

ORIGINAL RESEARCH

An energy-reduced dietary pattern, including moderate protein and increased nonfat dairy intake combined with walking promotes beneficial body composition and metabolic changes in women with excess adiposity: a randomized comparative trial

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Abstract

Moderate protein and nonfat dairy intake within an energy-reduced diet (ERD) may contribute to health benefits achieved with body weight (BW) loss. The current study examined the effectiveness of a weight-loss/weight-loss maintenance intervention using an ERD with moderate dietary protein (30% of kcals) and increased nonfat dairy intake (4–5 svg/d), including yogurt (INT group) and daily walking compared to an ERD with standard protein (16–17% of kcals) and standard nonfat dairy intake (3 svg/d) (COM group) with daily walking. A randomized comparative trial with 104 healthy premenopausal women with overweight/obesity was conducted in a university setting. Women were randomized to INT group or COM group. Anthropometric measurements, as well as dietary intake, selected vital signs, resting energy expenditure, blood lipids, glucose, insulin, and selected adipose-derived hormones were measured at baseline, and weeks 2, 12, and 24. Targets for dietary protein and nonfat dairy intake, while initially achieved, were not sustained in the INT group. There were no significant effects of diet group on anthropometric measurements. Women in the INT group and COM group, respectively, reduced BW (-4.9 ± 3.2 and -4.3 ± 3.3 kg, $P < 0.001$) and fat mass (-3.0 ± 2.2 and -2.3 ± 2.3 kg, $P < 0.001$) during the 12-week weight-loss phase and maintained these losses at 24 weeks. Both groups experienced significant decreases in body mass index, fat-free soft tissue mass, body fat percentage, waist and hip circumferences and serum triglycerides, total cholesterol, and leptin (all $P < 0.001$). Healthy premenopausal women with excess adiposity effectively lost BW and fat mass and improved some metabolic risk factors following an ERD with approximately 20% protein and 3 svg/d of nonfat dairy intake.

Introduction

Weight loss achieved by following an energy-restricted dietary pattern promotes metabolic benefits (Fontana et al. 2007; Coppola et al. 2009; Lefevre et al. 2009). In addition to favorable outcomes conferred by reduced body weight (BW), research suggests additive beneficial effects

of certain dietary components on various weight-related outcomes including body composition, insulin sensitivity, and risk factors for cardiovascular disease (Parker et al. 2002; Appel et al. 2003; Layman et al. 2003a).

Dietary protein intake has been widely studied, and benefits beyond BW loss have been reported with typical energy-restricted diets (Layman et al. 2008; Abete et al.

2010). Higher protein diets have resulted in enhancement of BW (Skov *et al.* 1999; Brehm and D'Alessio 2008) and fat mass losses (Layman *et al.* 2008, 2009), preservation of fat-free soft tissue mass (Leidy *et al.* 2007; Brehm and D'Alessio 2008) and improvement in blood lipids (Skov *et al.* 1999; Farnsworth *et al.* 2003; Layman *et al.* 2003b, 2005, 2009; Noakes *et al.* 2005). These results have been partially attributed to an increased thermic effect of food (Lejeune *et al.* 2006), increased satiety (Lejeune *et al.* 2006; Veldhorst *et al.* 2008) and maintenance of fat-free soft tissue mass (Layman *et al.* 2003b).

Similarly, increased dairy intake has been implicated in body composition improvements (5–10% decrease in fat mass) with corresponding BW loss of 5–10% (Zemel *et al.* 2005, 2009; Josse *et al.* 2011). Several studies have shown an inverse relationship between total dairy intake and body mass index (Pereira *et al.* 2002), nonfat dairy intake and fat mass (Zemel *et al.* 2005), body fat percentage (Poddar *et al.* 2009), truncal fat (Poddar *et al.* 2009) and waist circumference (Newby *et al.* 2003), and calcium intake and body fat percentage (Zemel *et al.* 2000). Dietary calcium (Zemel *et al.* 2004) as well as the branched-chain amino acid leucine (Koopman *et al.* 2005; Layman and Walker 2006) found in dairy products have been regarded as contributors to the reported link between dairy food consumption and weight-related outcomes. Specifically, nonfat yogurt combined with energy restriction has been shown to produce greater fat mass losses (~4.5 kg) than energy restriction alone (~2.8 kg) (Zemel *et al.* 2005). Healthy premenopausal women reduced fat mass and body fat percentage and increased fat-free soft tissue mass to a greater extent when consuming an energy-restricted diet with 30% of energy as protein and 6–7 servings of low-fat dairy per day with exercise, compared to women consuming an energy-restricted diet with adequate protein (15% of kcal) and 3–4 servings of low-fat dairy per day with exercise or adequate protein and <1 serving of low-fat dairy per day with exercise (Josse *et al.* 2011). Similarly, adults consuming roughly 30% of energy from protein who walked 5 days per week over a 4-month weight-loss period lost more fat mass and maintained that loss over 1 year, compared to adults consuming a conventional weight-loss diet with lesser protein (Layman *et al.* 2009). The higher protein group also experienced a greater reduction in triglycerides and elevation of high-density lipoprotein cholesterol concentrations (Layman *et al.* 2009).

Given that many of the previous studies have been cross-sectional, very short-term, laboratory-based feeding trials and/or not tested in free-living women (Skov *et al.* 1999; Parker *et al.* 2002; Farnsworth *et al.* 2003; Layman *et al.* 2003a,b; Noakes *et al.* 2005; Lejeune *et al.* 2006; Leidy *et al.* 2007; Zemel *et al.* 2009; Josse *et al.* 2011), the current study evaluated effects of moderate dietary

protein and increased nonfat dairy intake as part of an energy-reduced diet (ERD) within a 6-month comprehensive lifestyle intervention in free-living premenopausal women with overweight/obesity. It was hypothesized that an ERD including moderate protein (30% of kcals) and increased nonfat dairy intake (4–5 *svg/d*) combined with daily walking (INT group) would induce greater reductions in BW, body mass index, fat mass, body fat percentage, waist and hip circumferences and maintenance of fat-free soft tissue mass, compared to an ERD, including standard protein (16–17% of kcals) and standard nonfat dairy intake (3 *svg/d*) (i.e., control diet) combined with daily walking (COM group), during 12 weeks of weight loss followed by 12 weeks of weight-loss maintenance. Effects on selected vital signs (i.e., resting heart rate and blood pressure), serum lipids, glucose and insulin concentrations, and selected adipose-derived hormones were further explored.

Methods

Participants, screening, enrollment and informed consent

Participants were recruited by newsletter, newspaper and radio advertisements, posted flyers, mailed advertisements, and by word-of-mouth. Selection criteria included: women aged 20–45 years with a body mass index of ≥ 25 and ≤ 36 kg/m^2 ; limited physical activity (≤ 2 h of planned exercise/week); eumenorrhea; stable BW during the past 6 months; a score of < 50 on the Zung Self-Rating Depression Scale/Status Inventory (Zung 1972); absence of yogurt intolerance, aversion, or allergy; consumption of < 24 oz. of yogurt per day, ≤ 16 oz. of caffeinated beverages per day; ≤ 1 alcoholic beverage per day (i.e., ≤ 12 fl. oz. of regular beer or ≤ 5 fl. oz. wine or ≤ 1.5 fl. oz. 80-proof distilled spirits); and a desire to lose weight. Exclusion criteria included current smoking; pregnancy; diagnosed metabolic or health conditions; use of medications or supplements for metabolic or health conditions or for BW loss; health or orthopedic conditions limiting musculoskeletal activity; gastric bypass surgery; hysterectomy and ovariectomy without hormone replacement therapy; and/or oral contraceptive use of < 2 years duration (if used). A flow diagram of response, screening, randomization and attrition throughout the study is displayed in Figure 1.

This study was approved by the Institutional Review Board for Research Involving Human Subjects at The Pennsylvania State University, University Park, PA. All participants provided implied consent for initial screening and written informed consent before review of complete eligibility screening materials.

