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Less-than-expected weight loss in normal-weight women undergoing caloric restriction and exercise is accompanied by preservation of fat-free mass and metabolic adaptations

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Abstract

Background/Objectives: Normal-weight women frequently restrict their caloric intake and exercise, but little is known about the effects on body weight, body composition and metabolic adaptations in this population.

Subjects/Methods: We conducted a secondary analysis of data from a randomized controlled trial in sedentary normal-weight women. Women were assigned to a severe energy deficit (SEV: -1062 ± 80 kcal per day; $n = 9$), a moderate energy deficit (MOD: -633 ± 71 kcal per day; $n = 7$) or energy balance (BAL; $n = 9$) while exercising five times per week for 3 months. Outcome variables included changes in body weight, body composition, resting metabolic rate (RMR) and metabolic hormones associated with energy conservation.

Results: Weight loss occurred in SEV (-3.7 ± 0.9 kg, $P < 0.001$) and MOD (-2.7 ± 0.8 kg; $P = 0.003$), but weight loss was significantly less than predicted (SEV: -11.1 ± 1.0 kg; MOD: -6.5 ± 1.1 kg; both $P < 0.001$ vs actual). Fat mass declined in SEV ($P < 0.001$) and MOD ($P = 0.006$), whereas fat-free mass remained unchanged in all groups ($P = 0.33$). RMR decreased by $-6 \pm 2\%$ in MOD ($P = 0.020$). In SEV, RMR did not change on a group level ($P = 0.66$), but participants whose RMR declined lost more weight ($P = 0.020$) and had a higher baseline RMR ($P = 0.026$) than those whose RMR did not decrease. Characteristic changes in leptin ($P = 0.003$), tri-iodothyronine ($P = 0.013$), insulin-like growth factor-1 ($P = 0.016$) and ghrelin ($P = 0.049$) occurred only in SEV. The energy deficit and adaptive changes in RMR explained 54% of the observed weight loss.

Conclusions: In normal-weight women, caloric restriction and exercise resulted in less-than-predicted weight loss. In contrast to previous literature, weight loss consisted almost exclusively of fat mass, whereas fat-free mass was preserved.

Introduction

Caloric restriction, exercise or a combination thereof is essential for successful weight loss.^{1–3} Past research on caloric restriction and exercise has focused on overweight and obese populations, but many normal-weight individuals and particularly young adult women also use caloric restriction and exercise to remain lean or to lose additional weight.^{4,5} However, when compared with their overweight and obese counterparts, normal-weight individuals undergoing weight loss are more likely to regain weight and typically accumulate more body fat during weight regain.^{6,7} Therefore, controlled studies on the effects of caloric restriction and exercise are much needed in normal-weight populations.

Traditional recommendations are based on the assumption that weight loss is proportional to the energy deficit,⁸ whereas more recent predictions acknowledge that additional factors, including the tissue composition of weight loss and metabolic adaptations, also modulate weight loss.⁹ On average, ~25% of weight loss consist of lean mass,¹⁰ but normal-weight individuals tend to lose proportionally more lean mass than overweight or obese individuals,¹¹ even though exercise may attenuate this loss of lean mass.¹² Because the energy content of fat mass is almost five times greater than that of lean mass,¹³ actual weight loss may be misestimated substantially when the composition of weight loss is unaccounted for. Moreover, metabolic adaptations that lower energy expenditure, such as a reduction in

resting metabolic rate (RMR) or the suppression of expendable metabolic functions,^{14–18} may explain why individuals in a putative energy deficit lose less weight than expected or even become weight stable.¹⁹ Understanding the effects of caloric restriction and exercise on body composition and metabolic adaptations in normal-weight individuals is also important because these outcomes may explain the greater propensity of weight regain and overproportional fat gain in this demographic.^{6,7} This knowledge may further expand our understanding of the metabolic aberrations in patients with anorexia nervosa and other eating disorders, who usually start restricting their caloric intake at a normal weight, and not while being overweight. Research in this population is typically limited to observational studies as well as interventions aimed at returning to a normal weight.

