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Maintenance of resting energy expenditure after weight loss in premenopausal women: potential benefits of a high-protein, reduced-calorie diet

Stefan M. Pasiakos, Jodi B. Mettel, Kristy West, Ingrid E. Lofgren, Maria Luz Fernandez, Sung I. Koo, Nancy R. Rodriguez*
Department of Nutritional Sciences, University of Connecticut, Storrs, CT 06269, USA
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Abstract

The number of contemporary diet plans promoting high protein intakes for weight management has increased dramatically. Complementing this dietary approach with increased physical activity has proven to be beneficial. Recent studies have suggested that protein intakes in excess of the current Recommended Dietary Allowance (0.8 g/kg) may be of metabolic benefit during weight loss. This investigation assessed changes in resting energy expenditure and substrate oxidation in overweight and obese premenopausal women in response to a weight loss intervention that combined a high-protein, reduced-calorie diet with increased physical activity. Thirty-nine overweight and obese premenopausal women (age, 30.9 ± 1.5 years; body mass index, 30.2 ± 0.5 kg/m^2) participated in a 10-week weight loss program in which they ate a reduced-calorie diet for which protein provided 30% of total energy and approximated 1.4 g/kg. Subjects incrementally increased physical activity (ie, steps walking) throughout the diet intervention period. Resting energy expenditure, substrate oxidation, and body composition were assessed before (PRE) and after (POST) the 10-week weight loss program. Subjects experienced a 5% decrease in body weight, with significant decreases in both fat mass (PRE, 35.5 ± 1.2 kg; POST, 32.4 ± 1.1 kg; $P < .0001$) and fat-free mass (PRE, 44.6 ± 0.7 kg; POST, 43.6 ± 0.7 kg; $P < .0001$). Changes in body weight or body composition did not alter resting energy expenditure. Protein oxidation increased (PRE, 18% ± 1%; POST, 20% ± 1%; $P < .05$) and fat oxidation decreased (PRE, 37% ± 3%; POST, 30% ± 3%; $P < .05$) after the 10-week intervention. These findings illustrate that a weight loss intervention combining consumption of a high-protein, reduced-calorie diet with increased physical activity promotes weight loss without negatively impacting resting energy expenditure in this population of women.

1. Introduction

Overweight and obesity have reached epidemic proportions in the United States. According to recent reports from the Centers for Disease Control and Prevention, more than 66% and 32% of Americans are either overweight or obese, respectively [1]. Obese and overweight individuals are at increased risk for developing cardiovascular disease, metabolic syndrome, and diabetes mellitus. These health risks, as well as the psychosocial ramifications of being overweight, have more than two thirds of adults in the United States trying to lose weight [2,3].

Various weight loss programs are marketed to the American public [4]. These programs offer a variety of methods to achieve weight loss, including diet—particularly changes in macronutrient composition, exercise, behavior modification, herbal remedies, and pharmacotherapy [4]. The dietary components of these programs vary in their recommended energy content and macronutrient composition [5-8]. Exercise recommendations vary in the frequency and mode of exercise as well as exercise intensity and duration [9,10].

The American Heart Association, American Dietetics Association, American College of Sports Medicine, Institute of Medicine, Centers for Disease Control and Prevention,

* Corresponding author. Tel.: +1 860 486 0120.
E-mail address: nancy.rodriguez@uconn.edu (N.R. Rodriguez).

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Table 1
An example of a typical menu provided to the subjects of the 1400-kcal group

Meal	
Breakfast	Omelet:
	½ Cup egg substitute, ¼ cup chopped onion
	¼ Cup chopped green pepper, ¼ cup chopped tomato
	1 Teaspoon olive oil
	Sauté onion, pepper, and tomato in oil. Add egg and let cook.
	Optional: salt and pepper to taste
Snack	1 Medium slice of wheat bread
Lunch	Chef salad:
	2 Cups chopped lettuce, ¾ cup chopped tomato
	¼ Cup chopped green pepper, ¼ cup chopped onion
	2 Teaspoons olive oil, 2 teaspoons vinegar
	2 oz deli turkey, 1 oz deli ham
	Dessert:
	¼ Cup raisins
Snack	1 oz provolone cheese, 1 medium apple
Dinner	Entrée:
	Sauté chicken in oil mixed with BBQ sauce.
	6 oz chicken breast, 1 teaspoon olive oil, 2 teaspoons BBQ sauce
	1 Cup spinach (sauté or steam)
	1 Cup asparagus (sauté or steam)
Snack	1 oz tortilla chips, 1 tablespoon salsa
	¼ Cup mozzarella cheese

