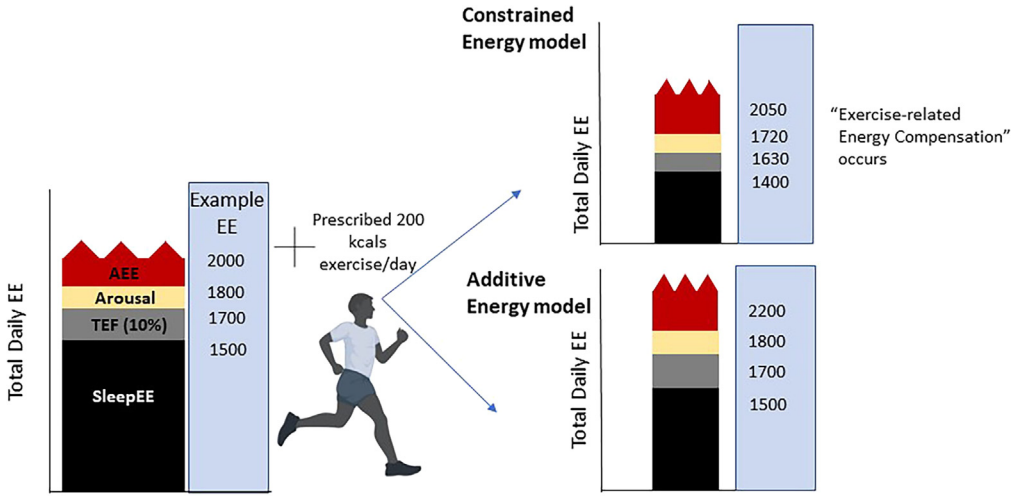


Figure 1. Example proposed effects of a 200 kcal/day exercise training program through the lens of the constrained energy model (top right) and additive energy model (bottom right)

In the constrained energy model, energy is seemingly "lost" whereas, in the additive energy model, all energy is accounted for.

disease-promoting cellular energetics, such as inflammation and decreased cellular energy utilization. Thus, if ExEC occurs in response to traditional exercise prescriptions for weight loss and weight loss maintenance, it is plausible that reductions to energy expenditures may be evidence of beneficial reductions to disease progression or that, due to more efficient utilization of cellular energy, exercise doses should be increased over time to ensure the desired effects on body weight are achieved.

The physiological response of metabolic adaptation has yet to be disentangled from ExEC, nor have these concepts been investigated in a randomized trial of supervised exercise prescriptions. One distinction of metabolic adaptation from ExEC is the apparent influence of body mass with metabolic adaptation, whereas ExEC is evident in studies in which tissue loss was minimal or non-existent.[17] It is unknown if observed energy compensation in response to exercise is a result of metabolic adaptation or an independent pathway. We hypothesize that ExEC is coupled with metabolic adaptation. The aims of this analysis were to identify and characterize phenotypes of individuals who exhibit ExEC in response to a supervised aerobic exercise intervention, determine if metabolic adaptations occur alongside ExEC, and observe if individuals with ExEC have different exercise-induced responses to downstream cardiometabolic risk factors compared to individuals without ExEC.

RESULTS

Subject characteristics

Of the 42 participants with complete data and thus included in the ancillary trial, 14 were randomized into the 8 kcal/kg of body weight per week (KKW) group and 15 into the 20 KKW group for a total of 29 participants included in the analysis (female = 20). According to their assigned exercise groups at baseline, there were no statistically significant differences with respect to baseline age (8 KKW: 46.3 $\pm$ 11.4 vs. 20 KKW: 49.4 $\pm$ 13.8 years), weight (8 KKW: 95.8 $\pm$ 13.4 vs. 20 KKW: 94.6 $\pm$ 12.3 kg), BMI (8 KKW: 34.3 $\pm$ 3.7 vs. 20 KKW: 33.9 $\pm$ 2.9 kg/m^2), cardiorespiratory fitness (8 KKW: 21.9 $\pm$ 3.3 mL kg·min vs. 20 KKW: 21.9 $\pm$ 3.2 mL kg·min), or biomarkers of cardiometabolic health.

Exercise intervention

By design, the prescribed exercise energy expenditure (8 KKW: 18,420 $\pm$ 2,866 vs. 20 KKW: 43,625 $\pm$ 5,954 kcals/week, $p < 0.001$) and the exercise energy expenditure achieved (8 KKW: 18,857 $\pm$ 3,170 vs. 20 KKW: 44,074 $\pm$ 5,970 kcals/week, $p < 0.001$) were both higher in the 20 KKW group compared to the 8 KKW group. Across the 24 weeks, session adherence to the prescribed exercise was 92% $\pm$ 3%, with no statistically significant differences between 8 and 20 KKW groups (92.9% and 92.2%, respectively).

ExEC

Of the total 29 participants, 14 exhibited ExEC while 15 did not (Figure 2). Individuals with ExEC expended -308 ± 158 kcals per day less than predicted whereas non-compensators expended 94 $\pm$ 124 kcals per day more than predicted, $p < 0.001$. There was no statistically significant difference in sex, age, baseline BMI or weight, or prescribed exercise intensity group between those who compensated and those who did not (Table 1).

Weight change

The mean weight loss was -1.2 ± 3.0 kg (range: -9.8 through +7.5 kg). There was no statistically significant difference in weight change between compensators and non-compensators (compensators: -1.5 ± 3.4 and non-compensators: -0.85 ± 2.7 kg, $p = 0.59$).

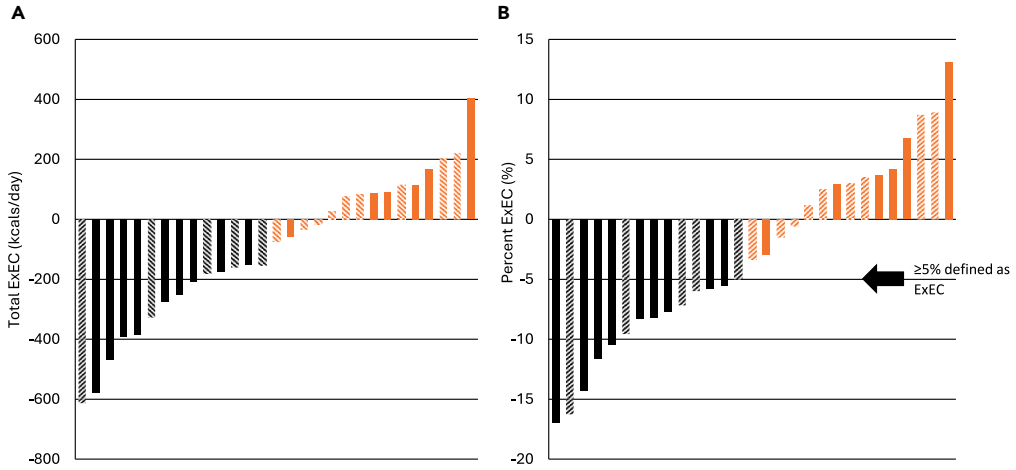


Figure 2. Observed exercise related energy compensation

Exercise-related energy compensation (ExEC) expressed as (A) Absolute ExEC (kcals/day) and (B) Percent ExEC. Black indicates energy compensation, and orange bars indicate no energy compensation. Hashed bars indicate 8 KKW, and solid bars indicate 20 KKW.