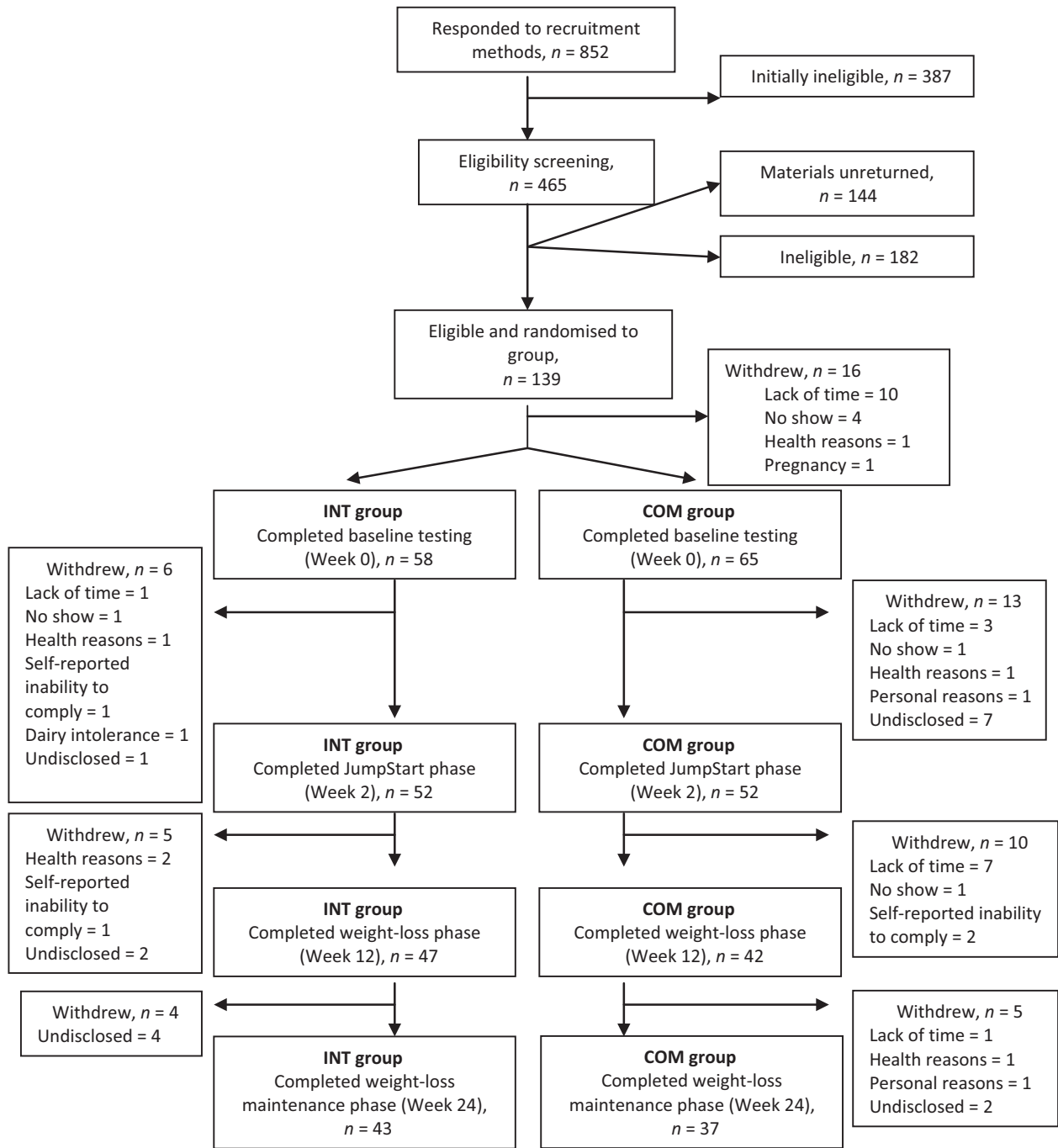


Figure 1. Diagram of recruitment, enrollment, randomization, and attrition of participants in a study examining changes in body composition and metabolic parameters in premenopausal women with overweight/obesity following an energy-reduced dietary pattern (ERD), including moderate protein and increased nonfat dairy intake (INT group) or an ERD with standard protein and standard nonfat dairy intake (COM group) combined with walking. Primary reasons for study withdrawal included lack of time (n = 22), undisclosed (n = 16), and no return for subsequent testing (n = 7).

Experimental approach and intervention

This randomized intervention was conducted from December 2009 to March 2011. Participants were stratified by baseline age, body mass index and self-reported physi

cal activity and then randomized, using a random number generator, into one of two groups. Women in each group completed the JumpStart phase (weeks 0–2), the weight-loss phase (weeks 3–12; total of 12 weeks), and the weight-loss maintenance phase (weeks 13–24; total of 12 weeks).

Women in the intervention (INT) group followed an ERD (1200 kcal/d) that included nonfat yogurt (Yoplait Light®, General Mills, Inc., Minneapolis, MN) as part of two standard meals per day (breakfast and lunch) during the JumpStart phase. Each standard meal consisted of one, 6 oz. nonfat yogurt choice (100 kcal), one cup cereal (100–120 kcal), or one granola bar (90–95 kcal) (Nature Valley, General Mills, Inc.), and one fruit serving. For dinner, women were instructed to consume foods based on an exchange system (American Diabetes Association and American Dietetic Association 2008). Women in the INT group were instructed to consume an additional three servings of nonfat dairy intake each day, for a total of five servings per day. During the weight-loss phase, women in the INT group consumed an ERD (1500 or 1700 kcal/d) that included nonfat yogurt as part of a standard meal for breakfast and nonfat yogurt as a snack for a total of 4–5 servings of nonfat dairy intake per day. During the weight-loss maintenance phase, women in the INT group consumed an energy-balanced diet, adjusted for current BW that included nonfat yogurt twice each day for a total of 4–5 servings of nonfat dairy per day. Women in the INT group were provided with weekly supplies of nonfat yogurt, cereal, and granola bars throughout the study.

Women in the comparison (COM) group followed an ERD (1500 or 1700 kcal/d, based on individual estimated energy needs) (Mifflin *et al.* 1990) during the JumpStart phase. Women in the COM group were instructed to consume a total of three servings of nonfat dairy intake per day. During the weight-loss phase, women in the COM group continued their assigned ERD (1500 or 1700 kcal/d), with three servings of nonfat dairy intake per day. During the weight-loss maintenance phase, women in the COM group consumed an energy-balanced diet, adjusted for current BW, with three servings of nonfat dairy intake per day. Women in the COM group were instructed not to consume yogurt throughout the entire study.

For both groups, total energy was divided into food group exchanges, and women were instructed to follow their exchange patterns (Table 1). By design, the intervention (INT group) was expected to produce a slightly greater energy deficit compared to the COM group during the JumpStart phase. Proportions of macronutrients are provided in Table 1. It was expected that the ERD combined with walking would decrease BW by around one pound per week. All women were provided with dietary patterns, sample menus and exchange list information (American Diabetes Association and American Dietetic Association 2008).

Intake of calcium and vitamin D were normalized across all ERD patterns in both groups, using the 1700 kcal per

day INT diet as the standard, as dietary calcium has been suggested to influence BW (Zemel *et al.* 2000). Supplementation was designed to match targets of 1500 mg of calcium and 10–20 µg of vitamin D per day (Table 1). Investigators dispensed nutrient supplements (Wegmans Calcium Citrate + Vitamin D, Wegmans Food Market, Rochester, NY) to women at weekly educational sessions.

Women in both groups walked 30–40 min per day at a moderate pace (~3 min/h) during all three phases of the study. Weekly educational sessions were held for both INT and COM groups throughout the 6-month study and included lessons on basic nutrition knowledge, exchange patterns of eating, portion size and control, purchasing and preparing food and modifying recipes as well as motivational lessons on outcome expectations, self-regulation and monitoring, problem-solving, lifestyle modification, emotion eating and motivation for walking. Throughout the 24-week intervention, women in both groups received items, such as insulated lunch bags, measuring cups and food storage containers, to encourage motivation. These items were provided approximately once per month to women. Women were compensated for their time with \$20 US after each testing session; women who completed the full study were compensated with an additional \$50 US for their time.

