VOLUME 30 · NUMBER 19 · JULY 1 2012

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Reduced-Calorie Dietary Weight Loss, Exercise, and Sex Hormones in Postmenopausal Women: Randomized Controlled Trial

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Submitted July 19, 2011; accepted February 21, 2012; published online ahead of print at www.jco.org on May 21, 2012.

Supported by National Cancer Institute at the National Institutes of Health (Grants No. R01 CA102504; 5KL2RR025015-03 to K.F.S.; R25 CA94880 and 2R25CA057699 to A.K.); and Canadian Institutes of Health Research (Fellowships to K.L.C. and C.M.). None of the funding agencies were involved in the trial design or conduct.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Clinical Trials repository link available on JCO.org.

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0732-183X/12/3019-2314/$20.00

DOI: 10.1200/JCO.2011.37.9792

ABSTRACT

Purpose
Estrogens and androgens are elevated in obesity and associated with increased postmenopausal breast cancer risk, but the effect of weight loss on these biomarkers is unknown. We evaluated the individual and combined effects of a reduced-calorie weight loss diet and exercise on serum sex hormones in overweight and obese postmenopausal women.

Patients and Methods
We conducted a single-blind, 12-month, randomized controlled trial from 2005 to 2009. Participants (age 50 to 75 years; body mass index > 25.0 kg/m^2, exercising < 100 minutes/wk) were randomly assigned using a computer-generated sequence to (1) reduced-calorie weight loss diet ("diet"; n = 118), (2) moderate- to vigorous-intensity aerobic exercise ("exercise"; n = 117), (3) combined reduced-calorie weight loss diet and moderate- to vigorous-intensity aerobic exercise ("diet + exercise"; n = 117), or (4) control (n = 87). Outcomes were estrone concentration (primary) and estradiol, free estradiol, total testosterone, free testosterone, androstenedione, and sex hormone–binding globulin (SHBG) concentrations (secondary).

Results
Mean age and body mass index were 58 years and 30.9 kg/m^2, respectively. Compared with controls, estrone decreased 9.6% ($P = .001$) with diet, 5.5% ($P = .01$) with exercise, and 11.1% ($P < .001$) with diet + exercise. Estradiol decreased 16.2% ($P < .001$) with diet, 4.9% ($P = .10$) with exercise, and 20.3% ($P < .001$) with diet + exercise. SHBG increased 22.4% ($P < .001$) with diet and 25.8% ($P < .001$) with diet + exercise. Free estradiol decreased 21.4% ($P < .001$) with diet and 26.0% ($P < .001$) with diet + exercise. Free testosterone decreased 10.0% ($P < .001$) with diet and 15.6% ($P < .001$) with diet + exercise. Greater weight loss produced stronger effects on estrogens and SHBG.

Conclusion
Weight loss significantly lowered serum estrogens and free testosterone, supporting weight loss for risk reduction through lowering exposure to breast cancer biomarkers.

J Clin Oncol 30:2314-2326. © 2012 by American Society of Clinical Oncology

INTRODUCTION

Overweight, obesity, and a sedentary lifestyle are associated with an increased risk of postmenopausal breast cancer,[1-3] possibly through adiposity-induced excess levels of sex hormones.[1,4] Serum concentrations of estrogens and androgens have been positively associated with risk for breast cancer in several cohort studies.[5-9] Adipose tissue is the main storage site in postmenopausal women for aromatase and 17β-hydroxysteroid dehydrogenases, enzymes that catalyze the formation of estrone, estradiol, and testosterone.[10]

In observational studies, lower body weight/body fat and higher levels of physical activity are associated with lower circulating postmenopausal blood estrogens and androgens and higher sex hormone–binding globulin (SHBG), which reduces their bioactivity.[11-17] Previous randomized controlled exercise trials reported modest or no reductions in estradiol and free estradiol, with little change in androgens,[18-21] although one found significant

reductions in exercisers who lost body fat.[18,19] Low-fat dietary interventions with minimal or no weight loss have also modestly reduced estrogens and increased SHBG.[22-29] To our knowledge, no previous randomized controlled trials have tested the effects of a weight loss intervention on sex hormones in postmenopausal women, a group at elevated risk for breast cancer.