Energy expenditure

For the whole sample, baseline TDEE was 2,678 ± 491 kcals/day and increased to 2,734 ± 445 in response to exercise ($p = 0.04$). Baseline 24 hour energy expenditure (24hrEE) was 2,003 ± 255 kcals/day and remained unchanged at the end of the intervention (1,989 ± 267 kcal/day, $p = 0.62$). Similarly, EE at rest and during sleep did not change in response to the exercise intervention (Figure 3).

Baseline TDEE significantly differed between compensators and non-compensators (2,914 ± 416 and 2,458 ± 463 kcal/day, respectively; $p = 0.01$) and was negatively associated with the amount of exercise energy compensation ($r = -0.50$, $p = 0.016$, Figure 4A). Despite differences at baseline in REE between compensators and non-compensators (1,985 ± 169 and 1,791 ± 282 kcals/day, respectively; $p = 0.04$), there

Table 1. Descriptive statistics and baseline phenotypic data

	Total	Compensation	No compensation	Difference *p*
Demographics				
BMI (m/kg^2)	34.1 ± 3.2	34.1 ± 3.6	34.0 ± 3.0	0.98
Weight (kg)	95.1 ± 12.6	99.3 ± 13.3	91.2 ± 11.0	0.09
Age (y)	47.9 ± 12.6	45.1 ± 12.5	50.5 ± 12.5	0.26
Sex[a] (n)				0.18
Male	9	6	3	
Female	20	8	12	
Cardiometabolic markers				
SBP (mmHg)	121 ± 9	122 ± 10	121 ± 8	0.87
DBP (mmHg)	79 ± 7	80 ± 5	79 ± 8	0.61
Cholesterol (mg/dL)	204 ± 37	200 ± 37	207 ± 37	0.63
Triglycerides (mg/dL)	120 ± 59	108 ± 47	131 ± 68	0.31
Glucose (mg/dL)	93 ± 7	92 ± 6	94 ± 8	0.70
HDL (mg/dL)	55.9 ± 17.1	54.3 ± 14.0	57.4 ± 20.0	0.63
LDL (mg/dL)	124.0 ± 25.0	124.5 ± 25.9	123.6 ± 25.1	0.94
WBC (× 10^3 cells/uL)	6.5 ± 1.8	6.5 ± 1.7	6.6 ± 2.0	0.87
Neutrophils (× 10^3 cells/uL)	3.8 ± 1.4	4.0 ± 1.5	3.7 ± 1.2	0.54
Eosinophils (× 10^3 cells/uL)	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.2	0.25

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoproteins; LDL, low-density lipoproteins; WBC, white blood cells; KG, kilograms; DLW, doubly labeled water; kcal, kilocalories; L, liters; mL, milliliters; mmHg, millimeters mercury; mg/dL, milligrams per deciliter; uL, microliter. Data are presented as mean ± SD.

[a]Chi-squared analysis.

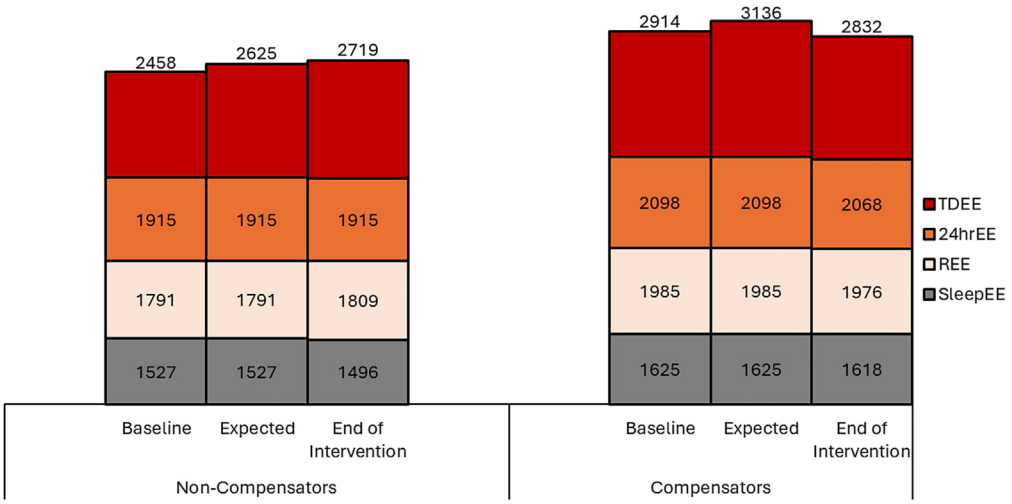


Figure 3. Observed and predicted changes to components of energy expenditure with exercise intervention

TDEE, total daily energy expenditure obtained by doubly labeled water; SEE, sleeping energy expenditure measured with metabolic chamber; REE, resting energy expenditure measured with metabolic chamber; 24hrEE, cumulative 24-h energy expenditure within metabolic chamber.

were no statistically significant associations among any components of sedentary energy expenditure measured with the metabolic chamber and energy compensation (Figures 4B–4D).

Cardiovascular fitness was higher in compensators compared to non-compensators (absolute maximal oxygen uptake in compensators: 2.3 ± 0.5 compared to non-compensators: 1.9 ± 0.4 L/min, $p = 0.02$ Cohen's d = −0.94; mass-adjusted maximal oxygen uptake in compensators: 23.3 ± 3.4 compared to non-compensators: 20.6 ± 2.4, $p = 0.02$ Cohen's d = −0.90; Table 2). Those with a higher maximal oxygen uptake at baseline tended to compensate more (absolute maximal oxygen uptake: r = −0.33, $p = 0.08$; mass-adjusted maximal oxygen uptake: r = −0.30, $p = 0.11$; Figures 4E and 4F).

Physical activity

Energy expended through spontaneous physical activity (SPA) was 218 ± 17 kcals/day at baseline and remained unchanged at the end of the intervention (203 ± 77 kcal/day, $p = 0.25$). There were no statistically significant differences in SPA at baseline or at the end of the intervention between compensators and non-compensators (baseline in compensators: 218 ± 97 kcals/day compared to non-compensators: 217 ± 90 kcals/day, $p = 0.97$, Cohen's d = 0.1; end of intervention in compensators: 205 ± 80 compared to non-compensators: 201 ± 78 kcals/day, $p = 0.88$, Cohen's d = 0.6).