Testing sessions

During this 24-week parallel-arm study, women completed testing sessions (between 0700 and 0930 h) at baseline (pre-intervention) and after 2, 12, and 24 weeks of the intervention. Women fasted for at least 12 h and avoided strenuous physical activity and alcohol intake before each testing session. Women completed 4-day food and physical activity records before each testing session and submitted these records upon arrival. Women completed anthropometric, selected vital signs and body composition measurements. Fasting venous whole blood samples were collected from women at weeks 0, 2, 12, and 24. Whole blood was centrifuged at $1252 \times g$ for 10 min, and serum was aliquotted into cryovials and stored at -80°C for later analysis.

Dietary and physical activity assessment

Before the study began, participants were trained to accurately complete a 4-day food and physical activity record. To facilitate accuracy in recording of foods and beverages, participants were provided with handouts that contained pictures of common items and standard portion sizes. Training minimized over- and under-reporting by women, as implausible intakes (<500 or >5000 kcal/d) were not

Table 1. Food exchange patterns by group during the JumpStart, weight-loss and weight-loss maintenance phases in a study examining changes in body composition and metabolic parameters with an energy-reduced dietary pattern (ERD) including moderate protein and increased non-fat dairy intake (INT group) or an ERD with standard protein and standard nonfat dairy intake (COM group), combined with walking.

Energy-level ^a (Macronutrient distribution)	Pattern ^{b,c}	Intervention (INT) group combined with walking ^d (n = 52)			Comparison (COM) group combined with walking (n = 52)	
		1200 kcal/d (29% PRO, 24% FAT, 47% CHO)	1500 kcal/d (30% PRO, 25% FAT, 45% CHO)	1700 kcal/d (30% PRO, 25% FAT, 45% CHO)	1500 kcal/d (17% PRO, 24% FAT, 59% CHO)	1700 kcal/d (16% PRO, 25% FAT, 59% CHO)
Baseline Testing: Anthropometric and body composition measurements; selected vital signs; serum lipids, glucose and insulin concentrations; selected adipose-derived hormones						
JumpStart Phase (Weeks 0–2)	BREAKFAST					
	Standard Meal ^e	1	–	–	0	0
	EXCHANGES					
	Dairy					
	Nonfat	1 (nonfat yogurt) ^f	–	–	1 ^{g,h}	1 ^{g,h}
	1% fat	0	–	–	0	0
	2% fat	0	–	–	0	0
	Starch	1 (select from list) ⁱ	–	–	1	2
	Fruit	1	–	–	1	1
	Vegetable	0	–	–	0	0
	Meat					
	Lean	0	–	–	0	0
	Medium fat	0	–	–	0	0
	Fat	0	–	–	0	0
	LUNCH					
	Standard Meal	1	–	–	0	0
	Exchanges					
	Dairy					
	Nonfat	1 (nonfat yogurt) ^f	–	–	1	1
	1% fat	0	–	–	0	0
	2% fat	0	–	–	0	0
	Starch	1 (select from list) ⁱ	–	–	2	2
	Fruit	1	–	–	1	1
	Vegetable	0	–	–	2	2
	Meat					
	Lean	0	–	–	0	0
	Medium fat	0	–	–	1	1
	Fat	0	–	–	2	2
	SNACK					
	Standard Meal	0	–	–	0	0
	Exchanges					
	Dairy					
	Nonfat	1 ^h	–	–	0	0
	1% fat	0	–	–	0	0
	2% fat	0	–	–	0	0
	Starch	0	–	–	1	1
	Fruit	0	–	–	1	1
	Vegetable	2	–	–	0	1
	Meat					
	Lean	0	–	–	0	0
	Medium fat	0	–	–	0	0
	Fat	0	–	–	0	0
	DINNER					
	Standard Meal	0	–	–	0	0
	Exchanges					
	Dairy					
	Nonfat	2 ^h	–	–	1	1
	1% fat	0	–	–	0	0
	2% fat	0	–	–	0	0
	Starch	0	–	–	2	3

Table 1. Continued

Energy-level ^a (Macronutrient distribution)	Pattern ^{b,c}	Intervention (INT) group combined with walking ^d (n = 52)			Comparison (COM) group combined with walking (n = 52)	
		1200 kcal/d (29% PRO, 24% FAT, 47% CHO)	1500 kcal/d (30% PRO, 25% FAT, 45% CHO)	1700 kcal/d (30% PRO, 25% FAT, 45% CHO)	1500 kcal/d (17% PRO, 24% FAT, 59% CHO)	1700 kcal/d (16% PRO, 25% FAT, 59% CHO)
	Fruit	0	–	–	1	0
	Vegetable	2	–	–	2	3
	Meat					
	Lean	6	–	–	0	0
	Medium fat	0	–	–	2	2
	Fat	1	–	–	2	3
Week 2 Testing: Anthropometric measurements						
Weight-Loss Phase (Weeks 2–12)						
	BREAKFAST					
	Standard Meal	–	1	1	0	0
	Exchanges					
	Dairy					
	Nonfat	–	1 (nonfat yogurt) ^f	1 (nonfat yogurt) ^f	1 ^{g,h}	1 ^{g,h}
	1% fat	–	0	0	0	0
	2% fat	–	0	0	0	0
	Starch	–	1 (select from list) ⁱ	1 (select from list) ⁱ	1	2
	Fruit	–	1	1	1	1
	Vegetable	–	0	0	0	0
	Meat					
	Lean	–	0	0	0	0
	Medium fat	–	0	0	0	0
	Fat	–	0	0	0	0
	LUNCH					
	Standard Meal	–	0	0	0	0
	Exchanges					
	Dairy					
	Nonfat	–	1 ^h	1 ^h	1	1
	1% fat	–	0	0	0	0
	2% fat	–	0	0	0	0
	Starch	–	1	1	2	2
	Fruit	–	1	1	1	1
	Vegetable	–	1	2	2	2
	Meat					
	Lean	–	3	3	0	0
	Medium fat	–	0	0	1	1
	Fat	–	1	1	2	2
	SNACK					
	Standard Meal	–	0	0	0	0
	Exchanges					
	Dairy					
	Nonfat	–	1 (nonfat yogurt) ^f	1 (nonfat yogurt) ^f	0	0
	1% fat	–	0	0	0	0
	2% fat	–	0	0	0	0
	Starch	–	0	0	1	1
	Fruit	–	1	1	1	1
	Vegetable	–	1	0	0	1
	Meat					
	Lean	–	3	3	0	0
	Medium fat	–	0	0	0	0
	Fat	–	0	0	0	0
	DINNER					
	Standard Meal	–	0	0	0	0

Table 1. Continued

Energy-level ^a (Macronutrient distribution)	Pattern ^{b,c}	Intervention (INT) group combined with walking ^d (n = 52)			Comparison (COM) group combined with walking (n = 52)	
		1200 kcal/d (29% PRO, 24% FAT, 47% CHO)	1500 kcal/d (30% PRO, 25% FAT, 45% CHO)	1700 kcal/d (30% PRO, 25% FAT, 45% CHO)	1500 kcal/d (17% PRO, 24% FAT, 59% CHO)	1700 kcal/d (16% PRO, 25% FAT, 59% CHO)
	Exchanges					
	Dairy					
	Nonfat	–	1 ^h	2 ^h	1	1
	1% fat	–	0	0	0	0
	2% fat	–	0	0	0	0
	Starch	–	0	1	2	3
	Fruit	–	0	0	1	0
	Vegetable	–	2	2	2	3
	Meat					
	Lean	–	0	0	0	0
	Medium fat	–	3	3	2	2
	Fat	–	2	3	2	3
Week 12 Testing: Anthropometric and body composition measurements; selected vital signs; serum lipids, glucose and insulin concentrations; selected adipose-derived hormones						
Weight-Loss	BREAKFAST					
Maintenance	Standard Meal	–	0	0	0	0
Phase (Weeks 13–24)	Exchanges					
	Dairy					
	Nonfat	–	1 ^j	1 ^j	1 ^{g,h}	1 ^{g,h}
	1% fat	–	0	0	0	0
	2% fat	–	0	0	0	0
	Starch	–	1	1	1	2
	Fruit	–	1	1	1	1
	Vegetable	–	0	0	0	0
	Meat					
	Lean	–	0	0	0	0
	Medium fat	–	0	0	0	0
	Fat	–	0	0	0	0
	LUNCH					
	Standard Meal	–	0	0	0	0
	Exchanges					
	Dairy					
	Nonfat	–	1 ^j	1 ^j	1	1
	1% fat	–	0	0	0	0
	2% fat	–	0	0	0	0
	Starch	–	1	1	2	2
	Fruit	–	1	1	1	1
	Vegetable	–	1	2	2	2
	Meat					
	Lean	–	3	3	0	0
	Medium fat	–	0	0	1	1
	Fat	–	1	2	2	2
	SNACK					
	Standard Meal	–	0	0	0	0
	Exchanges					
	Dairy					
	Nonfat	–	1 ^j	1 ^j	0	0
	1% fat	–	0	0	0	0
	2% fat	–	0	0	0	0
	Starch	–	0	0	1	1
	Fruit	–	1	1	1	1
	Vegetable	–	1	0	0	1
	Meat					
	Lean	–	3	3	0	0
	Medium fat	–	0	0	0	0
	Fat	–	0	0	0	0