The goal of the present study was to assess the impact of caloric restriction and exercise in a normal-weight population on body weight, body composition and physiologic markers of energy conservation. For this purpose, we retrospectively analyzed data from a randomized controlled trial that assessed the impact of caloric restriction and exercise on reproductive function.²⁰ Primary outcomes included body weight, body composition, RMR and metabolic hormones linked to energy conservation and future weight regain, including leptin,^{16,21,22} ghrelin,^{21,23} triiodothyronine (T3)^{24,25} and insulin-like growth factor-1 (IGF-1).²⁶ We hypothesized that 3 months of caloric restriction combined with exercise would result in significant weight loss, but that exercise

would preserve lean mass and promote loss of fat mass such that actual weight loss would be less than predicted. We further hypothesized that women exposed to a greater energy deficit would demonstrate more pronounced metabolic adaptations indicative of energy conservation when compared with women in a smaller energy deficit, and that these metabolic adaptations would further attenuate actual weight loss.

Subjects and Methods

Experimental design

This is a secondary analysis of data from a randomized, prospective study in young normal-weight women. The study was originally designed to assess the impact of energy deficiency on reproductive function and was conducted over the course of three academic years.²⁰ Because primary outcome measures were related to reproductive function, the study duration was adjusted to each participants' menstrual cycle. Following a baseline cycle (~28 days), participants were randomly assigned to experimental groups for three menstrual cycles (~3 months).

Participants

Inclusion criteria were (1) female, (2) 18–30 years of age, (3) body weight between 45–75 kg, (4) body mass index between 18 and 25 kg/m², (5) 15–35% body fat, (6) nonsmoking, (7) no serious medical condition, (8) no current evidence or history of an eating disorder, (9) no use of medication affecting study outcomes, (10) no significant weight fluctuations (± 2.3 kg) during the past year, (11) <1 h per week of aerobic exercise, (12) no hormonal contraceptives in the past 6 months and (13) documentation of at least two ovulatory menstrual cycles. The study was approved by our Institutional Review Board, and all participants provided written informed consent. Screening included medical and menstrual histories, assessment of eating attitudes and behavior, a complete blood count, basic chemistry and endocrine panels, an interview to assess the risk of eating and other psychiatric disorders, and verification of menstrual status via calendars and confirmation of ovulation.²⁰

Study groups

Following screening, participants were randomly assigned to study groups. To minimize the confounding effect of varying exercise regimen, the present analysis is limited to groups with the same exercise prescription: (1) a severely calorie-restricted group (SEV) where caloric intake was reduced by 30% from baseline; (2) a moderately calorie-restricted group (MOD) where caloric intake was maintained at baseline levels; and (3) a balanced control group (BAL) where caloric intake was increased to match the increased exercise expenditure. All groups conducted aerobic exercise on 5 days per week designed to increase energy expenditure on exercise days by 30%.

Baseline

During baseline, participants were kept in energy balance and weight stable. Baseline caloric intake matched baseline energy expenditure, calculated as baseline RMR adjusted for physical activity measured during the first week of baseline using accelerometry (RT3, Stayhealthy, Monrovia, CA, USA).^{20,27} Minor adjustments in caloric intake (± 100 kcal) were made if fasting body weight changed by 40.5 kg during the first week.^{28,29}

Intervention

Following completion of baseline, food intake was adjusted per group assignment and participants conducted supervised exercise on 5 days per week. Exercise modes included treadmill running, elliptical, stair stepping and stationary bicycling. Participants wore heart rate monitors (S610, Polar, Kempele, Finland), and intensity was maintained at 70–80% of maximal heart rate. Twice per week, heart rate monitors were calibrated with the most recent body weight, maximum heart rate, maximal oxygen uptake and age.³⁰ Exercise duration was increased gradually and was adjusted individually such that energy expenditure on exercise days increased by 30% over baseline energy expenditure.

Food intake

During the first week of baseline and throughout the intervention, all food was prepared and weighed in our Clinical Research Center's metabolic kitchen. The macronutrient composition of the diet was 55% energy from carbohydrates, 30% energy from fat and 15% energy from protein. On weekdays, participants consumed two meals per day in the lab. Dinner, snacks and weekend meals were packed out. Participants were instructed to consume all food as prepared, and, when necessary, to weigh and record uneaten food and additional food not prescribed. Caloric intake was analyzed using Nutritionist Pro software (First Data Bank, Indianapolis, IN, USA). Participants met regularly with a clinical dietician to ensure compliance to diet prescriptions.