The purpose of this investigation was to assess the independent and combined effects of reduced-calorie weight loss and moderate-to-vigorous aerobic exercise interventions with achievable goals on serum sex hormones. We hypothesized that the combined reduced-calorie weight loss diet and moderate-to-vigorous aerobic exercise intervention would produce a greater reduction in estrogens and androgens, and a greater increase in SHBG, compared with either condition alone or with controls. Because the association between obesity and breast cancer risk could be multifactorial, we also report weight loss and exercise effects on other breast cancer biomarkers: fasting insulin, C-reactive protein, adiponectin, and leptin.[30-34]

PATIENTS AND METHODS

The Nutrition and Exercise for Women randomized controlled trial, conducted in Seattle, WA, from 2005 to 2009, tested the effects of three 12-month-long weight loss and exercise interventions on estrone, estradiol, free estradiol, total testosterone, free testosterone, androstenedione, and SHBG. Study procedures were approved by the Fred Hutchinson Cancer Research Center institutional review board in Seattle, WA. All participants provided signed informed consent.

Participant Recruitment and Inclusion and Exclusion Criteria

Participants were postmenopausal (no menstrual cycles for ≥ 1 year or follicle-stimulating hormone level of > 23.0 IU/L for women 50 to 59 years of age without a uterus), age 50 to 75 years, body mass index (BMI) ≥ 25.0 kg/m^2 (≥ 23.0 kg/m^2 if Asian-American), and participating in less than 100 minutes/wk in moderate-intensity physical activity. Exclusion criteria included use of estrogen, progesterone, or testosterone hormones (past 3 months); history of breast cancer or other serious medical conditions; diabetes, fasting glucose ≥ 126 mg/dL, or use of diabetes medications; more than two alcohol drinks/d; currently smoking; or current use of weight loss medications or programs.