The subjects were provided 90% of the food. The remaining 10% were considered perishable, such as condiments and milk, and were the choice and responsibility of the subjects.

and National Institutes of Health all promote diet modification, physical activity, and behavior modification as important components of a successful weight loss and maintenance program [1,8-12]. The ideal macronutrient composition and energy intake needed to successfully lose weight while maintaining fat-free mass (FFM) remain elusive. Physical activity has been shown to positively influence loss of fat mass (FM) and to decrease the loss of FFM typically seen after weight loss [13]. Recently, Layman and coworkers [14] demonstrated that a reduced-calorie diet providing approximately 1.5 g protein per kilogram per day imparted metabolic benefits to women during weight loss, particularly with simultaneous participation in programmed exercise. These researchers showed a preservation of FFM during weight loss when protein provided approximately 30% of total energy [14]. Theoretically, either maintenance of or a reduced loss of FFM with weight loss would prevent changes in resting energy expenditure (REE) frequently seen in these situations [15]. Therefore, the primary objective of the present study was to evaluate the impact of a combined reduced-calorie diet for which protein provided 30% of the total energy and increased physical activity on REE and substrate oxidation in overweight and obese premenopausal women. We hypothesized that habitual consumption of a reduced-calorie diet for which protein provided a greater proportion of calories in conjunction with increased physical activity would promote maintenance of REE subsequent to weight loss. We also predicted that changes in substrate oxidation would reflect changes in macronutrient composition consequent to the implementation of the high-protein diet intervention.

2. Methods

2.1. Study design

After approval by the University of Connecticut Institutional Review Board, subjects were recruited from the University of Connecticut and surrounding communities. Exclusionary criteria included history of cardiovascular disease, kidney or liver problems, or diabetes mellitus. Women who were pregnant or lactating were also excluded. Thirty-nine women between the ages of 20 and 45 years with a body mass index (BMI) between 25 and 37 kg/m^2 and who reported themselves as sedentary to moderately active using the International Physical Activity Questionnaire [16] participated in this study. Criterion measures (ie, REE, substrate oxidation, anthropometry, body composition, and urinary creatinine) were assessed during an initial baseline period before the study intervention (PRE) and during the 10th week of the weight loss intervention (POST). Therefore, each subject served as their own control.

2.2. Diet intervention

Total energy expenditure (TEE) was estimated using the Harris-Benedict equation corrected for physical activity by using an activity factor of 1.2 [17]. Energy intake was set at 85% of TEE; and carbohydrates (CHO), proteins (PTN), and total fat (FAT) provided 40%, 30%, and 30% of energy intake, respectively. Subjects were assigned to one of 5 groups (1400, 1500, 1600, 1700, or 1800 kcal) based on the estimated energy intake for each individual. Assigned energy intake specific for each group was maintained for the duration of the study.

Subjects received daily menus from alternating, 2-week–cycle menus created by the researchers specific to each energy group. Researchers provided each individual with 90% of the groceries specific to the participant's specific menu plan for her energy group assignment each week throughout the study. Perishable groceries, such as milk, butter, cooking oil, and condiments (ie, mustard and ketchup), were the responsibility and choice of the subjects. To keep subjects adherent to their specific energy and daily food plans, subjects were allowed to choose foods from a list of acceptable items of appropriate portions provided by the researchers. An example of a 1-day menu is provided in Table 1. To determine individual adherence, subjects maintained diet records of actual foods consumed on forms specific to each daily menu throughout the 10-week intervention. To ensure accurate reporting of energy intake, subjects were also instructed to record any modifications made to their daily menus.

Estimates of baseline nutrient intake were self-reported by the subjects completing a 120-item food-frequency questionnaire developed by the Fred Hutchinson Cancer Research Center (Seattle, WA). Participants determined serving size by comparing their portions with pictures of small, medium, and large food items. Participants recorded the average number of times in the previous 3 months they had consumed each food listed in the questionnaire. During the intervention, the participants' menus for 3 nonconsecutive weeks (15 weekdays and 6 weekend days) were analyzed for estimates of energy and nutrient intake using the Nutrient Database Systems for Research version 4.05_33 (Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN) nutrient analysis software.