Body weight and body composition

Fasting body weights were taken at least once per week. Body composition was assessed by hydrostatic weighing during baseline (pre) and after study completion (post). Measurements were repeated until three tests yielded a difference of <0.5%. Body density, corrected for residual lung volume, was used to calculate fat and fat-free mass.³¹

Resting metabolic rate

RMR was assessed at pre and post. Following an overnight fast (≥ 12 h) and abstention from exercise (≥ 48 h), participants rested in a supine position for 20–30 min. Thereafter, a ventilated hood was placed over their head, and CO₂ (URAS 4, Hartmann & Braun, Frankfurt, Germany) and O₂ exchange (Magnos 4G, Hartmann & Braun, Frankfurt, Germany) were measured for 30 min. RMR was calculated from steady-state data using the Weir equation.³² To characterize adaptive changes in RMR that were independent of body composition changes, RMR was adjusted for fat-free mass.³³

Blood sampling and biochemical analyses

Assays were performed on fasting (≥ 10 h) blood samples obtained at pre, mid-study (mid) and at post. Mid-study blood samples were collected after completion of the first intervention cycle (~30 days) in study year 1, and after completion of the second intervention cycle (~60 days) in study years 2 and 3. Serum aliquots were stored at -80°C until analysis. Assays were run in duplicate and samples from one participant were analyzed within one batch. Leptin and ghrelin were assayed with radioimmunoassays from Linco Research (St Charles, MO, USA) with a sensitivity of 0.5 ng/ml (leptin) and 100 pg/ml (ghrelin), and intra-assay and inter-assay coefficients of variation of <8.3 and <6.2% (leptin) and <2.7 and <16.7% (ghrelin). Radioimmunoassay for total T3 (Diagnostics Products, Los Angeles, CA, USA) and IGF-1 (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA) had sensitivities of 7 ng/dl (T3) and 0.06 ng/ml (IGF-1), and intra-assay and inter-assay coefficients of variation of <8.9 and <10% (T3) and <3 and <8.4% (IGF-1).

Calculations

Total energy expenditure was calculated as the sum of RMR, thermic effect of food, exercise expenditure and 24 h non-exercise physical activity. The thermic effect was defined as 10% of caloric intake.³⁴ Exercise expenditure was obtained from heart rate monitors,³⁵ and non-exercise activity was assessed by triaxial accelerometry (RT3, Stayhealthy).^{36,37} Weight loss as well as changes in fat and fat-free mass were predicted using the National Institute of Health Body Weight Planner,³⁸ which utilizes mathematical modeling of human metabolism to simulate adaptations in energy expenditure during weight loss. The model combines data on changes in metabolism and body composition from a variety of populations across the body weight spectrum, and accounts for early- and late-phase weight changes, partitioning between adipose and lean tissue, and changes in RMR, and it has been validated against data from various weight-loss interventions.⁹ To predict weight loss, the following variables were entered for each participant: sex, weight, age, height, fat mass, RMR, physical activity level and duration of the intervention.

Statistical analyses

Statistical analyses were performed with R (version 3.2.2, R Foundation for Statistical Computing, Vienna, Austria). If not stated otherwise, data are reported as mean $\pm$ s.e.m. Normality was assessed using the

Table 1. Baseline characteristics of normal-weight women assigned to a severely calorie-restricted diet and exercise (SEV), a moderately calorie-restricted diet and exercise (MOD), and an energy-balanced diet and exercise (BAL)

Condition	N	Age (years)	Height (cm)	Weight (kg)	Body mass index (kg/m ²)	Body fat (%)	Physical activity level	Energy intake (kcal per day)	Carbohydrate intake (%)	Fat intake (%)	Protein intake (%)
SEV	9	20.1±0.6	163.7±1.9	60.9±1.2	22.8±0.6	29.8±1.4	1.64±0.06	1822±196	53±2	33±2	13±1
MOD	7	19.3±0.5	164.6±2.4	61.6±0.7	22.7±0.7	30.9±1.4	1.67±0.06	1954±225	55±3	29±2	12±1
BAL	9	20.4±0.3	165.4±2.4	59.1±2.3	21.7±1.2	27.0±2.2	1.64±0.05	1651±192	55±2	31±1	12±1

Data are presented as mean±s.e.m.