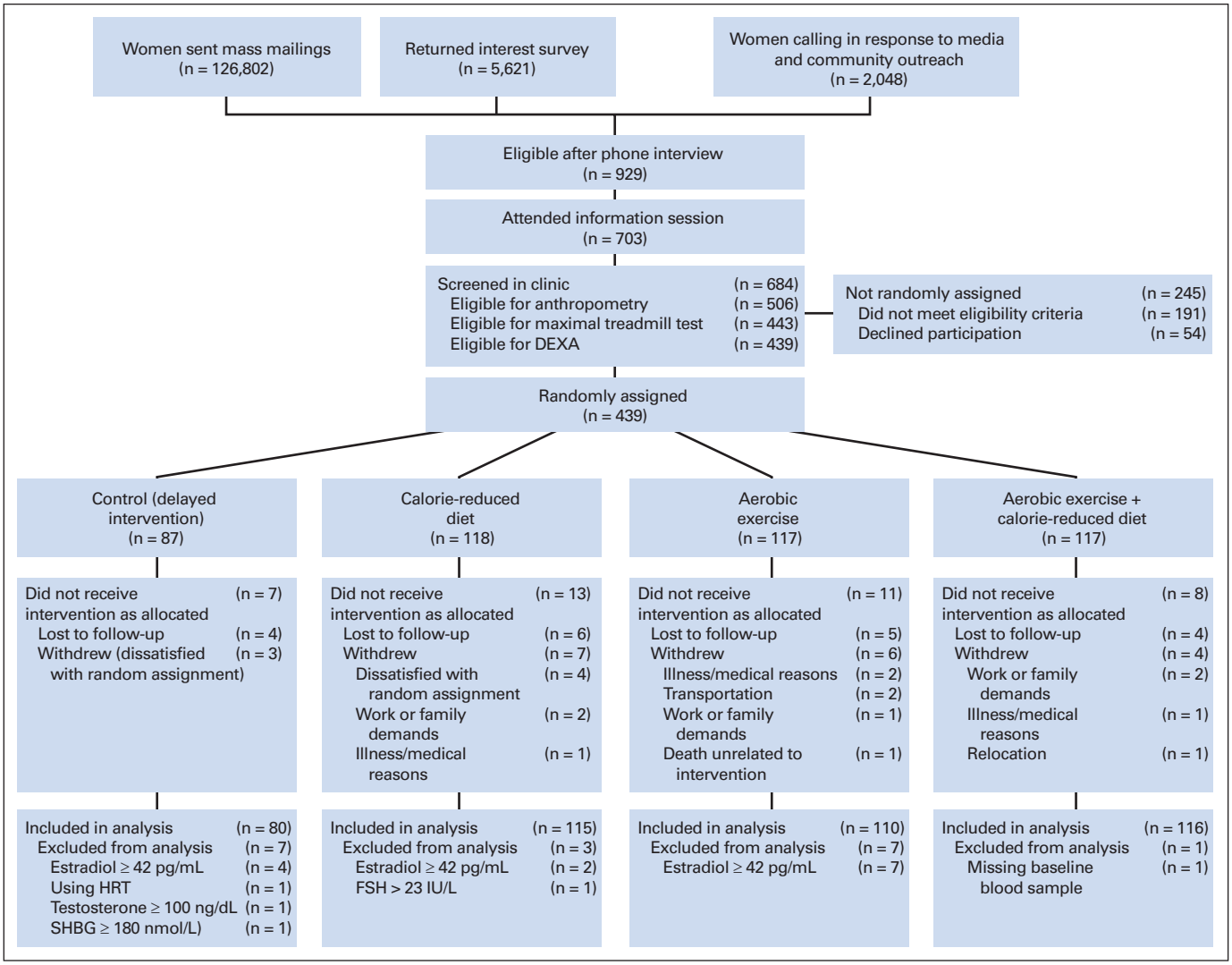

Fig 1. CONSORT diagram: Flow of participants through the Nutrition and Exercise for Women Trial. DEXA, dual x-ray absorptiometry; FSH, follicle-stimulating hormone; HRT, hormone-replacement therapy; SHBG, sex hormone–binding globulin.

Table 1. Baseline Characteristics of Randomly Assigned Participants

Characteristic	All (n = 439)		Control (n = 87)		Diet (n = 118)		Exercise (n = 117)		Diet + Exercise (n = 117)	
	No.	%	No.	%	No.	%	No.	%	No.	%
Age, years										
Mean	58.0		57.4		58.1		58.1		58.0	
SD	5.0		4.4		6.0		5.0		4.5	
Range	50-76		51-70		50-76		51-72		51-73	
Weight, kg										
Mean	83.6		84.2		84.0		83.7		82.5	
SD	11.8		12.5		11.8		12.3		10.8	
BMI, kg/m^2										
Mean	30.9		30.7		31.1		30.7		31.0	
SD	4.0		3.9		3.9		3.7		4.3	
Body fat, %										
Mean	47.2		47.3		47.0		47.3		47.4	
SD	4.3		4.4		4.3		4.1		4.5	
Body fat mass, kg										
Mean	39.8		40.1		39.8		39.9		39.4	
SD	8.1		8.5		8.1		8.2		7.9	
Lean mass, %										
Mean	48.5		48.5		48.7		48.3		48.3	
SD	4.2		4.3		4.3		3.9		4.4	
Lean mass, kg										
Mean	40.2		40.6		40.7		40.2		39.6	
SD	5.0		5.3		5.1		5.3		4.3	
VO_2max, L/min										
Mean	1.90		1.93		1.89		1.85		1.93	
SD	0.33		0.37		0.31		0.31		0.34	
Usual physical activity, MET min/wk										
Mean	151		109		160		173		151	
SD	208		196		230		202		197	
Total calories, kcal/d*										
Mean	1,934		1,988		1,884		1,986		1,890	
SD	638		669		661		589		638	
Alcohol, g/d*										
Mean	7.3		8.2		8.8		7.1		5.4	
SD	10.8		11.5		13.9		9.7		69	
Ethnicity										
Non-Hispanic white	373	85.0	74	85.1	101	85.6	98	83.8	100	85.4
African American	35	8.0	6	6.9	9	7.6	15	12.8	5	4.3
Asian/Pacific Islander	8	1.8	2	2.3	2	1.7	2	1.7	2	1.7
Hispanic/Latino	12	2.7	3	3.4	2	1.7	2	1.7	5	4.3
Other	11	2.5	2	2.3	4	3.4	0	0.0	5	4.3
Education										
Some high school or high school graduate	18	4.1	5	5.7	3	2.5	6	5.1	4	3.4
Some college	134	30.5	23	26.5	39	33.1	41	35.1	31	26.5
College graduate and above	287	65.4	59	67.8	76	64.4	70	59.8	82	70.1
Family history of breast cancer										
None	286	62.1	54	67.8	80	65.0	76	65.0	76	65.0
First degree	81	16.1	14	18.6	22	22.2	26	22.2	19	16.2
Second degree	72	21.8	19	13.6	16	12.8	15	12.8	22	18.8
Parity†										
Nulliparous	118	26.9	22	25.3	32	27.4	29	24.8	35	29.9
1	72	16.4	15	17.2	23	19.7	16	13.7	18	15.4
2	145	33.1	27	31.0	32	27.4	44	37.6	42	35.9
≥3	103	23.5	23	26.4	30	25.6	28	23.9	22	18.8
History of breastfeeding, months‡										
0	174	40.8	33	39.8	44	37.9	47	41.2	50	43.9
1-6	54	12.7	11	13.3	19	16.4	11	9.7	13	11.4
≥ 6	199	46.6	39	47.0	53	45.7	56	49.1	51	44.7