Table 1. Continued

Energy-level ^a (Macronutrient distribution)	Pattern ^{b,c}	Intervention (INT) group combined with walking ^d (n = 52)			Comparison (COM) group combined with walking (n = 52)	
		1200 kcal/d (29% PRO, 24% FAT, 47% CHO)	1500 kcal/d (30% PRO, 25% FAT, 45% CHO)	1700 kcal/d (30% PRO, 25% FAT, 45% CHO)	1500 kcal/d (17% PRO, 24% FAT, 59% CHO)	1700 kcal/d (16% PRO, 25% FAT, 59% CHO)
DINNER						
	Standard Meal Exchanges	–	0	0	0	0
	Dairy					
	Nonfat	–	1 ^j	2 ^j	1	1
	1% fat	–	0	0	0	0
	2% fat	–	0	0	0	0
	Starch	–	0	1	2	3
	Fruit	–	0	0	1	0
	Vegetable	–	2	2	2	3
	Meat					
	Lean	–	0	0	0	0
	Medium fat	–	3	3	2	2
	Fat	–	2	2	2	3

Week 24 Testing: Anthropometric and body composition measurements; selected vital signs; serum lipids, glucose and insulin concentrations; selected adipose-derived hormones

PRO, dietary protein; FAT, dietary fat; CHO, dietary carbohydrate. ^aEnergy-level included 1200 kcal/d in the INT group during the JumpStart phase only; 1500 kcal/d or 1700 kcal/d based on individual estimated energy needs during the weight-loss and weight-loss maintenance phases; energy-reduced diet included 500 fewer kcal/d than estimated needs. ^bPattern based on exchange system (American Diabetes Association and American Dietetic Association 2008); ^cCalcium and vitamin D normalized to 1700 kcal/d INT group; supplementation designed to meet targets of 1500 mg of calcium and 10–20 µg of vitamin D per day; ^dWalking included 30–40 min/d at a pace of ~3 mi/h during all three phases of the study in both groups; ^eStandard meal included 1 starch, 1, 6 oz. nonfat yogurt (nonfat dairy intake), and 1 fruit; ^fVariety of flavors provided; ^gNonfat yogurt not allowed in the COM group across all phases; ^hAll foods were participant's choice in COM group, foods were participant's choice in INT group, except where noted; ⁱ1 cup plain Cheerios, 1 cup Multi-grain Cheerios, 1 Cup Rice-Chex, or 1 Nature Valley Crunchy granola bar (choice of flavors) (General Mills, Inc., Minneapolis, MN); ^j2 of these were nonfat yogurt.

reported. Participants were provided with verbal and written directions for the recording of physical activities, using of pedometers and tracking of step counts. Women wore pedometers on their waistbands of clothing, above the hip and aligned with the length of the leg. Pedometers were worn during waking hours, except during showering or bathing, and step counts were recorded for each 24-h period of the 4-day interval. Food and beverage intakes, physical activity, and step counts were recorded during 3 weekdays and 1 weekend day, before each testing session.

Diet records were reviewed with participants upon receipt and verified by a registered dietitian. Records were analyzed for estimated average daily energy (kcal/d), macronutrient (g/d and % kcal/d) and calcium (mg/d) intakes (dietary and supplemental), as well as servings per day of total and nonfat dairy intake, using Nutrition Data System for Research (NDS-R version 2010, Minneapolis, MN) by a registered dietitian blinded to intervention assignment.

Physical activity records were reviewed with participants and used to estimate time (min) spent engaged in light, moderate, and vigorous physical activities, which

corresponded to metabolic equivalent levels. Duration of activity, metabolic equivalents, and participant BW were used to calculate total energy expended per day. Data from 4-day records were averaged to produce one estimated average daily energy expenditure value (kcal/d). The number of steps taken by women during each of the 4 days was estimated by pedometers (Accusplit AE120XL, Accusplit Inc., Pleasanton, CA) and averaged to produce one daily estimate. Records were analyzed across all study phases by one research assistant blinded to intervention assignment.

Anthropometric and body composition measurements

Baseline height was recorded to the nearest 0.1 cm, using a stadiometer (Seca 700, Seca North America East, Hanover, MD). Body weight was measured to the nearest 0.1 kg on a calibrated digital scale (TBF-410GS, Tanita Corporation, Arlington Heights, IL). Participants wore lightweight clothing with bare feet during height and weight measurements. Body mass index was calculated from BW and height. Total body fat mass (kg), fat-free

soft tissue mass (kg), body fat percentage, and central abdominal tissue percentage were measured by dual-energy X-ray absorptiometry (Hologic QDR4500A, Bedford, MA). Total body scans were conducted at baseline, week 12 and week 24, by one investigator blinded to intervention assignment. Test–retest reliability in 26 overweight/obese premenopausal women has produced coefficients of variation (CVs) of 1.87, 1.02, and 3.32% for fat mass, fat-free soft tissue mass, and central abdominal tissue percentage, respectively. Waist and hip circumferences were measured in duplicate (Gulik II tape measure, Country Technology, Gays Mills, WI), averaged and recorded to the nearest 0.1 cm.

Selected vital signs and resting energy expenditure measurements

Resting heart rate (beats/min) of the radial artery was measured by palpation, after a 5-min rest period. Seated systolic and diastolic blood pressure (mm Hg) was measured using a standard sphygmomanometer (Baumanometer® Desk Model, Copiague, NY) after a 5-min rest period. Two measurements were taken for both resting heart rate and blood pressure with a 2- to 3-min rest period between readings, and values were averaged. Resting heart rate and blood pressure measurements were completed by a registered nurse blinded to intervention assignment. Resting energy expenditure was estimated using the Mifflin–St. Jeor equation (Mifflin *et al.* 1990).

Biochemical markers of health

Serum was analyzed for triglycerides, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, glucose, insulin, leptin, resistin, and adiponectin concentrations. Triglycerides (TG) (mg/dL), total cholesterol (TC) (mg/dL), and high-density lipoprotein cholesterol (HDL-C) (mg/dL) concentrations were measured by spectrophotometry, using standard assay kits (Stanbio Labs, Boerne, TX). Low-density lipoprotein cholesterol (LDL-C) concentration (mg/dL) was calculated using the Friedewald equation: $LDL-C = TC - HDL-C - (TG/5)$ (Friedewald *et al.* 1972). Serum glucose (mg/dL) was measured by spectrophotometry (Stanbio Labs). Serum insulin ($\mu\text{U/mL}$) was measured by ultra-sensitive enzyme-linked immunosorbent assay (ALPCO Diagnostics, Salem, NH). Serum leptin (ng/mL), resistin (ng/mL), and adiponectin ($\mu\text{g/mL}$) were measured by enzyme-linked immunosorbent assay (Bio-Rad, Hercules, CA). Duplicate serum samples were analyzed for each biomarker at corresponding study intervals. Intra- and inter-assay CVs were 2.9 and 6.2% for triglycerides, 2.0 and 4.2% for total cholesterol, and 4.1 and 14.2% for high-density lipoprotein

cholesterol, respectively. Intra- and inter-assay CVs were 2.5 and 11.2% for glucose and 4.3 and 12.0% for insulin, respectively. Intra- and inter-assay CVs were 9.3 and 18.7% for leptin, 3.2 and 27.0% for resistin, and 8.4 and 20.9% for adiponectin.