Table 2. Caloric intake, expenditure and energy balance over the course of the study in normal-weight women assigned to a severely calorie-restricted diet and exercise (SEV), a moderately calorie-restricted diet and exercise (MOD), and an energy-balanced diet and exercise (BAL)

Condition	N	Caloric intake (kcal per day)	Total energy expenditure (kcal per day)	Exercise expenditure (kcal per day)	Energy deficit (kcal per day)	Energy deficit (%)
SEV	9	1308±20 ^{a,b}	2370±80	304±22 ^b	-1062±80 ^{a,b}	-44±2 ^{a,b}
MOD	7	2003±41 ^a	2636±82	386±23	-633±71 ^a	-24±2 ^a
BAL	9	2239±77	2399±84	330±15	-161±39	-6±2

a. Significantly different from BAL ($P<0.01$; $P<0.001$).

b. Significantly different from MOD ($P<0.05$; $P<0.001$). Data are presented as mean±s.e.m.

Shapiro–Wilk test, and group differences, changes over time and group × time interactions were assessed by two-way repeated measures analysis of variance or Kruskal–Wallis test, depending on normality. When effects or interactions were observed ($P<0.1$), *post hoc* analyses were conducted using Student's *t*-test or Mann–Whitney *U*-test, depending on normality. Significance was considered for $P<0.05$ and was Bonferroni-adjusted for multiple comparisons. On the basis of previously published data,²⁰ group sizes of $n = 7$ were required to detect a 0.5 kg difference in weight loss among study groups ($P<0.05$) with 80% power. Data from all study groups were combined to identify predictors of weight loss. Potential predictors included the energy deficit, baseline body mass index, body fat, RMR and metabolic hormones, and adaptive changes in RMR and metabolic hormones. Initially, correlation coefficients (Pearson's *r*) with weight loss were determined for each predictor, followed by stepwise linear regression, which included all factors with significant *r*. A sample size of $n = 25$ was sufficient to detect three factors accounting for ≥37% of weight loss ($P<0.05$) with 80% power.

Results

Participants and compliance

Of the 27 participants who entered the study in the three groups, 25 completed the intervention. Of these, nine participants had been assigned to SEV, seven to MOD and nine to BAL. At screening, participants were 20.0 ± 0.3 years old, their body mass index was 22.4 ± 0.5 kg/m² and their baseline physical activity level (total energy expenditure divided by RMR) was 1.66 ± 0.03 . Baseline anthropometrics, body composition, physical activity, caloric intake and macronutrient composition were not different among groups (Table 1). The intervention duration was on average 87 ± 2 days and did not differ among groups ($P>0.12$). Actual and prescribed caloric intake differed on average by $0.8 \pm 0.4\%$ from prescribed caloric intake, and all participants reached at least 95% of their prescribed exercise expenditure. The resulting energy deficits (Table 2) were $44 \pm 2\%$ (SEV), $24 \pm 2\%$ (MOD) and $6 \pm 2\%$ (BAL).

Changes in body weight and body composition

After ~3 months, women in SEV (-3.7 ± 0.9 kg, $P<0.001$) and MOD (-2.7 ± 0.8 kg, $P = 0.003$) had lost significant weight (Figure 1), whereas weight loss in BAL (-1.1 ± 0.4 kg, $P = 0.13$) was not significant. Weight loss was not significantly different between SEV and MOD ($P = 0.19$). Actual weight loss was significantly less than predicted in both SEV (-11.1 ± 1.0 , $P<0.001$ vs actual) and MOD (-6.5 ± 1.1 kg, $P = 0.017$ vs actual). Fat mass declined

significantly in SEV (-2.6 ± 0.7 kg, $P<0.001$) and MOD (-2.2 ± 0.6 kg, $P = 0.006$), but the loss of fat mass was significantly less than predicted only in SEV (-5.9 ± 0.5 kg, $P = 0.003$ vs actual). Fat-free mass remained unchanged in all groups ($P>0.33$), which was contrary to the NIH model, that predicted fat-free mass losses of -4.5 ± 0.8 kg (SEV) and -2.6 ± 0.7 kg (MOD).