(continued on following page)

Table 1. Baseline Characteristics of Randomly Assigned Participants (continued)

Characteristic	All (n = 439)		Control (n = 87)		Diet (n = 118)		Exercise (n = 117)		Diet + Exercise (n = 117)	
	No.	%	No.	%	No.	%	No.	%	No.	%
History of breast biopsy										
Yes	58	13.2	14	16.1	17	14.4	10	8.6	17	14.5
No	381	86.8	73	83.9	101	85.6	107	91.5	100	85.5
Age at randomization, years										
< 60	304	69.3	62	74.7	83	70.3	80	68.4	79	67.5
≥ 60	135	30.8	25	28.7	35	29.7	37	31.6	38	32.5
Age at menarche, years§										
< 13	209	48.0	44	50.6	54	46.2	55	47.0	56	48.7
≥ 13	227	52.0	43	49.4	63	53.9	62	53.0	59	51.3
Age at menopause, years‖										
< 50	178	42.4	28	33.3	52	46.0	42	38.2	56	49.6
≥ 50	242	57.6	56	66.7	61	54.0	68	61.8	57	50.4
Ever used hormone therapy										
No	178	40.5	37	42.5	53	44.9	49	41.9	39	33.3
Yes	261	59.5	50	57.5	65	55.1	68	58.1	78	66.7
History of hysterectomy										
No	345	78.6	67	77.0	93	78.8	92	78.6	93	79.5
Yes	94	21.4	20	23.0	25	21.2	25	21.4	24	20.5
History of bilateral oophorectomy										
No	416	94.8	83	95.4	112	94.9	109	93.2	112	95.7
Yes	23	5.2	4	4.6	6	5.1	8	6.8	5	4.3
Sex hormones¶										
Estrone, pg/mL										
Mean	37.2		35.8		38.1		37.4		36.9	
SD	15.5		17.1		15.5		14.8		15.3	
Estradiol, pg/mL										
Mean	12.7		12.4		12.7		12.6		12.8	
SD	5.8		6.1		5.4		5.4		6.3	
Testosterone, ng/dL										
Mean	26.5		26.3		26.6		26.8		26.2	
SD	12.9		15.2		13.5		11.3		12.1	
Androstenedione, ng/dL										
Mean	55.5		53.9		56.4		54.6		56.4	
SD	24.9		23.9		28.9		24.6		21.4	
SHBG, nmol/L										
Mean	39.3		38.1		39.3		43.4		36.2	
SD	17.6		16.5		16.9		22.2		12.8	
Free estradiol,# pg/mL										
Mean	0.35		0.34		0.35		0.34		0.36	
SD	0.17		0.16		0.16		0.16		0.18	
Free testosterone, pg/mL										
Mean	5.7		5.6		5.8		5.5		5.8	
SD	2.8		2.9		2.9		2.5		2.7	

NOTE. SI conversion factors: To convert estrone to pmol/L, multiply by 3.699; estradiol to pmol/L, multiply by 3.67; testosterone to nmol/L, multiply by 0.0347; androstenedione to nmol/L, multiply by 0.0349.