Minutes spent in physical activity measured through the SenseWear armband were lower at baseline in the non-compensators compared to the compensators (total minutes over 14 days 316 ± 37 and 593 ± 103, respectively; $p = 0.015$, d = 0.97). However, by the end of the intervention, there were no statistically significant differences in total minutes of activity between non-compensators and compensators (637 ± 56 and 588 ± 79, respectively; $p = 0.62$, d = 0.19). There were no statistically significant differences in steps at baseline or the end of the intervention between groups.

Energy compensation and metabolic adaptation

As previously reported, as a whole, the residual energy expenditure for 24hrEE, REE, and sleeping energy expenditure (SEE) did not differ from zero at follow-up.[19] Additionally, there were no statistically significant metabolic adaptations in 24hrEE, SEE, or REE in response to the exercise intervention between compensators and non-compensators (Table 3). When analyzed continuously, there were no statistically significant relationships observed between metabolic adaptations in 24hrEE, SEE, or REE and the degree of ExEC.

Downstream cardiometabolic implications

There were no statistically significant differences in changes in response to the exercise intervention to cholesterol, triglycerides, glucose, high- or low-density lipoproteins, or inflammatory markers between compensators and non-compensators (Table 4). When analyzed continuously, there were no statistically significant relationships observed between any changes to cardiometabolic outcome and the degree of ExEC.

DISCUSSION

ExEC is a relatively new theory which attempts to explain the non-proportional increase to TDEE in response to exercise. The present analysis of the "Examination of mechanisms of exercise-induced weight compensation (E-MECHANIC)" randomized trial supports that energy

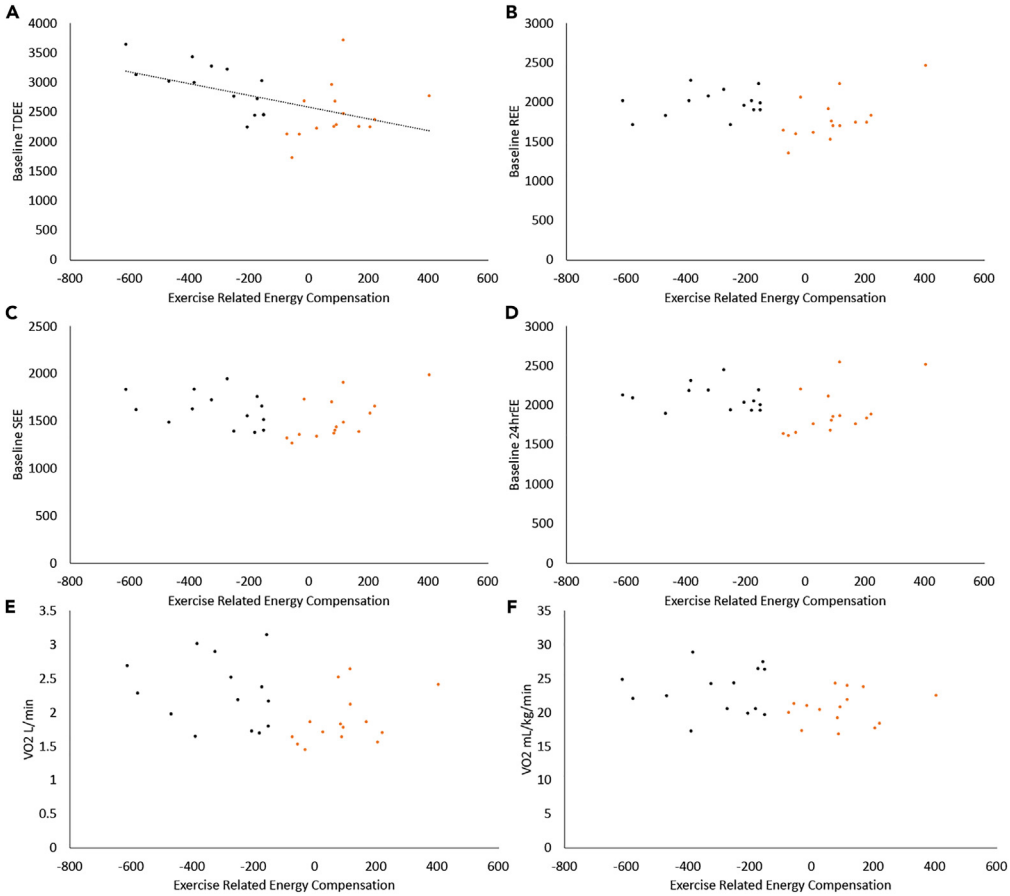


Figure 4. Exercise related energy compensation and baseline energy expenditures

(A) TDEE, total daily energy expenditure obtained by doubly labeled water; (B) REE, resting energy expenditure measured with metabolic chamber; (C) SEE, sleeping energy expenditure measured with metabolic chamber; (D) 24hrEE, cumulative 24-h energy expenditure within metabolic chamber; (E) VO2, maximum ventilatory oxygen in liters per minute; (F) VO_2 mL/kg/min, maximum ventilatory oxygen relative to body mass. Black indicates energy compensation, and orange indicate no energy compensation.

compensation is heterogeneous and occurs in some but not all people in response to aerobic exercise conditioning. The data also suggest that metabolic adaptation, a well-accepted energetic response to weight loss, is not implicated in ExEC. Lastly, we did not find evidence supporting that cardiometabolic outcomes are a downstream benefit of ExEC to exercise intensities necessary for weight loss and weight loss maintenance.

Human energetics is the product of complex networks of physiological systems. Unraveling where energy compensation occurs as a result of increased exercise EE is critical to understanding the metabolic response to exercise. ExEC was shown to occur in approximately half of participants after the 24-week aerobic exercise intervention. These findings align with other theories, such as the ActivityStat hypothesis, which proposes that approximately half of exercise interventions result in some degree of energy compensation.[20] In the context of the constrained energy model, it was anticipated that the higher workload and hence larger perturbations to energy balance would result in greater energy compensation due to the proposed "ceiling effect" of TDEE.[10] Contrary to our hypothesis, mean ExEC was not different between those who expended low versus high calories in structured exercise. Notably, while there were no differences in the degree of ExEC, more individuals in the higher calorie exercise group exhibited ExEC (high-calorie group n = 9; low-calorie group n = 5). While others have proposed that higher workloads result in greater ExEC,[21] our rigorous approach of phenotyping individuals contradicts these findings. These findings are in line with previous studies that have provided evidence for the non-linear relationship between exercise dose and the amount of ExEC.[22,23] Rather, a higher baseline TDEE was the primary driver for greater ExEC. These data indeed support a constrained energy model in which there may be an upper limit to the total amount of energy that can feasibly be expended on any given day. Instead of achieving this proposed upper limit through increased exercise workload, elevated baseline energy expenditure and higher baseline physical activity levels appear to contribute to maximizing available energy expenditures. We thus hypothesize that individuals with a higher baseline TDEE have less available energy to partition increased energetic demand of exercise and therefore demonstrate a greater ExEC.