Changes in RMR and metabolic hormones

Baseline RMR was higher in MOD when compared with BAL and SEV (both $P = 0.031$). After adjusting RMR for fat-free mass, this difference became insignificant ($P = 0.064$). Adjusted RMR (Figure 2) decreased by $6 \pm 2\%$ in MOD ($P = 0.019$) and increased by $10 \pm 3\%$ in BAL ($P = 0.006$). In SEV, adjusted RMR did not change on a group level ($P = 0.66$), but decreased by $13 \pm 4\%$ in 4 participants and increased by $21 \pm 7\%$ in the remaining 5 participants. Sub-analysis of participants in SEV based on their RMR change (Table 3) revealed that those participants whose adjusted RMR decreased had a significantly higher baseline RMR when compared with those whose RMR did not decrease ($P = 0.026$). Further, participants whose RMR decreased also lost more weight ($P = 0.020$) and fat mass ($P = 0.038$).

Serum concentrations of leptin ($-37 \pm 9\%$, $P = 0.005$), total T3 ($-12 \pm 4\%$, $P = 0.013$) and IGF-1 ($-13 \pm 4\%$, $P = 0.016$) were reduced in SEV at the study mid-point (Figure 3), but only T3 remained below baseline concentrations until the study end point ($-13 \pm 5\%$, $P = 0.032$). Ghrelin was elevated in SEV only at the study end point ($35 \pm 13\%$, $P = 0.049$). Changes in leptin, but not in T3, IGF-1 and ghrelin, differed significantly between participants in SEV whose RMR decreased when compared with those whose RMR increased ($P = 0.023$). Leptin, T3, ghrelin and IGF-1 did not change significantly in MOD or BAL.

Predictors of weight loss

Actual weight loss was correlated with the energy deficit ($r = 0.42$, $P = 0.038$), the change in RMR ($r = -0.57$, $P = 0.003$) and the change in leptin ($r = -0.41$, $P = 0.043$), and there were trends suggesting that weight loss was also correlated with baseline body fat percentage ($r = 0.34$, $P = 0.088$) and baseline RMR ($r = 0.36$, $P = 0.074$). Stepwise linear regression revealed that only the energy deficit ($P = 0.004$) and changes in RMR ($P<0.001$) independently predicted actual weight loss. The energy deficit explained 17% of actual weight loss, and including the change

Figure 1. Top: time course of weight loss in normal-weight women assigned to a severely calorie-restricted diet and exercise (SEV, $n=9$), a moderately calorie-restricted diet and exercise (MOD, $n=7$), or a diet designed to maintain energy balance while exercising (BAL, $n=9$). Bottom: comparison of actual weight loss (left), loss of fat mass (middle), and fat-free mass (right) and changes as predicted by the NIH model.³⁸ Data are presented as mean $\pm$ s.e.m.

##, ### Significantly different from 0 ($P<0.01$, $P<0.001$);

†, ††, ††† Significantly different from actual loss ($P<0.05$, $P<0.01$, $P<0.001$).

Figure 2. Changes in resting metabolic rate adjusted for fat-free mass from baseline (pre) to the end of the intervention (~90 days) in normal-weight women assigned to a severely calorie-restricted diet and exercise (SEV, $n=9$), a moderately calorie-restricted diet and exercise (MOD, $n=7$), or a diet designed to maintain energy balance while exercising (BAL, $n=9$).

in RMR as well as the interaction between the energy deficit and the change in RMR significantly improved the prediction of actual weight loss to 54%. The interaction between the energy deficit and the change in RMR was negatively associated with actual weight loss, suggesting that participants whose RMR dropped lost less weight than participants whose RMR did not change or increased.

DISCUSSION

The present study is the first in normal-weight women to report the effects of prolonged caloric restriction and exercise

on weight loss, body composition and metabolic adaptations. Even though caloric restriction led to weight loss, the amount of weight lost was substantially lower than expected. On average, women assigned to a caloric deficit lost only $42 \pm 13\%$ (MOD) and $35 \pm 8\%$ (SEV) of the weight predicted by the NIH model. Also contrary to NIH predictions, weight loss consisted almost exclusively of fat loss, whereas fat-free mass was preserved in the present study. Metabolic adaptations to caloric restriction included characteristic reductions in RMR and alterations in key metabolic hormones, and stepwise linear regression suggests that adaptive changes in RMR, but not changes in metabolic hormones, attenuated weight loss, likely through energy conservation.