Abbreviations: BMI, body mass index; SD, standard deviation; SHBG, sex hormone–binding globulin.

*N = 427 for total; n = 85 for control; n = 114 for diet, exercise, and diet + exercise groups.

†N = 438 for total; n = 87 for control; n = 117 for diet, exercise, and diet + exercise groups.

‡N = 427 for total; n = 83 for control; n = 116 for diet; n = 114 for exercise and diet + exercise groups.

§N = 436 for total; n = 87 for control; n = 117 for diet and exercise groups; n = 115 for diet + exercise.

‖N = 420 for total; n = 84 for control; n = 113 for diet; n = 110 for exercise; n = 113 for diet + exercise.

¶N = 421 for total; n = 80 for control; n = 115 for diet; n = 110 for exercise; n = 116 for diet + exercise groups.

#N = 420 for total; n = 79 for control; n = 115 for diet; n = 110 for exercise; n = 116 for diet + exercise group.

The trial design and recruitment are depicted in Figure 1.[35] Eligible women were randomly assigned, stratified according to BMI (< 30 kg/m^2 or ≥ 30 kg/m^2) and race/ethnicity (non-Hispanic white, black, other), into one of four groups: (1) reduced-calorie weight loss diet ("diet"; n = 118), (2) moderate- to vigorous-intensity aerobic exercise ("exercise"; n = 117), (3) combined reduced-calorie weight loss diet and moderate- to vigorous-intensity aerobic exercise ("diet + exercise"; n = 117), or (4) no diet or exercise change ("control"; n = 87). A permuted blocks, computer-generated randomization (ratio 0.75:1:1:1) was used to assign a proportionally smaller number of women to the control group.

Table 2. Adherence to the Interventions by Study Group

Outcome	Baseline		6-Month		12-Month		$\Delta_{0\text{-}6\text{-mo}}$	$\%\Delta_{0\text{-}6\text{-mo}}$	$\Delta_{0\text{-}12\ mo}$	$\%\Delta_{0\text{-}12\ mo}$	P^*
	Mean	SD	Mean	SD	Mean	SD					
Weight, kg											
Control	84.2	12.5	83.8	13.0	83.7	12.3	−0.5	−0.6	−0.5	−0.6	
Diet	84.0	11.8	77.0	12.8	74.9	12.3	−7.1	−8.4	−9.1	−10.8	$P_C < .001$† $P_E < .001$‡ $P_{D+E} = .17$‡
Exercise	83.7	12.3	82.1	12.3	80.9	12.2	−1.6	−1.9	−2.8	–3.3	$P_C = .02$† $P_D < .001$‡ $P_{D+E} < .001$‡
Diet + exercise	82.5	10.8	74.9	10.8	72.7	10.9	−7.7	−9.3	−9.8	−11.9	$P_C < .001$† $P_D = .10$‡ $P_E < .001$‡
Body fat, %							NA	NA			
Control	47.3	4.4			47.2	5.3			−0.3	−0.5	
Diet	47.0	4.3			42.1	6.4			−5.0	−10.6	$P_C < .001$† $P_E = .02$‡ $P_{D+E} < .001$‡
Exercise	47.3	4.1			45.5	5.0			−1.8	−3.8	$P_C < .001$† $P_D < .001$‡ $P_{D+E} < .001$‡
Diet + exercise	47.4	4.5			41.1	7.0			−6.4	−13.4	$P_C < .0001$† $P_D = .02$‡ $P_E < .001$‡
Pedometer, steps/wk§							NA	NA			
Control	5,605	2,334			6,136	2,909			530.9	9.5	
Diet	5,539	2,257			6,365	2,841			825.7	14.9	$P_C = .67$† $P_E < .001$‡ $P_{D+E} < .001$‡
Exercise	5,777	2,129			9,139	3,120			3362.6	58.2	$P_C < .001$† $P_D < .001$‡ $P_{D+E} = .06$‡
Diet + exercise	5,980	2,393			10,069	2,944			4088.5	68.4	$P_C < .001$† $P_D < .001$‡ $P_E = .06$‡
VO2max, L/min							NA	NA			
Control	1.93	0.37			1.91	0.33			−0.02	−0.9	
Diet	1.89	0.31			1.84	0.32			−0.04	−2.3	$P_C = .83$† $P_D < .001$‡ $P_{D+E} < .001$‡
Exercise	1.85	0.31			2.04	0.35			0.19	10.1	$P_C < .001$† $P_D < .001$‡ $P_{D+E} = .30$‡
Diet + exercise	1.93	0.34			2.07	0.41			0.15	7.6	$P_C < .001$† $P_D < .001$‡ $P_E = .30$‡
Total calories, kcal/d‖							NA	NA			
Control	1,988	669			1,733	616			−255	−13	
Diet	1,885	661			1,564	539			−320	−17	$P_C = .54$† $P_E = .30$‡ $P_{D+E} = .75$‡
Exercise	1,987	589			1,768	501			−219	−11	$P_C = .73$† $P_D = .30$‡ $P_{D+E} = .17$‡
Diet + exercise	1,891	638			1,549	525			−342	−18	$P_C = .36$† $P_D = .75$‡ $P_E = .10$‡
Fat intake, % of kcal/d‖							NA	NA			
Control	35.6	6.9			33.3	6.9			−2.3	−6.3	
Diet	33.1	6.3			26.2	6.9			−6.9	−21.0	$P_C < .001$† $P_E < .001$‡ $P_{D+E} = .17$‡
Exercise	33.6	6.9			31.8	7.0			−1.8	−5.4	$P_C = .30$† $P_D < .001$‡ $P_{D+E} < .001$‡