2.3. Physical activity

Each participant received an Omron HJ-104 (Omron Healthcare, Vernon Hills, IL) pedometer for estimation of the number of daily steps taken during 1 week. Log sheets were provided for recording step data and to estimate daily physical activity. Baseline number of steps was determined by an average of the number of steps taken each day during the first week. Subjects were instructed to maintain this activity level for the baseline period (PRE). After the baseline assessment, subjects were instructed to increase their daily steps by 1500 steps (~1 km/d) more at 3 different periods throughout the 10-week study (weeks 2, 4, and 6). As a result, an increase of 4500 steps or approximately 3 km/d over baseline was expected to occur by week 10 of the study. To verify the accuracy of the number of steps reported by the subjects, as well as subject adherence, log sheets were provided to the subjects for recording the number of steps taken at the end of each day. These sheets were collected at the beginning of each week. In addition, the pedometers were equipped with a 7-day memory function. Subjects were selected at random to verify the accuracy of the reported number of steps taken per day compared with those recorded on the pedometer.

2.4. Anthropometry

Anthropometric assessments were performed consistently the morning after a 12-hour fast for all subjects. Weight and height measurements were performed using standardized techniques and equipment. Weight was measured to the nearest 0.5 lb and height to the nearest 0.5 in on a portable stadiometer scale [18]. These measurements were converted into metric measures to calculate BMI (in kilograms per square meter).

2.5. Body composition

Body composition was assessed PRE and POST by a trained individual using dual-energy x-ray absorptiometry (DEXA) (DPX-MD densitometer; LUNAR, Madison, WI). All body composition assessments were performed at similar times the morning after a 12-hour fast. Fat mass, FFM, and percentage of body fat were determined using composite body mass obtained from DEXA scans.

2.6. Resting energy expenditure

Resting energy expenditure was assessed by open-circuit spirometry and indirect calorimetry using a metabolic cart (MedGraphics CPX/D; Medical Graphics, St Paul, MN) PRE and POST the weight loss intervention. Subjects were driven to the Metabolic Assessment Laboratory between 6:00 AM and 9:00 AM after an approximately 12-hour overnight fast. Predicted REE was calculated using the Harris-Benedict equation. Subjects rested in the supine position for 10 to 15 minutes before beginning the test. Resting energy expenditure was assessed while lying in a quiet, temperature-regulated room for approximately 20 minutes. The test was discontinued when 20 minutes of steady-state oxygen consumption (VO_2) and carbon dioxide production (VCO_2) was recorded.

2.7. Nitrogen excretion

Urinary nitrogen was measured from a single pooled 24-hour sample for each subject during PRE and POST. Urine was collected in bottles provided to the subjects and stored (on 15 mL of 0.1 N HCl) in refrigerated containers to preserve urinary ammonia. Urine volume was measured, and aliquots were stored at −20°C until analyzed. Total nitrogen content of the urine was determined using a micro-Kjeldahl apparatus (Tecator Kjeltec System, Hoganas, Sweden).

2.8. Substrate oxidation

Nitrogen values determined by the 24-hour urinary nitrogen measurement that coincided with the respective REE determinations were used to estimate substrate oxidation according to Ferrannini [19]. Equations modified for use for estimates of substrate oxidation were as follows (MedGraphics CPX/D, Medical Graphics):

$$\text{Protein oxidation (in grams per day)} \\ = 6.25 \times \text{urinary nitrogen } (N)$$

$$\text{Glucose oxidation (in grams per day)} \\ = 4.12\, VCO_2 - 2.91\, VO_2 - 2.56N$$

$$\text{Fat oxidation (in grams per day)} \\ = 1.69\, VCO_2 - 1.69\, VO_2 - 1.92\, N$$

Substrate oxidation rates were expressed as a percentage of TEE.

2.9. Urinary creatinine

To determine completeness of urine collections, urinary creatinine concentration was determined for each subject from a 24-hour pooled urine sample that coincided with the respective REE PRE and POST. Creatinine excretion was estimated using in vitro colorimetric method for the quantitative determination of creatinine in serum or in urine (Wako Clinical Diagnostic Reagents creatinine kit 277-10501; Wako Chemicals USA, Richmond, VA). Briefly,

Table 2
Anthropometric, physical activity, REE, total energy, and macronutrient intake of premenopausal women at baseline and after the 10-week intervention

	PRE	POST
Weight (kg)	80.2 ± 1.6	76 ± 1.6 ***
BMI (kg/m^2)	30.2 ± 0.5	28.6 ± 0.5 *
No. of steps per day	10 205 ± 981	13 157 ± 786 **
REE (kcal)	1402 ± 41	1364 ± 33
Energy intake (kcal)	2145 ± 133	1519 ± 15 ***
Carbohydrates (% energy)	51 ± 1.7	42 ± 0.2 ***
Total fat (% energy)	32 ± 1.4	32 ± 0.2
Protein (% energy)	17 ± 0.4	28 ± 0.2 ***
Protein (g/d)	87 ± 5.0	107 ± 1 ***
Protein (g/kg)	1.1 ± 0.07	1.4 ± 0.03 ***

Values are mean ± SEM (n = 39).
* $P < .05$, significantly different from PRE.
** $P < .01$, significantly different from PRE.
*** $P < .0001$, significantly different from PRE.