Table 2. Energy expenditure and body composition before and after exercise intervention

	Compensation		No compensation		Baseline difference		End difference	
	Baseline	End	Baseline	End	p	Effect size (d)	p	Effect size (d)
Energy expenditures								
TDEE (DLW)	2914 ± 416	2832 ± 331	2458 ± 463	2719 ± 537	0.10	1.03	0.50	0.25
24hrEE (kcal/day; metabolic chamber)	2098 ± 159	2068 ± 232	1915 ± 298	1615 ± 284	0.05	0.76	0.13	0.59
REE (kcal/day; metabolic chamber)	1985 ± 169	1976 ± 214	1791 ± 282	1809 ± 270	0.04	0.83	0.08	0.68
SEE (kcal/day; metabolic chamber)	1625 ± 180	1618 ± 226	1527 ± 223	1496 ± 200	0.21	0.48	0.13	0.057
Maximal oxygen uptake (absolute; L O_2)	2.3 ± 0.5	2.5 ± 0.5	1.9 ± 0.4	2.1 ± 0.4	0.02	0.94	0.03	0.87
Maximal oxygen uptake (relative; mL O_2/kg/min)	23.4 ± 3.4	24.9 ± 4.6	20.6 ± 2.4	22.7 ± 2.7	0.03	0.90	0.06	0.61
Body composition								
Body mass	99.2 ± 13.3	97.8 ± 15.1	91.2 ± 11.0	90.4 ± 12.0	0.09	0.66	0.15	0.55
Fat mass	44.1 ± 9.8	43.8 ± 10.0	41.8 ± 4.9	41.3 ± 5.8	0.44	0.29	0.62	0.19
Fat-free mass	55.2 ± 11.1	55.0 ± 11.7	49.4 ± 8.5	49.1 ± 8.8	0.13	0.58	0.14	0.57
Total metabolizable energy (kcal)	470,860 ± 89,831	458,774 ± 93,361	443,498 ± 49,230	438,324 ± 57,284	0.31	0.38	0.48	0.27
Lean metabolizable energy (kcal)	60,679 ± 12,214	60,455 ± 12,837	54,334 ± 9,314	53963 ± 9,728	0.13	0.59	0.14	0.57
Fat metabolizable energy (kcal)	410,181 ± 91,366	398,320 ± 92,983	389,164 ± 45,560	384,360 ± 53,514	0.44	0.29	0.62	0.19

TDEE, total daily energy expenditure; 24hrEE, 24h energy expenditure; REE, resting energy expenditure; SEE, sleeping energy expenditure; DLW, doubly labeled water; kcal, kilocalories; L, liters; mL, milliliters. Metabolizable energy computed from Sanghvi et al.[18] with coefficients fat mass = 9,300 kcal/kg and lean mass = 1,100 kcal/kg. Data are presented as mean ± SD. Effect sizes are calculated using Cohen's d.

Metabolic adaptation, a change in metabolic rate that is greater than that expected on the basis of tissue loss, is another form of energy compensation. Metabolic adaptation is associated with optimized health outcomes, namely improvements in cardiometabolic response and reduced oxidative damage.[5,8] In a previous study examining the energetic response to exercise in conjunction with dietary calorie restriction, we observed no change to TDEE while sedentary components of energy expenditure (i.e., SEE) decreased.[5,6] Therefore, we hypothesized that, if energy compensation did occur in response to a 24-week exercise intervention, it would likely be a downstream occurrence as part of metabolic adaptation. In the present study, we observed that metabolic adaptation did not occur in parallel to energy compensation. In those that exhibited ExEC, there was no observed metabolic adaptation among any measured component of sedentary expenditure. This may be attributed to lack of weight change observed with the exercise intervention, given that metabolic adaptation typically occurs in the presence of tissue loss. In previously observed metabolic adaptation with exercise, participants lost an average of 10% body weight (range 5%–10%). In the present study, while weight loss was minimal (average 1.1%), it ranged from 12% loss to 6% gain. Half of the intervention was designed to induce weight loss; however it was previously shown that weight loss was not observed in most participants due to compensatory increases in energy intake. A sensitivity analysis was conducted in those who lost weight in response to the intervention (N = 20; data not shown) and revealed no relationship between ExEC and metabolic adaptation.

If reductions to sedentary expenditures are not present, one must look toward activity components of energy expenditures. We speculate that the observed ExEC evident with aerobic exercise at 8 KKW and 20 KKW is likely attributable to increases in exercise efficiency and/or behavioral adaptations, allowing participants to maintain current levels of exercise while expending less activity-related energy.[24] Notably, individuals with less cardiorespiratory fitness and lower levels of physical activity at baseline exhibited less ExEC, indicating a greater potential to increase their exercise energy expenditure and hence TDEE before bolstering exercise efficiency. Exercise efficiency is higher in active individuals,[25] and while all participants were sedentary prior to the exercise intervention, at baseline there was a wide range in cardiorespiratory fitness (1.2–3.8 L/min) and measured average physical activity determined by accelerometry (4–116 min/day). These differences, at least in part, explain why individuals with a higher baseline $VO_{2\ max}$ (maximum ventilatory oxygen uptake relative to body mass) and free-living physical activity tended to exhibit greater ExEC. This relationship between ExEC and baseline energy expenditure is supported by similar findings

Table 3. Metabolic adaptations to sedentary energy expenditures

Residuals	Total	Compensation	No compensation	Difference *p*	Effect size (d)
24hrEE (kcals/day)	−6 ± 155	3 ± 92	−14 ± 199	0.77	0.11
REE (kcals/day)	−107 ± 72	−97 ± 80	−115 ± 65	0.50	0.25
SEE (kcals/day)	−18 ± 137	−9 ± 121	−27 ± 154	0.73	0.13

24hrEE, 24 h energy expenditure; REE, resting energy expenditure; SEE, sleep energy expenditure. Data are presented as mean ± SD. Effect sizes are calculated using Cohen's d.

in postmenopausal women.[15] While efforts were made to account for changes in exercise efficiency during exercise sessions by measuring exercise oxygen consumption every four weeks, acute changes to mechanical and metabolic physiology underpin improvements to exercise efficiency. Indeed, a similar six-month exercise intervention, consisting of running/walking and biking in sedentary adults, showed to improve exercise efficiency by 17%.[24] It was also shown that those who were less efficient at the start of the intervention showed larger increases in exercise efficiency. These differences indicate that individuals with a higher cardiorespiratory fitness may already have higher efficiency, despite being sedentary at the start of the intervention. While unmeasured, exercise efficiency may manifest as improved mitochondrial function, enhanced recruitment of type I muscle fiber types, and skeletal-muscle synergy. Regarding the potential involvement of behavioral factors, despite differences at baseline, at the completion of the intervention there were no differences in free-living physical activity as measured by accelerometry. Furthermore, SPA measured within the metabolic chamber or mass-adjusted cardiorespiratory fitness between compensators and non-compensators did not differ, thus, indicating that behavioral compensation to reduce non-exercise, free-living physical activity may be present.