(continued on following page)

Table 2. Adherence to the Interventions by Study Group (continued)

	Baseline		6-Month		12-Month						
Outcome	Mean	SD	Mean	SD	Mean	SD	$\Delta_{0\text{-}6\text{-mo}}$	$\%\Delta_{0\text{-}6\text{-mo}}$	$\Delta_{0\text{-}12\ mo}$	$\%\Delta_{0\text{-}12\ mo}$	P^*
Diet + exercise	35.3	7.3			27.0	6.6			−8.4	−23.7	$P_C < .001$† $P_D = .17$‡ $P_E < .001$‡

Abbreviations: SD, standard deviation; Δ, change.
*Change from baseline to 12 months.
†P_C, P values for comparing the changes between control group and three intervention groups.
‡P_D or P_E, P values for comparing the changes between exercise group and diet group; P_{D+E}, P values for comparing the changes between diet + exercise group and two other intervention groups (exercise group or diet group).
§N = 428 for total; n = 82 for control; n = 117 for diet; n = 114 for exercise; and n = 115 for diet + exercise groups.
‖N = 427 for total; n = 85 for control; n = 114 for diet; n = 114 for exercise; n = 114 for diet + exercise groups.

Interventions and Control Group

The weight loss diet intervention[35] was a modification of the dietary component of the Diabetes Prevention Program[36] and the Look AHEAD (Action for Health in Diabetes)[37] lifestyle intervention programs, with a goal of daily energy intake of 1200 to 2000 kcal/d based on baseline weight, less than 30% daily energy intake from fat, and a 10% reduction in body weight by 6 months with maintenance to 12 months. In months 1 to 6, participants met individually with a study dietitian at least twice, attended weekly group dietitian-led meetings, and kept daily food logs. In each of months 7 to 12, participants had one face-to-face individual or group contact and one phone or e-mail contact with a dietician.

The exercise intervention goal was ≥ 45 minutes of moderate- to vigorous-intensity aerobic exercise, 5 days per week (225 minutes/wk).[18,19] Each week, participants attended three monitored exercise sessions at our study facility and two at home. The program progressed to the maintenance target of 70% to 85% maximal heart rate for 45 minutes by week 7. Activities with four or more metabolic equivalents,[38] such as brisk walking, were counted toward the prescribed exercise target.

Women randomly assigned to the diet + exercise intervention received both interventions, but with separate diet sessions and instructions not to discuss the diet intervention at the exercise facility.

Women randomly assigned to the control group were requested not to change their diet or exercise habits and were offered four weight loss classes and 8 weeks of facility exercise training at study end.