0.5 mL of urine was combined with 3.0 mL deproteinizing reagent. Two-milliliter aliquots of the solution were pipetted out and incubated for 5 minutes in a 25°C to 30°C water bath. One milliliter of picric acid and 1.0 mL of 0.75 N NaOH were added, and the solution was incubated for 20 minutes in a 25°C to 30°C water bath. The sample solution was then read at 520 nm on a DU-640 UV spectrophotometer (Beckman Coulter, Fullerton, CA).

2.10. Statistical analysis

Repeated-measures analysis of variance was used to evaluate overall study differences before and after the 10-week intervention on all respective criterion measures (mean ± SEM). When significant differences were found, post hoc analyses were performed using paired Student *t* tests. The α level of significance was set at .05. All data were analyzed using SPSS version 11.5 (SPSS, Chicago, IL).

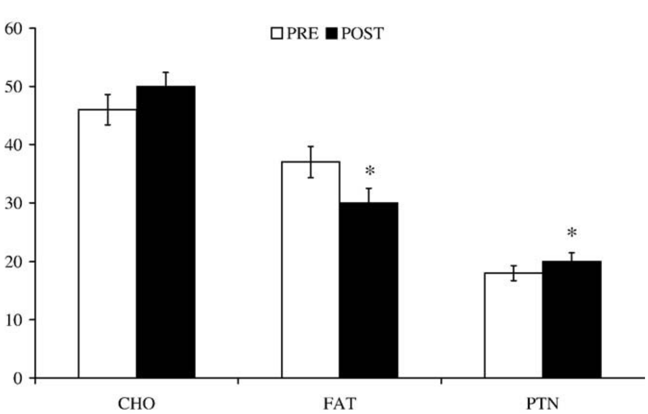

Fig. 1. Changes in percentage of substrate oxidation of premenopausal women at baseline and after the 10-week intervention. Values are mean ± SEM (n = 39). *$P < .05$, significantly different from PRE.

3. Results

3.1. Subject characteristics and anthropometrics

Subjects' mean age, height, and weight were 30.9 ± 1.5 years, 64 ± 0.4 in, and 80.2 ± 1.6 kg, respectively. Before the weight loss intervention, mean BMI was 30.2 ± 0.5 kg/m^2 (Table 2). Body weight significantly decreased from PRE to POST, with subjects losing approximately 4.2 kg during the weight loss intervention. As a result, BMI decreased an average of 1.6 kg/m^2 ($P < .05$).

3.2. Total energy intake and macronutrient composition of the 10-week diet intervention

Data for total energy intake and macronutrient composition of the diet are presented in Table 2. Total energy intake decreased from PRE (2145 ± 133 kcal/d) to POST (1519 ± 15 kcal/d, $P < .0001$). In addition, changes in macronutrient composition reflected those of the prescribed 10-week diet intervention. Absolute and relative protein intake increased from PRE (87 g/d and 1.1 g/kg) to POST (107 g/d and 1.4 g/kg, $P < .0001$), respectively.

3.3. Physical activity, REE, and substrate oxidation

Data for physical activity and REE are presented in Table 2. Subjects averaged approximately 10 200 steps per week during the baseline period (PRE), which significantly increased by nearly 3000 steps ($P < .01$) during the intervention. The REE did not change from PRE to POST. Protein oxidation increased ($P < .05$) with a significant decrease ($P < .05$) in fat oxidation after the weight loss intervention. Carbohydrate oxidation did not change. Average substrate oxidation was 46% CHO, 37% FAT, and 18% PTN for PRE and 50% CHO, 30% FAT, and 20% PTN for POST (Fig. 1). Nitrogen excretion (mean ± SEM) tended to increase from PRE (8.9 ± 0.7 mg/L) to POST (9.9 ± 0.7 mg/L, $P = .07$). No differences in urinary creatinine concentrations (mean ± SEM) from PRE (1.06 ± 0.06 mg/dL) to POST (1.06 ± 0.06 mg/dL) were noted, indicating complete 24-hour urine collections for participants.