The constrained energy model proposes that reductions to non-essential energy expenditures, such as those required for disease progression, occur in concert with dampened daily energy expenditures in response to exercise.[22] Reducing energy expenditure allocated to inflammation, detrimental immune system activity, and non-exercise metabolic activity (e.g., glucose kinetics, mitochondrial efficiency) is proposed to be one of the beneficial responses to chronic exercise. Thus, at mild to moderate exercise volumes, as those prescribed in the E-MECHANIC trial, ExEC may be implicated in improvements to cardiometabolic risk factors. In the present study, we found that ExEC does not appear to induce direct downstream benefits to cardiometabolic health. Compensators and non-compensators did not differ in the exercise-induced weight loss or fasting levels of glucose, lipids, or inflammatory markers. We speculate that this is likely explained by ExEC being a product of exercise efficiency, and not basal metabolism, which reflects cellular metabolic activity during non-exercise. It is unknown how downstream cardiometabolic factors would be differentially influenced in the presence of concomitant weight loss.

Indeed, exercise that increases energy expenditure beyond what is needed for essential functions may result in more harmful adaptations, particularly as seen by reductions to sympathetic nervous system (SNS), reproduction function, and immune response. Perhaps the most researched of these are perturbations in the hypothalamic-pituitary-ovarian axis. Data have shown that energy imbalance, as a result of dietary restriction or excess exercise, perturbs the hypothalamic-pituitary-ovarian access slowing gonadotropin hormone-releasing hormone (GnRH) pulsatility, inducing anovulation, and amenorrhea.[26] Yet, evidence suggests that menstrual cycle disturbances also occur in energy balance, again pointing toward a constrained energy model where energy expenditure, rather than energy balance, may influence essential physiological systems.[27,28] In the present analysis, we lacked the ability to time study visits to allow for rigorous assessments of reproductive function. Furthermore, we lacked measurement of SNS activity and immune response and therefore are unable to quantify downstream effects to these physiological systems.

Phenotyping individuals based on energy compensation may prove to be clinically relevant for weight loss and management. It is generally accepted that exercise alone does not induce significant weight loss.[2] The presence of energy compensation may provide avenue to phenotype individuals who may be able to successfully lose weight in response to an exercise intervention. This understanding would allow for more personalized and precise weight-loss therapy. While the non-compensators in the current study did not have different weight loss than the individuals who compensated, it is possible that they would have success when paired with behavioral counseling.

Limitations of the study

The present analysis has several strengths including the use of the metabolic chamber to precisely quantify components of sedentary energy expenditure in conjunction with the use of doubly labeled water to measure free-living TDEE. The study is potentially limited by the duration of the exercise intervention. The aerobic exercise intervention was 24 weeks, and it has been proposed that energy compensation is, at least in part, attributable to study duration.[22] It has been shown that exercise energy compensation occurs to a greater extent in exercise interventions of at least 26 weeks in length.[23] It is possible that individuals who did not demonstrate exercise energy compensation may have ultimately compensated with a more prolonged exercise duration. It is notable that these data are the first to present rigorous physiological phenotyping and gold-standard measures of energy expenditure alongside ExEC in a randomized clinical trial. Sample size calculations based upon the 8 KKW exercise group reveal that as few as 11 participants per group (compensation versus non-compensation) were needed to identify differences in sedentary components of energy expenditure, measured by the metabolic chamber. However, we recognize that this is a secondary analysis and all conclusions may be limited by sample size, as evident by several smaller effect sizes. Furthermore, some analyses presented with non-significance but with medium-sized effects, indicating the possibility that future controlled trials recruiting a larger

Table 4. Percent changes to cardiometabolic biomarkers

	Compensation	No compensation	p	Effect size (d)
SBP (mmHg)	−1.9 ± 6.3	1.6 ± 7.9	0.21	0.48
DBP (mmHg)	−0.44 ± 6.0	2.5 ± 7.8	0.27	0.42
Cholesterol (mg/dL)	−0.35 ± 8.8	−3.5 ± 8.1	0.32	0.38
Triglycerides (mg/dL)	1.6 ± 21.2	0.31 ± 31.9	0.90	0.05
Glucose (mg/dL)	−2.7 ± 6.6	−0.43 ± 5.6	0.33	0.37
HDL (mg/dL)	1.4 ± 10.6	0.5 ± 11.7	0.85	0.07
LDL (mg/dL)	−1.3 ± 11.0	−3.0 ± 13.1	0.71	0.14
WBC (× 10^3 cells/uL)	−0.4 ± 21.2	3.2 ± 10.2	0.58	0.21
Neutrophils (× 10^3 cells/uL)	−3.1 ± 29.5	3.9 ± 15.7	0.45	0.29
Eosinophils (× 10^3 cells/uL)	10.7 ± 52.5	−5.8 ± 18.7	0.28	0.42

SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoproteins; LDL, low-density lipoproteins; WBC, white blood cells; KG, kilograms; DLW, doubly labeled water; kcal, kilocalories; L, liters; mL, milliliters; mmHg, millimeters mercury; mg/dL, milligrams per deciliter; uL, microliter. Data are presented as mean ± SD. Data are presented as Mean ± SD. Effect sizes are calculated using Cohen's d.

sample may reveal differences. In the present dataset we were unable to measure all proposed indicators in the constrained energy model. Moreover, more precise markers of inflammation as well as measures of SNS activity and reproductive functionality were not available.[22] Lastly, it is important to acknowledge that the computation of energy expenditures to estimate ExEC does not include an adjustment for body size before and after the exercise intervention. Indeed, there were no differences in body weight change between compensators and non-compensators, and compensation did not differ between exercise workloads. Thus, if TDEE was to decrease in response to exercise training-induced weight loss, compensation might be underestimated in some people.