Statistical analyses

Participants completing the JumpStart phase ($n = 104$) were included in the intention-to-treat analysis. This analysis was conducted using the last observation carried forward approach. All baseline data were normally distributed. Related variables were analyzed by multivariate analysis of covariance with repeated measures on the time factor. Cohort and baseline values were used as covariates in all statistical models. Sample *t*-tests using Bonferroni adjustments for multiple comparisons were completed when significant group or group \times time interactions were found. A probability of $P < 0.01$ (two-tailed) was considered statistically significant. Data are reported as means \pm standard deviation (SD), unless otherwise noted. Data analyses were conducted using the Statistical Package for the Social Sciences (version 19.0, 2010, SPSS Inc, Chicago, IL). Power analysis using effect size of 0.80 within each group for BW and fat mass in the current study indicated that a sample size of 30 participants per group yielded >0.85 observed power.

Results

A total of 852 women responded to recruitment methods between July 2009 and September 2010. Of these, 465 women met prescreening criteria (appropriate age, body mass index, and limited self-reported physical activity) and received screening materials, including a Medical History Form, Zung Self-Rating Depression Scale/Status Inventory (Zung 1972) and Informed Consent Form. A total of 321 women returned screening materials which were reviewed by investigators to further determine enrollment eligibility. Of these, 139 women were eligible and obtained medical clearance for participation from their health-care providers.

Baseline testing was completed by 123 healthy premenopausal women with overweight/obesity (Caucasian, $n = 98$; African American, $n = 11$; Other, $n = 3$; No response, $n = 11$). After the JumpStart phase, 104 (INT, $n = 52$; COM, $n = 52$) women (age 33.7 ± 7.3 years; body mass index 29.2 ± 3.1) remained and were included in data analyses (Fig. 1). There were no significant differences in baseline age, BW, or body mass index between the 123 women who began the intervention and the 104 women who completed the JumpStart phase (or the 80 women who completed the entire intervention). There were no

significant differences in baseline age, BW, or body mass index for participants who completed the intervention ($n = 80$) and participants who withdrew ($n = 43$).

Class attendance was 83% for the INT group and 78% for the COM group. Nonfat yogurt consumption was 73% of prescribed intake in the INT group. Approximately 60% of all women completed the walking protocol on at least 4 days of each week for at least 19 of 24 weeks.

Dietary and physical activity assessments

Dietary intake data are presented in Table 2. Complete food records were available for 71 women. Compared to baseline, estimated average energy intake was reduced by 35.0 and 31.2% for women in the INT and COM groups, respectively, at week 2 and by 25.2 and 30.4% at week 12 and by 18.4 and 27.0% at week 24. As expected, due to study design, estimated energy intake significantly differed between groups only at week 2.

In the INT group, estimated total carbohydrate intake significantly decreased from week 0 to week 2, 12, and 24, while estimated carbohydrate intake as a percentage of total energy intake remained relatively constant, between 49 and 52%. In the COM group, the estimated carbohydrate intake as a percentage of total energy intake significantly increased from baseline to weeks 2, 12, and 24. The estimated protein intake as a percentage of total energy intake increased over time in both groups. Though protein intake did not reach 30% of total energy intake as designed in the INT group, estimated average percentage protein intake was significantly greater in the INT group compared to COM group at week 2 (26.1 vs. 18.6%, respectively, $P < 0.001$) and 12 (23.9 vs. 19.6%, respectively, $P < 0.001$). Both groups reduced the estimated total fat intake and percentage of energy intake as dietary fat over time; the reduction in dietary fat as a percentage of total energy intake was significantly different between groups only at week 2.

Servings of total ($P < 0.01$) and nonfat ($P < 0.001$) dairy intake were greater in the INT group at weeks 2, 12, and 24 compared to the COM group. The estimated total and nonfat dairy intake significantly increased from baseline to weeks 2, 12, and 24 in the INT group. The estimated nonfat dairy intake significantly increased from baseline to weeks 2, 12, and 24 in the COM group. Mean calcium intakes significantly increased from baseline to weeks 2, 12, and 24 in both groups. The calcium intake was greater ($P < 0.001$) in the COM group at week 2 compared to the INT group.

Energy expenditure from physical activity data are presented in Table 2. Complete physical activity records were available for 64 women, and complete pedometer step counts were available for 42 women. There was no effect of the diet group or time on the estimated energy

expenditure from physical activity. There was no effect of the diet group on the estimated number of step counts. Both groups significantly increased estimated step counts from baseline at weeks 2, 12, and 24.

Anthropometric and body composition measurements

There was no significant effect of diet group on body composition or anthropometric measurements (Table 3). Women in both groups significantly reduced BW, body mass index, and waist and hip circumferences during the JumpStart phase. Women in the INT and COM groups had significant decreases in BW (-4.9 ± 3.2 and -4.3 ± 3.3 kg), body mass index (-1.8 ± 1.2 and -1.6 ± 1.2 kg/m²), fat mass (-3.0 ± 2.2 and -2.3 ± 2.3 kg), fat-free soft tissue mass (-1.5 ± 1.6 and -1.7 ± 2.1 kg), body fat percentage (-1.7 ± 1.7 and $-1.2 \pm 2.0\%$), waist circumference (-4.4 ± 2.9 and -3.8 ± 3.1 cm), hip circumference (-4.7 ± 3.5 and -4.3 ± 3.6 cm), and central abdominal tissue percentage (-3.0 ± 3.2 and $-2.8 \pm 2.9\%$), respectively, from baseline to week 12. These losses were maintained from week 12 through week 24. A group \times time interaction was not observed for any of these outcome variables.

Selected vital signs and resting energy expenditure measurements

There was no significant effect of the diet group on resting heart rate, systolic, or diastolic blood pressure, or resting energy expenditure (Table 4). There were no significant differences in resting heart rate or systolic or diastolic blood pressure over time in either group. A group \times time interaction was not observed for any of these measures. There was a significant reduction in resting energy expenditure from baseline to week 12, and this reduction was maintained through week 24.

Biochemical markers of health

There were no significant diet group effects for biochemical markers of health (Table 5). Women in the INT and COM groups experienced significant reductions in triglycerides and total cholesterol concentrations at week 12 that were maintained to week 24. Serum low-density lipoprotein cholesterol significantly decreased at week 12, but this reduction was no longer significant at week 24. There were no other significant changes in groups over time, and a group \times time interaction was not observed in any of these secondary outcome measures.

A significant diet group effect was not found for serum leptin, resistin, or adiponectin concentrations (Table 6). Leptin concentration significantly decreased in both groups

Table 2. Estimated energy and macronutrient intakes and energy expenditure from physical activity of premenopausal women with overweight/obesity at baseline and Weeks 2, 12, and 24 in a study examining changes in body composition and metabolic parameters with an energy-reduced dietary pattern (ERD) including moderate protein and increased nonfat dairy intake (INT group) or an ERD with standard protein and standard nonfat dairy intake (COM group), combined with walking.