3.4. Body composition

Fat mass, FFM, and percentage of body fat from PRE to POST are presented in Table 3. There was a 9% decrease in total FM and a 2% decrease in total FFM from PRE to POST. Approximately 75% of the 4 kg of body mass lost during the

Table 3
Body composition of premenopausal women at baseline and after the 10-week intervention using DEXA

	Baseline	10-week
FM (kg)	35.5 ± 1.2	32.4 ± 1.1 *
FFM (kg)	44.6 ± 0.7	43.6 ± 0.7 *
% Body fat	44 ± 1	42 ± 1 *

Values are mean ± SEM (n = 39).
* $P < .0001$, significantly different from PRE.

intervention was FM (ie, 3 kg). Total body fat decreased 5% from PRE to POST.

4. Discussion

The optimal macronutrient composition for weight loss continues to be debated. The current study used a hypocaloric diet that provided 40% CHO, 30% PTN, and 30% FAT combined with an increase in physical activity. This 10-week intervention resulted in an average weight loss of 4 kg, which included significant decreases in both FM and FFM, with FM losses accounting for 75% of the reduction in body mass. No change was observed in REE irrespective of changes in body mass and FFM. With regard to substrate oxidation, a significant increase in protein oxidation and a decrease in fat oxidation were noted after the weight loss intervention.

Recent studies have clearly documented the effectiveness of reduced-calorie diets that provide protein in excess of the current Recommended Dietary Allowance, yet within the Acceptable Dietary Macronutrient Range [9], for weight loss [20,21]. More importantly, these studies illustrate similar changes in the composition of weight lost. Of significance to the present study is the greater reduction in FM relative to losses of FFM. Recently, studies by Layman et al [14,22] have suggested that consumption of higher-protein, reduced-calorie diets with or without increased physical activity can better spare FFM when compared with similar energy-restricted diets that are higher in carbohydrate. More specifically, a comparison between 2 isocaloric, exercise-free, weight loss diets for which protein provided either 30% or 16% of total energy yielded similar reductions in body weight. However, the higher-protein, reduced-energy diet resulted in greater partitioning of weight loss due to changes in FM vs FFM [22]. The percentages of change in body composition due to reductions in both FM and FFM are similar to those noted in the current study. In another study, Layman et al [14] implemented an exercise program in combination with a diet design similar to that noted in the aforementioned study. The addition of exercise to the high-protein, reduced-energy diet resulted in greater reductions in body weight and FM and preserved FFM compared with findings with the low-protein or low-protein-with-exercise groups. The authors suggested that exercise has an additive effect in preserving FFM during weight loss. Differences in the extent of FFM losses between the present study and that of Layman and coworkers [14] might be partially explained by differences in the exercise interventions used. In the present study, physical activity was increased by having participants increase the number of steps taken per day to reach an activity level that was actually similar to that of subjects in the high-protein, no-exercise group in the study of Layman et al. Interestingly, the reductions in total body weight and percentage of change in total body mass due to reductions in FM and FFM in our study are consistent with those reported for the high-protein, no-exercise group in the study of Layman et al [14]. Therefore, it would appear that a more structured, as well as intense, exercise program is needed for sustaining FFM during weight loss.

Fat-free mass is a major determinant of REE; and therefore, reductions in FFM are typically accompanied with a concurrent decrease in REE [15,23]. In light of decreases in FFM, there were no reductions in REE noted in the current study. Although statistically significant, reductions in FFM may not have been physiologically sufficient to induce changes in REE. Our findings support the contention that higher protein intakes may confer metabolic benefits in addition to the preservation of FFM, such as a prevention of the decline in REE frequently noted with weight loss [15,20]. Indeed, it is possible that habitual consumption of higher protein intakes during weight loss spares endogenous protein and thereby minimizes losses of FFM and reductions in REE [14,22].

Although participants did not achieve the projected additional 4500 steps by the end of the intervention, it is possible that the approximately 3000 additional steps (~2 km/d) consequent to the intervention, in combination with the high-protein, reduced-energy diet, may have also contributed to the maintenance of REE and prevented further losses in FFM [14]. Recently, Villanova et al [24] noted an increase in REE in response to a similar mode of increasing physical activity after weight loss. As a result, further weight loss was achieved, which the authors attributed to the exercise-induced changes in REE. Furthermore, Ebbeling and Rodriguez [25] noted that the addition of a similar exercise program to a reduced-calorie weight loss plan prevented further declines in FFM and REE in young children. These investigators credited these observations to the noted up-regulation of whole-body protein turnover in response to increased physical activity during a state of negative energy balance that was subsequent to consumption of a reduced-calorie diet. Although the study of Ebbeling and Rodriguez was conducted in children, the design was similar to that of the present investigation in that subjects served as his or her own control so that pre– and post–weight loss measures could be evaluated subsequent to the diet and exercise interventions [25].