Outcome Measures

The primary outcome of the trial was estrone, and secondary outcomes included the sex hormones estradiol, free estradiol, total testosterone, free testosterone, androstenedione, and SHBG. Twelve-hour fasting blood was collected at baseline and 12 months, processed within 1 hour, and stored at −70°C. Laboratory assays were performed at the Reproductive Endocrine Research Laboratory (University of Southern California, Los Angeles, CA). Estrone, estradiol, total testosterone, and androstenedione were quantified by radioimmunoassays after organic solvent extraction and Celite column partition chromatography.[39,40] SHBG was quantified via chemiluminescent immunometric assay using the Immulite Analyzer (Siemens Medical Solutions Diagnostics, Malvern, PA). Free estradiol and free testosterone were calculated using the measured values for estradiol, total testosterone, SHBG, and an assumed constant for albumin.[41-43] The interassay coefficients of variation (CVs) ranged from 8% to 13% for the steroid hormone assays and 5% to 7% for the SHBG assay. Insulin (quantified by a 48-hour, polyethylene glycol-accelerated, double antibody radioimmunoassay) and high-sensitivity C-reactive protein (assay kits from Siemens Healthcare Diagnostics Products, Tarrytown, NY) were analyzed at the University of Washington Clinical Nutrition Research Unit Laboratory (Seattle, WA), and intra-assay CVs were 4.5% and 4.1%, respectively.[44] Leptin and adiponectin (quantified by radioimmunoassays from Millipore [Billerica, MA] and Linco Research [St Charles, MO], respectively) were analyzed at the Northwest Lipid Metabolism and Diabetes Research Laboratories (Seattle, WA), and intra-assay CVs were 9.1% and 8.4%, respectively.

Covariate Measures

Study measures were obtained by trained study personnel blinded to randomization status. Participants completed questionnaires on demographic information, medical history, and food frequency dietary patterns[45] and a physical activity interview.[46] Height and weight were measured. Body composition was estimated using a dual x-ray absorptiometry scanner (GE Lunar, Madison, WI). All participants wore pedometers (Accusplit, Silicon Valley, CA) for 7 consecutive days to estimate average daily pedometer steps. Cardiorespiratory fitness (VO_2max reported in liters per minute[47]) was measured by a maximal graded treadmill test and a metabolic cart (MedGraphics, St Paul, MN).[48,49] Intervention women completed daily diet logs (for the first 6 months) and/or facility and home activity logs (for all 12 months), depending on assigned group.

Adverse Outcome Measures

Self-reported injuries and hot flash symptoms[50] were ascertained via standard questionnaires, and total bone mineral density (in grams per square centimeter) was measured with dual x-ray absorptiometry.[51] Participants were asked to report any musculoskeletal injuries to staff.

Statistical Analysis

Descriptive data are presented as means ± standard deviation (SD). Blood measures were log-transformed and presented as geometric means with 95% CIs. Intervention effects were examined based on the assigned treatment (ie, intent to treat). Mean 12-month changes in diet, exercise, and diet + exercise groups were compared with controls and with the other intervention groups using the generalized estimating equations (GEE) approach to random effects regression to account for the correlation within individuals over time.[35] The GEE analysis based on available data was conducted to deal with missing outcome values at 12 months. We used the Bonferroni correction (two-sided $\alpha = .05/6$; critical P value of $P = .008$) to adjust for the six comparisons of the four intervention arms.

We also assessed changes in sex hormones for each intervention arm using GEE models by preplanned subgroups reflecting intervention adherence, including (1) weight loss, (2) body fat loss, (3) change in pedometer steps per day, (4) change in VO_2max, (5) diet session attendance, and (6) exercise adherence (in minutes per day). For these subgroup analyses, a P value of .05 was considered significant. Incidence and severity of injuries and hot flashes were compared across groups using χ^2 tests. All statistical analyses were performed using SAS software version 9.1 (SAS Institute, Cary, NC).