Variable	Group	Baseline (Week 0)	Week 2	Week 12	Week 24	P-value
		← Mean ± standard deviation →				
Energy intake (kcal/d)	INT (n = 37)	1757 ± 387	1142 ± 182 ^a	1315 ± 308 ^{a,b}	1433 ± 309 ^{a,b}	Group < 0.01 Time < 0.001 G × T = 0.03
	COM (n = 34)	1974 ± 441	1358 ± 246 ^{a,d}	1374 ± 269 ^a	1442 ± 366 ^a	
Total carbohydrate (g/d)	INT (n = 37)	219 ± 51	165 ± 27 ^a	177 ± 42 ^a	188 ± 35 ^a	Group = 0.013 Time < 0.001 G × T = 0.17
	COM (n = 34)	232 ± 57	198 ± 43 ^a	194 ± 43 ^a	195 ± 47 ^a	
Percentage carbohydrate (%kcal/d)	INT (n = 37)	49.2 ± 6.4	52.2 ± 5.8 ^a	52.2 ± 5.9	51.5 ± 6.4	Group = 0.97 Time < 0.001 G × T < 0.01
	COM (n = 34)	46.2 ± 4.6	54.5 ± 5.8 ^a	54.5 ± 5.8 ^a	53.4 ± 6.6 ^a	
Total protein (g/d)	INT (n = 37)	65.1 ± 14.0	72.7 ± 14.5	75.7 ± 18.6	71.9 ± 16.5	Group = 0.52 Time = 0.15 G × T < 0.001
	COM (n = 34)	75.0 ± 18.2	62.8 ± 12.5 ^a	66.8 ± 11.8	62.8 ± 14.3	
Percentage protein (%kcal/d)	INT (n = 37)	15.3 ± 2.8	26.1 ± 4.0 ^a	23.9 ± 4.6 ^a	20.8 ± 4.3 ^{a,b,c}	Group < 0.001 Time < 0.001 G × T < 0.001
	COM (n = 34)	15.6 ± 2.3	18.6 ± 2.8 ^{a,d}	19.6 ± 2.6 ^{a,d}	17.8 ± 2.8	
Total fat (g/d)	INT (n = 37)	69 ± 20	24 ± 10 ^a	36 ± 15 ^{a,b}	44 ± 17 ^{a,b}	Group = 0.01 Time < 0.001 G × T = 0.01
	COM (n = 34)	82 ± 21	38 ± 11 ^{a,d}	40 ± 14 ^a	46 ± 19 ^a	
Percentage fat (%kcal/d)	INT (n = 37)	34.0 ± 5.4	17.7 ± 5.5 ^a	22.9 ± 5.6 ^{a,b}	25.9 ± 5.6 ^{a,b}	Group = 0.01 Time < 0.001 G × T = 0.10
	COM (n = 34)	36.2 ± 3.5	24.4 ± 5.7 ^{a,c}	24.9 ± 5.7 ^a	27.0 ± 6.3 ^a	
Calcium (mg/d)	INT (n = 37)	718 ± 205	1189 ± 309 ^{a,d}	1218 ± 330 ^a	1141 ± 344 ^a	Group < 0.001 Time < 0.001 G × T = 0.47
	COM (n = 34)	929 ± 315	1589 ± 361 ^a	1528 ± 446 ^a	1335 ± 421 ^a	
Dairy intake, total (svg/d)	INT (n = 37)	1.8 ± 0.87	4.0 ± 1.0 ^{a,d}	3.7 ± 0.93 ^{a,d}	3.3 ± 0.96 ^{a,b,d}	Group < 0.01 Time < 0.001 G × T < 0.001
	COM (n = 34)	2.2 ± 0.96	2.8 ± 1.0	2.5 ± 1.0	2.3 ± 1.2	
Dairy intake, nonfat (svg/d)	INT (n = 37)	0.20 ± 0.41	3.4 ± 1.2 ^{a,d}	3.0 ± 0.94 ^{a,d}	2.5 ± 0.91 ^{a,b,d}	Group < 0.001 Time < 0.001 G × T < 0.001
	COM (n = 34)	0.47 ± 0.57	1.8 ± 1.0 ^a	1.8 ± 1.0 ^a	1.5 ± 0.90 ^a	
Energy expenditure (kcal/d)	INT (n = 32)	556.0 ± 425.4	600.0 ± 399.2	580.7 ± 456.7	544.5 ± 488.2	Group = 0.90 Time = 0.57 G × T = 0.15
	COM (n = 32)	457.1 ± 475.8	553.3 ± 415.3	575.2 ± 442.3	467.2 ± 400.1	
Step counts (steps/d)	INT (n = 17)	8522 ± 3512	12394 ± 3558 ^a	11972 ± 5012 ^a	12153 ± 5544 ^a	Group = 0.03 Time < 0.001 G × T = 0.21
	COM (n = 25)	6388 ± 2647	10148 ± 3447 ^a	9335 ± 3954 ^a	8772 ± 3340 ^a	

Dietary intake records analyzed using Nutrition Data System for Research (NDS-R, version 2010, Minnesota, MN) and reported for 71 women who provided full records for the entire 24-week study. Calcium intake reports include supplemental intake. Energy expenditure estimated using metabolic equivalent level calculations, duration of activity and participant body weight. Energy expenditure is reported for 64 women who provided full records for the entire 24-week study. Step counts are estimated using participant self-reports from pedometers and are reported for 42 women who provided full records for the entire 24-week study.

^adifferent from baseline;

^bdifferent from week 2;

^cdifferent from week 12;

^ddifferent between groups, using $P < 0.01$ for statistical significance;

P-values from multivariate analysis of covariance with repeated measures on the time factor.

G × T = group × time interaction.

at week 12, and this reduction was maintained through week 24. There were no significant changes in resistin concentration over time for either group. There was a

significant increase in adiponectin between weeks 12 and 24 in both groups. Significant group × time interactions were not observed in these adipose-derived hormones.

Table 3. Anthropometric and body composition measurements of premenopausal women with overweight/obesity at baseline and weeks 2, 12, and 24 in a study examining changes in body composition and metabolic parameters with an energy-reduced dietary pattern (ERD) including moderate protein and increased nonfat dairy intake (INT group) or an ERD with standard protein and standard nonfat dairy intake (COM group), combined with walking.

Variable	Group	Baseline (Week 0)	Week 2	Week 12	Week 24	P-value
		← Mean ± standard deviation →				
Body weight (kg)	INT (n = 52)	84.1 ± 12.5	81.7 ± 12.5 ^a	79.2 ± 12.9 ^{a,b}	79.2 ± 13.1 ^{a,b}	Group = 0.28 Time < 0.001 G × T = 0.49
	COM (n = 52)	80.8 ± 10.2	79.1 ± 9.9 ^a	76.5 ± 9.5 ^{a,b}	76.1 ± 9.5 ^{a,b}	
Body mass index (kg/m ²)	INT (n = 52)	30.3 ± 3.2	29.4 ± 3.2 ^a	28.5 ± 3.2 ^{a,b}	28.5 ± 3.4 ^{a,b}	Group = 0.88 Time < 0.001 G × T = 0.51
	COM (n = 52)	29.9 ± 3.2	29.2 ± 3.1 ^a	28.3 ± 3.2 ^{a,b}	28.2 ± 3.3 ^{a,b}	
Fat mass (kg)	INT (n = 52)	30.9 ± 7.4	–	28.0 ± 7.6 ^a	28.2 ± 7.8 ^a	Group = 0.80 Time < 0.001 G × T = 0.42
	COM (n = 52)	29.7 ± 6.1	–	27.4 ± 6.4 ^a	27.2 ± 6.6 ^a	
Fat-free soft tissue mass (kg)	INT (n = 52)	49.0 ± 5.7	–	47.5 ± 5.8 ^a	47.3 ± 5.5 ^a	Group = 0.05 Time < 0.001 G × T = 0.67
	COM (n = 52)	47.2 ± 5.3	–	45.5 ± 4.7 ^a	45.4 ± 4.7 ^a	
Body fat percentage (%)	INT (n = 52)	37.2 ± 4.1	–	35.4 ± 4.3 ^a	35.7 ± 4.5 ^a	Group = 0.38 Time < 0.001 G × T = 0.43
	COM (n = 52)	37.2 ± 4.4	–	36.1 ± 5.0 ^a	35.9 ± 5.3 ^a	
Waist circumference (cm)	INT (n = 52)	89.2 ± 8.7	87.1 ± 8.6 ^a	84.8 ± 8.4 ^{a,b}	84.7 ± 8.4 ^{a,b}	Group = 0.78 Time < 0.001 G × T = 0.55
	COM (n = 52)	87.7 ± 7.9	86.2 ± 7.8 ^a	84.0 ± 7.5 ^{a,b}	83.6 ± 7.9 ^{a,b}	
Hip circumference (cm)	INT (n = 52)	113.7 ± 8.6	111.8 ± 8.2 ^a	109.0 ± 8.5 ^{a,b}	108.8 ± 8.8 ^{a,b}	Group = 0.60 Time < 0.001 G × T = 0.84
	COM (n = 52)	112.2 ± 8.0	110.7 ± 7.9 ^a	107.9 ± 7.9 ^{a,b}	107.8 ± 7.8 ^{a,b}	
Central abdominal tissue percentage (%)	INT (n = 52)	36.9 ± 6.3	–	33.9 ± 7.0 ^a	34.1 ± 7.2 ^a	Group = 0.77 Time < 0.001 G × T = 0.77
	COM (n = 52)	36.1 ± 5.2	–	33.3 ± 5.9 ^a	33.2 ± 6.7 ^a	

Fat mass, fat-free soft tissue mass, body fat percentage and central abdominal tissue percentage analyzed by dual-energy X-ray absorptiometry at baseline and Weeks 12 and 24 only.