Although diet-induced thermogenesis was not measured in the present study, it is possible that the habitual consumption of a high-protein diet may have contributed to the maintenance of REE. Diet-induced thermogenesis is generally accepted to account for approximately 10% of total energy expended during a 24-hour period [26]. In controlled-feeding studies, consumption of high-protein diets has been shown to increase diet-induced thermogenesis in comparison with low-protein, high-carbohydrate or high-fat diets, thus impacting TEE [27-31]. Whitehead et al [31] found that a weight loss diet for which protein provided 36% of total energy was associated with a smaller decline in 24-hour REE than 2 isocaloric weight loss diets for which protein provided 15% of total energy. Baba et al [32] reported a similar maintenance in REE as a result of high-protein, reduced-energy diets. Therefore, it is possible that the additional energy cost of habitual consumption of higher protein intakes during weight loss in combination with increased physical activity may have abated any changes possibly seen in REE resulting from losses of FFM in the present study.

Substrate oxidation can be affected by diet, body composition, and physical activity [33-39]. The noted changes in substrate oxidation reflect changes in diet composition from that noted at baseline to that used for the weight loss intervention (51% CHO, 17% PTN, and 32% FAT vs 42% CHO, 28% PTN, and 32% FAT; for baseline and weight loss intervention, respectively). Although the percentage of energy derived from fat during the intervention was equivalent to the percentage of energy before the intervention, absolute fat intake decreased from 76 to 54 g/d during the intervention because of reduced energy intake. This difference likely contributed to a decrease in fat oxidation. With regard to the changes noted in protein oxidation, Gaine et al [35] found that increasing protein intake from 0.8 to 1.8 and 3.6 g/kg per day resulted in a parallel increase in protein oxidation. Increased protein oxidation in the current study is consistent with findings reported in the study of Gaine et al such that a significant increase in protein intake from 1.1 to 1.4 g/kg per day corresponded with an increase in protein oxidation. The increase in both protein intake and oxidation is concurrent with the noted increase in urinary nitrogen excretion. In the present study, subjects lost significant amounts of both FM and FFM. Studies have shown that weight loss, as well as the distribution of weight loss, influences substrate oxidation [38,40]. Therefore, increased protein oxidation concomitant with FFM losses suggests that protein was preferentially used as fuel to support energy expenditure in response to this particular weight loss intervention.

In contrast to other studies, the diet intervention used in combination with increased physical activity decreased oxidation of fat. Short- and long-term exercise has been shown to influence substrate oxidation, with studies finding increased fat oxidation in humans undertaking exercise programs [39,41]. In another study, Gaine et al [42] showed no effect of endurance exercise training on substrate utilization at rest in previously unfit men and women. Differences in study findings could be due to differences in exercise intensity as well as a decreased reliance on fat for fuel subsequent to the higher intake of protein in our study. In addition, it may have been necessary to increase the level of physical activity more significantly for a longer period.

The present study used a contemporary approach to weight loss that used a higher-protein, reduced-calorie diet intervention combined with a practical means of increasing physical activity in a free-living population of overweight and obese premenopausal women. In response to the weight loss intervention, the women lost weight while maintaining REE. The latter observation could be of physiological significance because reductions in REE noted in other weight loss studies [15] may hinder weight maintenance, prevent further weight loss, and perhaps precipitate weight gain [23]. The noted changes in substrate oxidation suggest the use of protein as an alternative fuel when a reduced-energy diet providing a greater percentage of energy from protein (vs carbohydrate) is habitually consumed. Finally, a higher rate of protein turnover consequent to increased protein intake [35] or increased physical activity [43] may have contributed to the maintenance of REE. The maintenance of REE provides additional evidence in support of higher protein intakes for the nutritional management of overweight and obesity in this population. The noted losses in FFM that accompanied weight loss warrant further research focused on determining the amount of dietary protein needed to complement various degrees of energy restriction, as well as different levels of physical activity, for weight loss for longer periods of time during which FFM can be maintained.

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