Composition of weight loss

The discrepancy between actual and predicted weight loss following caloric restriction and exercise is partially explained by the tissue composition weight loss. In contrast to the NIH model, which predicted fat-free mass to account for more than one-third of weight lost, fat-free mass was almost completely preserved in the present study. This finding is surprising considering that lean individuals typically lose a greater proportion of fat-free mass when compared with their overweight or obese counterparts,¹¹ and is in contrast to previous reports in overweight and obese individuals, who lost 5–9% of lean mass during 3 months of caloric restriction and exercise.^{14,15,39} However, as exercise, and particularly strenuous exercise, attenuates the degradation of lean mass during weight loss,¹² it is likely that our exercise regimen of 5 days per week was more protective of fat-free mass than the three exercise sessions per week that have been used previously in overweight and obese individuals.^{14,15} Because fat mass requires a much greater energy deficit to lose the same amount of weight when compared with weight loss of mixed composition,¹³ it is not surprising that the NIH model overestimated actual weight loss, which was composed almost

Figure 3. Time course of changes in leptin (top left), total T3 (top right), ghrelin (bottom left), and IGF-1 (bottom right) in normal-weight women assigned to a severely calorie-restricted diet and exercise (SEV, *n*=7), a moderately calorie-restricted diet and exercise (MOD, *n*=7), or a diet designed to maintain energy balance while exercising (BAL, *n*=9). Data are presented as mean ± s.e.m. #, ## Significantly different from pre (*P*<0.05, *P*<0.01).

Table 3. Comparison of normal-weight women whose RMR^a responded with a decrease following 3 months of severe calorie restriction and exercise ('RMR responders') vs those whose RMR^a did not decrease ('RMR nonresponders')

Variable	RMR responders (<i>n</i> = 4)	RMR nonresponders (<i>n</i> =4)	P-value
Baseline RMRa (kcal per day)	1398±77	1121±63	0.026
Change in RMRa (kcal per day)	-188±69	219±67	0.012
Baseline BMI (kg/m ²)	22.7±1.1	22.8±0.7	0.90
Baseline weight (kg)	61.7±2.0	60.6±2.1	0.72
Change in weight (kg)	-5.8±1.0	-1.9±0.8	0.020
Weight loss (% of NIH model)	54.6±4.9	19.6±8.0	0.010
Baseline fat mass (kg)	19.6±0.9	17.2±1.5	0.24
Change in fat mass (kg)	-4.2±1.1	-1.3±0.6	0.038
Baseline fat-free mass (kg)	40.8±1.5	42.4±1.8	0.53
Change in fat-free mass (kg)	-0.2±0.7	0.8±0.5	0.24
Baseline leptin (ng/ml)	13.5±3.7	10.2±1.0	0.36
Change in leptin (%)	-57.2±8.7	4.4±17.5	0.023
Baseline total T3 (ng/dl)	110.0±4.1	97.4±7.4	0.21
Change in total T3 (%)	-20.4±5.2	-6.1±6.8	0.16

Abbreviations: BMI, body mass index; RMR, resting metabolic rate; T3, triiodothyronine.

a. Adjusted for fat-free mass. Data are presented as mean ±s.e.m.

exclusively of fat mass. However, adjusting energy balance for the energy equivalents of changes in fat mass and fat-free mass, an approach that has previously been used to quantify adherence to dietary interventions in overweight women,⁴⁰ resulted in only a minimal improvement in actual vs predicted weight loss in MOD (55±26%) and no improvement in SEV (29 ±7%).

Compliance

Even though great efforts were made to maximize compliance, we cannot rule out that failure to adhere to diet prescriptions contributed to overestimation of weight loss. However, our approach, which involved two supervised meals per day on weekdays, and packing out all other food and weekend meals, was

virtually identical to what has been applied in seminal weight-loss studies such as the CALERIE study,^{14,15} where overall adherence was considered very good.⁴¹ Further, our finding that weight loss was almost equally overestimated in MOD and SEV despite distinctly different degrees of calorie restriction between these groups is in disagreement with previous reports that noncompliance is predicted by the magnitude of the calorie deficit,⁴⁰ and suggests that other metabolic factors may be responsible for the limited agreement between actual and predicted weight loss.