^adifferent from baseline;

^bdifferent from Week 2, using $P < 0.01$ for statistical significance; P-values using multivariate analysis of covariance with repeated measures on the time factor.

G × T = group × time interaction.

Table 4. Resting heart rate, blood pressure and resting energy expenditure measurements of premenopausal women with overweight/obesity at baseline and weeks 2, 12, and 24 in a study examining changes in body composition and metabolic parameters with an energy-reduced dietary pattern (ERD) including moderate protein and increased nonfat dairy intake (INT group) or an ERD with standard protein and standard nonfat dairy intake (COM group), combined with walking.

Variable	Group	Baseline (Week 0)	Week 2	Week 12	Week 24	P-value
		← Mean ± standard deviation →				
Resting heart rate (bpm)	INT (n = 52)	71.4 ± 7.5	72.4 ± 8.0	71.5 ± 8.6	73.0 ± 9.5	Group = 0.39 Time = 0.23 G × T = 0.79
	COM (n = 52)	72.7 ± 7.2	72.1 ± 7.0	73.1 ± 8.1	75.2 ± 9.7	
Resting systolic blood pressure (mm Hg)	INT (n = 52)	116.4 ± 9.0	116.7 ± 8.7	115.7 ± 10.4	117.4 ± 9.2	Group = 0.85 Time = 0.24 G × T = 0.94
	COM (n = 52)	116.5 ± 8.3	115.6 ± 9.3	114.7 ± 10.6	117.8 ± 12.6	
Resting diastolic blood pressure (mm Hg)	INT (n = 52)	73.7 ± 7.8	73.5 ± 6.7	73.2 ± 7.5	75.4 ± 7.6	Group = 0.43 Time = 0.02 G × T = 0.99
	COM (n = 52)	72.9 ± 8.0	72.1 ± 8.0	72.3 ± 7.6	75.1 ± 9.0	
Resting energy expenditure (kcal/d)	INT (n = 52)	1549 ± 168	1525 ± 168 ^a	1499 ± 174 ^{a,b}	1499 ± 175 ^{a,b}	Group = 0.25 Time < 0.001 G × T = 0.49
	COM (n = 52)	1508 ± 122	1491 ± 120 ^a	1465 ± 115 ^{a,b}	1461 ± 116 ^{a,b}	

^adifferent from baseline;

^bdifferent from Week 2, using $P < 0.01$ for statistical significance; P-values using multivariate analysis of covariance with repeated measures on the time factor.

G × T = group × time interaction.

Table 5. Blood lipid, glucose and insulin concentrations of premenopausal women with overweight/obesity at baseline and weeks 2, 12, and 24 in a study examining changes in body composition and metabolic parameters with an energy-reduced dietary pattern (ERD) including moderate protein and increased nonfat dairy intake (INT group) or an ERD with standard protein and standard nonfat dairy intake (COM group), combined with walking.

Variable	Group	Baseline (Week 0)	Week 2	Week 12	Week 24	P-value
		← Mean ± standard deviation →				
Triglycerides (mg/dL)	INT (n = 52)	104.5 ± 48.4	–	84.3 ± 42.0 ^a	84.9 ± 35.1 ^a	Group = 0.30 Time < 0.001 G × T = 0.52
	COM (n = 52)	107.8 ± 52.8	–	91.3 ± 46.1 ^a	91.1 ± 44.0 ^a	
Total cholesterol (mg/dL)	INT (n = 52)	185.2 ± 34.4	–	177.3 ± 33.5 ^a	176.2 ± 29.8 ^a	Group = 0.73 Time < 0.001 G × T = 0.38
	COM (n = 52)	188.2 ± 29.0	–	173.3 ± 28.9 ^a	177.5 ± 27.1 ^a	
High-density lipoprotein cholesterol (mg/dL)	INT (n = 52)	49.1 ± 12.5	–	49.6 ± 12.8	50.1 ± 12.9	Group = 0.42 Time = 0.13 G × T = 0.14
	COM (n = 52)	53.4 ± 15.8	–	51.2 ± 14.9	53.8 ± 14.3	
Low-density lipoprotein cholesterol (mg/dL)	INT (n = 52)	115.1 ± 30.0	–	110.7 ± 28.8 ^a	109.1 ± 26.0	Group = 0.67 Time < 0.01 G × T = 0.52
	COM (n = 52)	113.2 ± 32.2	–	103.4 ± 29.9 ^a	105.0 ± 26.1	
Glucose (mg/dL)	INT (n = 52)	82.8 ± 10.6	81.6 ± 9.9	80.9 ± 12.7	81.1 ± 9.4	Group = 0.45 Time = 0.25 G × T = 0.80
	COM (n = 52)	81.1 ± 11.6	79.7 ± 9.4	81.1 ± 7.5	79.9 ± 7.4	
Insulin (μU/mL)	INT (n = 52)	12.7 ± 10.2	12.1 ± 14.0	9.5 ± 5.5	10.4 ± 8.8	Group = 0.42 Time = 0.02 G × T = 0.38
	COM (n = 52)	11.0 ± 6.8	9.9 ± 5.0	9.0 ± 5.2	9.4 ± 4.4	

Fasting serum used to measure biomarker concentrations; for triglycerides, total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol samples were analyzed at baseline and weeks 12 and 24 only.

^adifferent from baseline, using $P < 0.01$ for statistical significance; P-values using multivariate analysis of covariance with repeated measures on the time factor.

G × T = group × time interaction.

Table 6. Concentrations of adipose-derived hormones in premenopausal women with overweight/obesity at baseline and Weeks 12 and 24 in a study examining changes in body composition and metabolic parameters with an energy-reduced dietary pattern (ERD) including moderate protein and increased nonfat dairy intake (INT group) or an ERD with standard protein and standard nonfat dairy intake (COM group), combined with walking.

Variable	Group	Baseline (Week 0)	Week 12	Week 24	P-value
		← Mean ± standard deviation →			
Leptin (ng/mL)	INT (n = 52)	27.6 ± 15.6	21.2 ± 16.5 ^a	21.5 ± 15.1 ^a	Group = 0.12 Time < 0.001 G × T = 0.58
	COM (n = 52)	31.0 ± 16.1	23.5 ± 13.0 ^a	25.2 ± 15.0 ^a	
Resistin (ng/mL)	INT (n = 52)	15.4 ± 13.4	16.8 ± 17.2	20.5 ± 16.5	Group = 0.42 Time = 0.35 G × T = 0.08
	COM (n = 52)	14.9 ± 12.7	16.6 ± 15.2	14.9 ± 12.2	
Adiponectin (μg/mL)	INT (n = 52)	7.8 ± 4.4	7.7 ± 4.3	8.3 ± 4.7 ^{a,b}	Group = 0.57 Time < 0.01 G × T = 0.66
	COM (n = 51)	8.2 ± 4.2	8.2 ± 4.1	9.0 ± 4.3 ^{a,b}	

Fasting serum used to measure adipose-derived hormone concentrations; data missing from one participant in the COM group for adiponectin.

^adifferent from baseline, ^bdifferent from week 12, using $P < 0.01$ for statistical significance; P-values using multivariate analysis of covariance with repeated measures on the time factor.

G × T = group × time interaction.