In conclusion, this is the first study to investigate the interaction between metabolic adaptation in sedentary energy expenditures and exercise energy compensation. In support of the constrained energy model, we demonstrated that approximately half of sedentary individuals experience exercise compensation at both moderate and high doses of aerobic exercise after 24 weeks in a dichotomous manner. Furthermore, we provide evidence that ExEC is neither a product of metabolic adaptations to sedentary energy expenditures nor downstream improvements to cardiometabolic risk and therefore is likely contributed to increase exercise efficiency. Future research may examine longer-term interventions and include more deep phenotyping of exercise efficiency including measures at the level of the skeletal-muscle mitochondria. A greater interrogation of exercise energy compensation on downstream physiological systems is also warranted. It remains unknown how maximal energy constraint is determined, which factors predispose someone to a higher constraint, and the effects it presents on human health and longevity. However, this study in sedentary individuals provides new evidence that it indeed exists.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS
  - Subjects and study design
- METHOD DETAILS
  - Exercise intervention
  - Weight and body composition
  - Exercise oxygen consumption
  - Energy expenditure
  - Physical activity
  - Calculation of metabolic adaptation
  - Calculation of exercise-related energy compensation
  - Cardiometabolic biomarkers
- QUANTIFICATION AND STATISTICAL ANALYSIS
  - Statistical analyses

ACKNOWLEDGMENTS

This work was funded by the National Institutes of Health (R01HL102166) and supported in part by P30DK072476 (Pennington/Louisiana NORC). E.W.F. is supported by the Eunice Kennedy Shriver National Institute of Child Health and Human Development of the National Institutes of Health under award number F32HD108022. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

AUTHOR CONTRIBUTIONS

Writing – original draft, formal analysis, E.W.F. Conceptualization, H.P. and L.M.R. Methodology, E.W.F, G.S.-D., L.M.R., and H.P. Investigation, funding acquisition, L.M.R., E.R., and C.K.M. Writing – review and editing, E.W.F., G.S.-D., C.K.M., H.P., and L.M.R.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: September 28, 2023
Revised: January 8, 2024
Accepted: April 25, 2024
Published: April 29, 2024

REFERENCES

1. King, N.A., Hopkins, M., Caudwell, P., Stubbs, R.J., and Blundell, J.E. (2008). Individual variability following 12 weeks of supervised exercise: identification and characterization of compensation for exercise-induced weight loss. Int. J. Obes. *32*, 177–184. https://doi.org/10.1038/sj.ijo.0803712.
2. Swift, D.L., McGee, J.E., Earnest, C.P., Carlisle, E., Nygard, M., and Johannsen, N.M. (2018). The Effects of Exercise and Physical Activity on Weight Loss and Maintenance. Prog. Cardiovasc. Dis. *61*, 206–213. https://doi.org/10.1016/j.pcad.2018.07.014.
3. Blundell, J.E., Stubbs, R.J., Hughes, D.A., Whybrow, S., and King, N.A. (2003). Cross talk between physical activity and appetite control: does physical activity stimulate appetite? Proc. Nutr. Soc. *62*, 651–661. https://doi.org/10.1079/PNS2003286.
4. Martin, C.K., Johnson, W.D., Myers, C.A., Apolzan, J.W., Earnest, C.P., Thomas, D.M., Rood, J.C., Johannsen, N.M., Tudor-Locke, C., Harris, M., et al. (2019). Effect of different doses of supervised exercise on food intake, metabolism, and non-exercise physical activity: The E-MECHANIC randomized controlled trial. Am. J. Clin. Nutr. *110*, 583–592. https://doi.org/10.1093/ajcn/nqz054.
5. Heilbronn, L.K., de Jonge, L., Frisard, M.I., DeLany, J.P., Larson-Meyer, D.E., Rood, J., Nguyen, T., Martin, C.K., Volaufova, J., Most, M.M., et al. (2006). Effect of 6-month calorie restriction on biomarkers of longevity, metabolic adaptation, and oxidative stress in overweight individuals: a randomized controlled trial. JAMA *295*, 1539–1548. https://doi.org/10.1001/jama.295.13.1539.
6. Redman, L.M., Heilbronn, L.K., Martin, C.K., de Jonge, L., Williamson, D.A., Delany, J.P., and Ravussin, E.; Pennington CALERIE Team (2009). Metabolic and behavioral compensations in response to caloric restriction: implications for the maintenance of weight loss. PLoS One *4*, e4377. https://doi.org/10.1371/journal.pone.0004377.
7. Lane, M.A., Baer, D.J., Tilmont, E.M., Rumpler, W.V., Ingram, D.K., Roth, G.S., and Cutler, R.G. (1995). Energy balance in rhesus monkeys (Macaca mulatta) subjected to long-term dietary restriction. J. Gerontol. A Biol. Sci. Med. Sci. *50*, B295–B302. https://doi.org/10.1093/gerona/50a.5.b295.
8. Redman, L.M., Smith, S.R., Burton, J.H., Martin, C.K., Il'yasova, D., and Ravussin, E. (2018). Metabolic Slowing and Reduced Oxidative Damage with Sustained Caloric Restriction Support the Rate of Living and Oxidative Damage Theories of Aging. Cell Metab. *27*, 805–815.e4. https://doi.org/10.1016/j.cmet.2018.02.019.
9. Roth, G.S., Lane, M.A., Ingram, D.K., Mattison, J.A., Elahi, D., Tobin, J.D., Muller, D., and Metter, E.J. (2002). Biomarkers of caloric restriction may predict longevity in humans. Science *297*, 811. https://doi.org/10.1126/science.1071851.
10. Pontzer, H., Durazo-Arvizu, R., Dugas, L.R., Plange-Rhule, J., Bovet, P., Forrester, T.E., Lambert, E.V., Cooper, R.S., Schoeller, D.A., and Luke, A. (2016). Constrained Total Energy Expenditure and Metabolic Adaptation to Physical Activity in Adult Humans. Curr. Biol. *26*, 410–417. https://doi.org/10.1016/j.cub.2015.12.046.
11. Pontzer, H. (2015). Constrained Total Energy Expenditure and the Evolutionary Biology of Energy Balance. Exerc. Sport Sci. Rev. *43*, 110–116. https://doi.org/10.1249/JES.0000000000000048.
12. Melanson, E.L., Keadle, S.K., Donnelly, J.E., Braun, B., and King, N.A. (2013). Resistance to exercise-induced weight loss: compensatory behavioral adaptations. Med. Sci. Sports Exerc. *45*, 1600–1609. https://doi.org/10.1249/MSS.0b013e31828ba942.
13. Willis, E.A., Creasy, S.A., Honas, J.J., Melanson, E.L., and Donnelly, J.E. (2020). The effects of exercise session timing on weight loss and components of energy balance: midwest exercise trial 2. Int. J. Obes. *44*, 114–124. https://doi.org/10.1038/s41366-019-0409-x.
14. Donnelly, J.E., Hill, J.O., Jacobsen, D.J., Potteiger, J., Sullivan, D.K., Johnson, S.L., Heelan, K., Hise, M., Fennessey, P.V., Sonko, B., et al. (2003). Effects of a 16-month randomized controlled exercise trial on body weight and composition in young, overweight men and women: the Midwest Exercise Trial. Arch. Intern. Med. *163*, 1343–1350. https://doi.org/10.1001/archinte.163.11.1343.
15. Wang, X., Bowyer, K.P., Porter, R.R., Breneman, C.B., and Custer, S.S. (2017). Energy expenditure responses to exercise training in older women. Physiol. Rep. *5*, e13360. https://doi.org/10.14814/phy2.13360.
16. Goran, M.I., and Poehlman, E.T. (1992). Endurance training does not enhance total energy expenditure in healthy elderly persons. Am. J. Physiol. *263*, E950–E957. https://doi.org/10.1152/ajpendo.1992.263.5.E950.
17. Hall, K.D. (2022). Energy compensation and metabolic adaptation: "The Biggest Loser" study reinterpreted. Obesity *30*, 11–13. https://doi.org/10.1002/oby.23308.
18. Sanghvi, A., Redman, L.M., Martin, C.K., Ravussin, E., and Hall, K.D. (2015). Validation of an inexpensive and accurate mathematical method to measure long-term changes in free-living energy intake. Am. J. Clin. Nutr. *102*, 353–358. https://doi.org/10.3945/ajcn.115.111070.
19. Broskey, N.T., Martin, C.K., Burton, J.H., Church, T.S., Ravussin, E., and Redman, L.M. (2021). Effect of Aerobic Exercise-induced Weight Loss on the Components of Daily Energy Expenditure. Med. Sci. Sports Exerc. *53*, 2164–2172. https://doi.org/10.1249/MSS.0000000000002689.
20. Gomersall, S.R., Rowlands, A.V., English, C., Maher, C., and Olds, T.S. (2013). The ActivityStat hypothesis: the concept, the evidence and the methodologies. Sports Med. *43*, 135–149. https://doi.org/10.1007/s40279-012-0008-7.
21. Hand, G.A., Shook, R.P., O'Connor, D.P., Kindred, M.M., Schumacher, S., Drenowatz, C., Paluch, A.E., Burgess, S., Blundell, J.E., and Blair, S.N. (2020). The Effect of Exercise Training on Total Daily Energy Expenditure and Body Composition in Weight-Stable Adults: A Randomized, Controlled Trial. J. Phys. Act. Health *17*, 456–463. https://doi.org/10.1123/jpah.2019-0415.
22. Pontzer, H. (2018). Energy Constraint as a Novel Mechanism Linking Exercise and