Metabolic adaptations

After adjusting RMR for fat-free mass, we observed a 5% reduction in RMR in women who were in a moderate energy deficit while exercising, whereas adjusted RMR increased by 10% in women who conducted the same amount of exercise while being in energy balance. We failed to observe a reduction in RMR on a group level in women in a more severe energy deficit, but RMR dropped on average by 13% in four participants. These participants lost notably more weight and exhibited more pronounced changes in metabolic hormones when compared with five participants in SEV whose RMR did not decrease, indicating that about half of the participants in SEV responded to the caloric deficit as expected. However, in the other five participants in SEV whose RMR did not decrease, baseline RMR adjusted for fat-free mass was almost 20% lower, and fat mass, leptin and T3 were also reduced at baseline. This finding suggests that these women may have already been metabolically suppressed prior to the start of the study. In light of these findings, future studies on energy balance, metabolic adaptations and weight loss should aim to carefully control for baseline RMR, particularly in normal-weight women.

Despite the absence of a group effect for RMR in SEV, stepwise linear regression across the whole data set identified changes in RMR adjusted for fat-free mass as an independent predictor of actual weight loss. This finding complements previous reports of attenuated weight loss secondary to RMR suppression in overweight populations^{39,42} as well as literature in anorexia nervosa patients, whose RMR appears to be chronically suppressed.⁴³ The reduction in RMR beyond what is accounted for by changes in fat-free mass is also referred to as adaptive thermogenesis. Adaptive thermogenesis is considered to be a result of the downregulation of cellular thermogenesis via uncoupling protein 1 and other mechanisms, primarily in the liver and skeletal muscle, and changes in T3, leptin and sympathetic nervous system have been considered as the primary drivers of adaptive thermogenesis.⁴⁴ In support of this, we found that T3 and leptin closely mirrored changes in RMR adjusted for fat-free mass, which is also in agreement with data from anorexia nervosa patients, in whom T3, leptin and RMR are chronically suppressed, but return to normal levels following refeeding and weight gain.^{22,25} Although parallel changes in T3, leptin and RMR have also been reported in normal-weight men undergoing calorie-restricted weight loss and subsequent refeeding, it is noteworthy that neither changes in T3 or leptin nor sympathetic nervous system activity predicted the magnitude of adaptive thermogenesis in this population.⁴⁵ As such, further research is needed to establish the biological basis of adaptive thermogenesis in humans.⁴⁴

Taken together, the metabolic effects observed in the present study are generally in agreement with what has been reported in weight-loss interventions in overweight and obese populations as well as in observational studies in anorexia nervosa patients,

although weight loss was much less than expected, even after accounting for differences in the composition of weight loss and potential lack of compliance. This discrepancy suggests that other metabolic adaptations unique to normal-weight women may prevent them from becoming underweight. One such mechanism is the loss of menstrual function, which is understood as a common biological response to starvation that serves to conserve energy.⁴⁶ Our previous finding that the frequency of menstrual disturbances is predicted by the magnitude of the energy deficit,²⁰ as well as the high prevalence of menstrual disturbances among anorexia nervosa patients⁴⁷ and energy-deficient exercising women,⁴⁸ suggest that this adaptation is more likely to occur at the lower end of the adiposity spectrum. As such, prediction equations may need to be refined in normal-weight women to account for metabolic adaptations exclusive to normal-weight or underweight women, which also has important implications for our understanding of metabolic aberrations and weight management in eating disorder patients.

Conclusion

In normal-weight women, calorie restriction and exercise result in weight loss, loss of fat mass and characteristic metabolic adaptations indicative of energy conservation. However, weight loss was considerably less than expected from advanced prediction models, and contrary to previous literature, fat-free mass was almost completely preserved in our group of normal-weight women. Further research is needed in normal-weight populations to refine weight-loss predictions, and to identify how caloric restriction and exercise contribute to long-term weight stability.

Conflict of Interest — The authors declare no conflict of interest.

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