Discussion

Healthy premenopausal women with excess adiposity significantly reduced BW and fat mass as well as triglycerides

and total cholesterol across a 12-week JumpStart + weight-loss phase and maintained these changes across a 12-week weight-loss maintenance phase of a lifestyle intervention, including diet modification and walking. The lack of

detectable differences in main outcome variables between diet groups at week 12 suggests that these two approaches to BW loss may have been equally effective. Both interventions significantly increased the number of nonfat dairy servings participants were consuming per day and met recommendations for protein intake within the acceptable macronutrient distribution range (National Academies Press 2005). Although protein comprised a greater proportion of total energy in the INT group, estimated dietary protein intake did not reach the goal of 30% of total energy and fell within the range of 0.89–0.96 g of protein per kg BW per day. Estimated protein intake in the COM group was within the range of 0.78–0.87 g of protein per kg BW per day. Absolute protein (g) consumed during energy restriction is often less than the amount consumed during energy balance, though protein may comprise the same percentage of overall energy intake (Pasiakos *et al.* 2014). During energy restriction, grams of protein intake per kg BW may fall below 0.8. Within the context of an ERD (i.e., lowered total energy intake), dietary protein as a percentage of total energy should increase to meet a minimally recommended amount (National Academies Press 2005).

In order to maintain fat-free soft tissue mass, protein intake should increase or at least be maintained as BW declines due to reduced overall caloric intake (Layman 2004). Interventions citing improved body composition and blood lipid outcomes within experimental higher-protein diet groups compared to standard-protein diet groups with or without exercise often include dietary protein at 1.3–1.6 g of protein per kg BW per day (approximately 30% of the total energy consumed; Layman *et al.* 2003b, 2005; Leidy *et al.* 2007; Meckling and Sherfey 2007; Josse *et al.* 2011). This level of protein intake was not met in the current study, likely due to the study design leveraging a dietary exchange weight-loss program in free-living women. Studies that provide meals or utilize controlled feedings may better achieve a strict dietary protein goal.

Participants in both groups similarly improved triglycerides and total cholesterol irrespective of dietary intervention, indicating overall improvement in these metabolic parameters with energy restriction, moderate walking and BW loss (Blumenthal *et al.* 2010). This is in contrast to other studies in which larger reductions in triglycerides concentrations were found in participants with greater protein intake (Skov *et al.* 1999; Farnsworth *et al.* 2003; Layman *et al.* 2003b, 2005, 2009; Noakes *et al.* 2005). However, these previous studies differed in research design and participant characteristics and compared wider extremes of dietary protein intake than in the current study. Protein as a percentage of total energy intake differed by approximately 12% between intervention groups in previous studies (Skov *et al.* 1999; Farnsworth *et al.* 2003;

Layman *et al.* 2003b, 2005, 2009; Noakes *et al.* 2005), while the current study achieved an approximate 5% difference in dietary protein intake between groups. The difference in carbohydrate intake between the INT and COM groups was roughly two starch servings at week 2 and one at week 12, with no appreciable difference at week 24. This suggests that an approximate 2% difference in dietary carbohydrate intake between groups was insufficient to impact secondary outcomes. Systolic and diastolic blood pressure remained unchanged and within normal blood pressure ranges (Goldstein *et al.* 2011).

In the current study, the JumpStart phase was designed to accelerate BW loss within the INT group. Interventions show that a more substantial initial BW loss (within the first 5–12 weeks) predicts overall BW loss (Wadden *et al.* 1992; Guaraldi *et al.* 2011) and maintenance of BW loss over time (Wadden *et al.* 1992; Jeffery *et al.* 1998; Stotland and Larocque 2005; Guaraldi *et al.* 2011) provided that early BW loss is significant and followed by lifestyle changes that are monitored over time (Toubro and Astrup 1997; Astrup and Rössner 2000). Estimated energy intake was significantly lower in the INT group compared to the CON group, and though not significant at $P < 0.01$ in the context of the entire intervention, BW (and body mass index) was reduced to a greater extent ($P < 0.01$) in the INT group than the COM group at Week 2 (-2.4 ± 1.0 vs. -1.7 ± 1.1 kg). The 2-week JumpStart phase may not have been sufficient in length to induce significant differences in BW loss (at $P < 0.01$) between groups as part of the entire 12-week weight-loss phase plus 12-week weight-loss maintenance phase (Wadden *et al.* 1992; Stotland and Larocque 2005; Guaraldi *et al.* 2011).

Mechanisms for improved metabolic conditions with BW loss may be partially explained by beneficial changes in select adipose-derived hormones (Jung *et al.* 2008). Leptin, resistin, and adiponectin originate predominately in adipose tissue (Rajala and Scherer 2003; Klempel and Varady 2011) and are known to contribute to the inflammatory milieu (Ma *et al.* 2012). While leptin and resistin are positively correlated with body mass index (Friedman 2002; Pagano *et al.* 2005), low adiponectin levels are associated with obesity (Weyer *et al.* 2001). In their dysregulated states, these adipose-derived hormones may negatively impact other body systems. Indeed, adipose tissue-derived hormones have been implicated in the progression of cardio-metabolic disease (Allende-Vigo 2010). In the current study, reductions in leptin and increases in adiponectin are consistent with other studies demonstrating BW and fat mass loss (Miller *et al.* 2009; Varady *et al.* 2009; Josse *et al.* 2012). A review of adipose-derived hormone response to randomized control energy-restricted diets reports that fat mass losses of $<10\%$ may be insufficient to reduce circulating resistin and increase adiponectin levels; however,

studies examining resistin response to BW and fat mass loss are limited (Klempel and Varady 2011). Leptin is particularly responsive to BW loss induced by energy restriction (Klempel and Varady 2011), responding to both short term alterations in energy intake (Sartorio *et al.* 2003) and reductions in fat mass. Further insight into the function of these adipose-derived hormones and their part in the improvement of metabolic factors observed during BW and fat mass loss should be a research priority.

Strengths of the current study include using a research design with free-living women to examine the effectiveness of the intervention (Most *et al.* 2003). Findings from this study may be directly generalisable to women of similar characteristics and relevant regarding the challenges faced by women who attempt this approach to BW loss. Additionally, this study included a daily moderate walking component in both groups. As an accepted promoter of BW and fat mass losses with energy restriction (Jakicic and Davis 2011), increased physical activity was not among study variables but a component of the overall intervention strategy. Similarly, all women attended weekly nutrition-related educational sessions that did not differ between groups, as attendance at group sessions during weight-loss intervention has been strongly associated with weight loss (Sacks *et al.* 2009). This study employed a comparison group following a dietary pattern adequate in both protein and dairy/calcium, rather than inadequate in dairy/calcium as evaluated in past studies (Zemel *et al.* 2004, 2005). In addition, the current study employed a comparison group meeting protein and dairy/calcium recommendations for evaluation against the experimental diet, thereby examining effects of protein and dairy/calcium intake beyond general recommendations. Studies reporting that dairy intake or calcium supplementation may accelerate BW and fat mass loss in the context of energy restriction often begin with participants who are calcium deficient or use a low-calcium, energy-restricted control group (Zemel *et al.* 2004, 2005; Josse *et al.* 2011). Recruitment in the current study was not limited to women who were deficient in either protein or dairy intakes, making this design more generalisable. Additionally, calcium intake was controlled via supplementation. Finally, this study used highly conservative statistical methods, limiting the likelihood of type 1 error, though this may have inflated the rate of false negatives.

This study had several limitations. Studies involving free-living participants allow for more realistic examinations of intervention components (Most *et al.* 2003); however, the lack of complete food intake control in the current study limited the ability to address diet efficacy on BW and body composition measurements. For example, protein intake goals for the INT group were not achieved, likely due to the lack of a feeding trial study design.

Additionally, self-reported physical activity and pedometer counts are prone to error (Melanson *et al.* 2004; Ferrari *et al.* 2007). Clearly, many women elected not to complete the walking component of the intervention. Generalizations from this study may be limited to only healthy, premenopausal and primarily Caucasian women.

In conclusion, an ERD including moderate protein and increased nonfat dairy combined with walking exercise resulted in significant losses in BW and fat mass along with other beneficial changes in anthropometric measurements and some metabolic biomarkers in healthy premenopausal women with excess adiposity. An ERD that meets dietary protein (0.8 g protein/kg BW) and dairy intake (3 servings/d) recommendations is as effective as an ERD that includes greater protein and more servings of nonfat dairy intake per day in producing BW and fat mass losses and maintaining these beneficial changes over time. Additionally, the current study did not demonstrate any benefit of early BW loss on longer-term BW loss and maintenance of that BW loss. Either dietary approach to BW loss and maintenance used in this study may be applied in clinical practice with modest outcomes.

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Conflict of Interest

None declared.

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