Health. Physiology *33*, 384–393. https://doi.org/10.1152/physiol.00027.2018.

23. Riou, M.È., Jomphe-Tremblay, S., Lamothe, G., Stacey, D., Szczotka, A., and Doucet, É. (2015). Predictors of Energy Compensation during Exercise Interventions: A Systematic Review. Nutrients *7*, 3677–3704. https://doi.org/10.3390/nu7053677.
24. Woo, J.S., Derleth, C., Stratton, J.R., and Levy, W.C. (2006). The influence of age, gender, and training on exercise efficiency. J. Am. Coll. Cardiol. *47*, 1049–1057. https://doi.org/10.1016/j.jacc.2005.09.066.
25. Mogensen, M., Bagger, M., Pedersen, P.K., Fernström, M., and Sahlin, K. (2006). Cycling efficiency in humans is related to low UCP3 content and to type I fibres but not to mitochondrial efficiency. J. Physiol. *571*, 669–681. https://doi.org/10.1113/jphysiol.2005.101691.
26. Loucks, A.B., and Thuma, J.R. (2003). Luteinizing hormone pulsatility is disrupted at a threshold of energy availability in regularly menstruating women. J. Clin. Endocrinol. Metab. *88*, 297–311. https://doi.org/10.1210/jc.2002-020369.
27. Melin, A., Tornberg, Å.B., Skouby, S., Møller, S.S., Sundgot-Borgen, J., Faber, J., Sidelmann, J.J., Aziz, M., and Sjödin, A. (2015). Energy availability and the female athlete triad in elite endurance athletes. Scand. J. Med. Sci. Sports *25*, 610–622. https://doi.org/10.1111/sms.12261.
28. Lagowska, K., Kapczuk, K., Friebe, Z., and Bajerska, J. (2014). Effects of dietary intervention in young female athletes with menstrual disorders. J. Int. Soc. Sports Nutr. *11*, 21. https://doi.org/10.1186/1550-2783-11-21.
29. Myers, C.A., Johnson, W.D., Earnest, C.P., Rood, J.C., Tudor-Locke, C., Johannsen, N.M., Cocreham, S., Harris, M., Church, T.S., and Martin, C.K. (2014). Examination of mechanisms (E-MECHANIC) of exercise-induced weight compensation: study protocol for a randomized controlled trial. Trials *15*, 212. https://doi.org/10.1186/1745-6215-15-212.
30. Lam, Y.Y., Redman, L.M., Smith, S.R., Bray, G.A., Greenway, F.L., Johannsen, D., and Ravussin, E. (2014). Determinants of sedentary 24-h energy expenditure: equations for energy prescription and adjustment in a respiratory chamber. Am. J. Clin. Nutr. *99*, 834–842. https://doi.org/10.3945/ajcn.113.079566.
31. Ravussin, E., Lillioja, S., Anderson, T.E., Christin, L., and Bogardus, C. (1986). Determinants of 24-hour energy expenditure in man. Methods and results using a respiratory chamber. J. Clin. Invest. *78*, 1568–1578. https://doi.org/10.1172/JCI112749.
32. Nguyen, T., de Jonge, L., Smith, S.R., and Bray, G.A. (2003). Chamber for indirect calorimetry with accurate measurement and time discrimination of metabolic plateaus of over 20 min. Med. Biol. Eng. Comput. *41*, 572–578. https://doi.org/10.1007/BF02345320.
33. Rosenbaum, M., and Leibel, R.L. (2010). Adaptive thermogenesis in humans. Int. J. Obes. *34*, S47–S55. https://doi.org/10.1038/ijo.2010.184.
34. Leibel, R.L., Rosenbaum, M., and Hirsch, J. (1995). Changes in energy expenditure resulting from altered body weight. N. Engl. J. Med. *332*, 621–628. https://doi.org/10.1056/NEJM199503093321001.
35. Galgani, J.E., and Santos, J.L. (2016). Insights about weight loss-induced metabolic adaptation. Obesity *24*, 277–278. https://doi.org/10.1002/oby.21408.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Cholesterol	Beckman Coulter	DxC CHOL 467825
Triglycerides	Beckman Coulter	DxC TG 445850
Glucose	Beckman Coulter	DxC LX Glucose 472500
White blood cell count	Beckman Coulter	DxH diluent 628017 DxH cell lyse 628019
Neutrophil Count	Beckman Coulter	DxH DIFF PACK 628020
Eosinophil Count	Beckman Coulter	DxH DIFF PACK 628020

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Leanne Redman (leanne.redman@pbrc.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- De-identified human data have been deposited at https://my.pbrc.edu/NORC/NORCRepository and are available upon request.
- This paper does not report original code.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

This is a secondary analysis of a prospective randomized controlled trial designed to assess weight compensation in response to a 24-week aerobic exercise intervention.[29] The "Examination of mechanisms of exercise-induced weight compensation (E-MECHANIC)" trial was conducted at Pennington Biomedical Research Center alongside an ancillary study to assess components of sedentary energy expenditure using a metabolic chamber (NCT01264406). The parent and ancillary studies were approved by the institutional review board, and participants provided written informed consent for all study testing. Primary outcomes from the parent and ancillary trials including additional metabolic and energy phenotyping were recently reported.[4,19]

Subjects and study design

Sedentary individuals (n = 53) between the ages of 18–65 years of age with overweight or obesity (BMI $\geq$25 kg/m^2 and $\leq$45 kg/m^2) who were otherwise healthy (i.e., free of chronic conditions) completed both the parent and ancillary trial. Individuals were excluded if they reported concurrent engagement in a weight loss program, prior bariatric surgery, smoking in the past 6 months, and consumption of more than 14 alcoholic drinks per week.

METHOD DETAILS

Exercise intervention

A full description of the exercise intervention has been previously published.[29] In brief, participants were randomized in a 1:1:1 ratio into a no-exercise control group or one of two 24-week aerobic exercise interventions. The exercise interventions reflected recommendations for general health and weight maintenance (exercise prescription of 8 kcals/kg of body weight per week; 8KKW) or weight loss (exercise prescription of 20 kcals/kg of body weight per week; 20 KKW). Exercise sessions consisted of exercise on a motorized treadmill or stationary bike and were modified by intensity or volume to achieve target exercise energy prescriptions weekly over the course of 24 weeks. Adherence to exercise sessions were recorded and calculated as achieved exercise compared to prescribed exercise energy intensity via heartrate monitoring throughout exercise sessions.

Weight and body composition

Weight and body composition were measured after an overnight fast at baseline (14 days prior to randomization) and during the last week of the trial (week 24). Weight was measured twice using a calibrated scale and body composition (fat and fat-free masses) were assessed by dual-energy X-ray absorptiometry (iDXA encore software version 13.60, GE Healthcare).

Exercise oxygen consumption

At baseline and during the last week of the intervention, exercise testing was conducted using a standardized graded exercise testing protocol on a treadmill or a cycle ergomenter. Gas exchange was measured with a ParvoMedics True Max 2400 Metabolic Measurement Cart (Salt Lake City, UT). The exercise protocol began at 2.8 METs and increased approximately 1.2 METs per stage every 2 min. Gas exchange and heart rate were monitored continuously throughout the exercise test.

In attempt to maintain the caloric exercise prescription with exercise induced adaptions (e.g., exercise efficiency), exercise energy expenditure was measured using a metabolic cart during the first week of the intervention and every four weeks thereafter. Adjustments to the exercise time to more accurately reflect the target kcal goal were made, as needed.

Energy expenditure

Free living TDEE was measured via doubly labeled water over two weeks at baseline (14 days prior to randomization) and during the last two weeks of the exercise intervention. Briefly, subjects provided two urine samples before being dosed with 2.0 g of 10% enriched H_2 ^{18}O and 0.12 g of 99.9% enriched $^{2}H_2O$ per kg of estimated total body water. Post-dose samples were collected at 4.5 and 6 h and days 7 and 14.[4]

Sedentary energy expenditures were assessed at baseline and during post-testing using a whole room indirect calorimeter. Participants entered the chamber at 8:00 a.m. after an overnight fast and left the chamber at 7:00 a.m. the next morning. Since no structured exercise was allowed during the chamber stay, this procedure was performed within 72 h of the final exercise session of the intervention. While in the room calorimeter, subjects were maintained in energy balance by estimating projected 24EE after 3 and 7 h to adjust the calories provided at lunch and dinner meals when necessary.[30] A detailed description of metabolic chamber procedures can be found elsewhere.[19]

Energy expenditure during sleep (SEE) was assessed between 2:00 a.m. and 5:00 a.m. for those minutes during which activity recorded by infrared motion detectors was less than 1%. Resting energy expenditure (REE) was calculated in the chamber as the y-intercept of the relationship between EE and % activity (by radar motion detector). 24hrEE was calculated as all valid minutes in the chamber.

Physical activity

Non-exercise activity thermogenesis was computed during the overnight chamber stay. Activity within the chamber was recorded by infrared motion detectors and spontaneous physical activity (SPA) was calculated. Energy cost of SPA was calculated by regressing the percent of time that the participant was active during the chamber stay against energy expenditure data for the corresponding time periods.[31,32]

Free-living physical activity was obtained by SenseWear armbands worn on the upper arm. Steps and total minutes of physical activity were obtained over two weeks at baseline and the last two weeks of the intervention. Physical activity was defined as time spent at $\geq$3 metabolic equivalents. Measurement spanned 24 h per day, except for water-based activities.

Calculation of metabolic adaptation

Metabolic adaptation for sedentary energy expenditures (24hrEE, SEE, REE) were calculated from linear regression models of energy expenditure at baseline in all participants (n = 53) using fat-free mass, fat mass, age, and sex as covariates. Individual data for each covariate measured at week 24 was entered into the baseline EE models, and the difference between the measured EE variable and EE variable predicted from the model, coined residual EE, was considered a metabolic adaptation to the intervention.[19,33–35]

Calculation of exercise-related energy compensation

ExEC was calculated as the difference between free-living TDEE at week 24 and the expected TDEE, determined as baseline TDEE plus the prescribed exercise energy expenditure per week. Expected TDEE was adjusted for individual adherence to the exercise prescription. Individuals whose measured energy expenditure was 5% or more below the expected energy expenditure at the completion of the trial were considered to have experienced ExEc and were termed 'compensators'. Compensation between 0 and 5% were within the precision of the EE and body composition measurements and therefore considered to be 'non-compensators'.

Cardiometabolic biomarkers

To investigate downstream cardiometabolic implications of energy compensation, blood pressure and a venous blood draw data were also evaluated. These data were obtained in the morning after a 12-h overnight fast. A complete blood count was performed, and blood were additionally analyzed for cholesterol, triglycerides, glucose, and high- and low-density lipoproteins. Inflammation was assessed by total white blood cell, neutrophil, and eosinophil count.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analyses

Only participants randomized into the exercise intervention were included in the analysis (i.e., no-exercise controls were not included). First, we computed energy compensation (*ExEC = TDEE Measured at Wk 24 – Predicted TDEE*). Second, participants were grouped into energy compensators (ExEC >5%) and non-compensators (ExEc $\leq$5%). To assess changes in non-exercise energy expenditures, independent samples t tests were used to test group differences for continuous variables between compensators and non-compensators. Chi-squared analyses were utilized to determine differences in categorical variables between compensators and non-compensators. To evaluate the relationship between baseline energy expenditures and ExEC, Pearson correlation coefficients were computed. Independent samples t tests were additionally used to determine differences downstream cardiometabolic risk factors between compensators and non-compensators. Effect size was estimated using Cohen's d, where 0.2, 0.5, and 0.8 represent a small, moderate, and large effect, respectfully. All analyses were conducted using SPSS Statistics version 28 (IBM Corp., Armonk, NY). Data are reported as mean (SD), and $\alpha \leq 0.05$ was the predetermined level of significance.