RESULTS

At 12 months, 399 participants (91%) completed physical examinations and provided blood (Fig 1). Women whose respective estradiol (n = 2) or estrone (n = 1) levels were below the limit of detection, less than 3 pg/mL and less than 5 pg/mL, respectively, were assigned a value halfway between 0 and the limit of detection (1.5 and 2.5 pg/mL) and included in the analyses. One participant was missing baseline

Table 3. Change in Sex Hormones by Study Group, Geometric Mean, and 95% CI

Biomarker	Baseline		12 Months		Δ*	%Δ	P†
	Mean	95% CI	Mean	95% CI			
Estrone, pg/mL							
Control	32.0	28.6 to 35.8	34.6	31.4 to 38.1	2.6	8.1	
Diet	35.2	32.7 to 37.9	31.8	29.4 to 34.4	−3.4	−9.6	$P_C = .001$‡ $P_E = .30$§ $P_{D+E} = .17$§
Exercise	34.8	32.4 to 37.4	32.9	30.5 to 35.5	−1.9	−5.5	$P_C = .01$‡ $P_D = .30$§ $P_{D+E} = .01$§
Diet + exercise	33.9	31.5 to 36.6	30.2	28.0 to 32.5	−3.8	−11.1	$P_C < .001$‡ $P_D = .17$§ $P_E = .01$§
Estradiol, pg/mL							
Control	10.9	9.6 to 12.3	11.4	10.2 to 12.8	0.5	4.9	
Diet	11.6	10.7 to 12.5	9.7	8.9 to 10.6	−1.9	−16.2	$P_C < .001$‡ $P_E = .002$§ $P_{D+E} = .07$§
Exercise	11.5	10.6 to 12.5	11.0	10.1 to 11.9	−0.6	−4.9	$P_C = .10$‡ $P_D = .002$§ $P_{D+E} < .001$§
Diet + exercise	11.5	10.6 to 12.5	9.2	8.4 to 10.0	−2.3	−20.3	$P_C < .001$‡ $P_D = .07$§ $P_E < .001$§
Total testosterone, ng/dL							
Control	22.8	20.2 to 25.7	23.2	20.9 to 25.7	0.4	1.8	
Diet	23.9	21.9 to 26.0	23.6	21.6 to 25.8	−0.2	−0.9	$P_C = .40$‡ $P_E = .67$§ $P_{D+E} = .07$§
Exercise	24.8	23.0 to 26.7	23.6	21.6 to 25.7	−1.2	−4.9	$P_C = .24$† $P_D = .67$§ $P_{D+E} = .24$§
Diet + exercise	23.9	22.1 to 25.8	22.5	20.8 to 24.3	−1.4	−5.9	$P_C = .02$‡ $P_D = .07$§ $P_E = .24$§
Androstenedione, ng/dL							
Control	48.7	43.9 to 54.0	49.4	45.4 to 53.7	0.7	1.5	
Diet	51.1	47.1 to 55.3	51.8	47.7 to 56.2	0.7	1.4	$P_C = .83$‡ $P_E = .93$§ $P_{D+E} = .26$§
Exercise	50.2	46.6 to 54.1	49.6	45.6 to 54.0	−0.6	−1.2	$P_C = .75$‡ $P_D = .93$§ $P_{D+E} = .25$§
Diet + exercise	52.6	49.1 to 56.4	50.8	47.1 to 54.7	−1.9	−3.5	$P_C = .22$‡ $P_D = .26$§ $P_E = .25$§
SHBG, nmol/L							
Control	34.7	31.5 to 38.2	33.7	30.3 to 37.5	−1.0	−2.7	
Diet	35.8	33.0 to 38.8	43.8	40.4 to 47.5	8.0	22.4	$P_C < .001$‡ $P_E < .001$§ $P_{D+E} = .48$§
Exercise	39.1	35.9 to 42.6	38.8	35.6 to 42.4	−0.3	−0.7	$P_C = .41$‡ $P_D < .001$§ $P_{D+E} < .001$§
Diet + exercise	34.1	31.9 to 36.4	42.9	40.2 to 45.6	8.8	25.8	$P_C < .001$‡ $P_D = .48$§ $P_E < .001$§
Free estradiol, pg/mL‖							
Control	0.30	0.27 to 0.34	0.33	0.30 to 0.36	0.02	6.3	
Diet	0.31	0.28 to 0.34	0.24	0.22 to 0.27	−0.07	−21.4	$P_C < .001$‡ $P_E < .001$§ $P_{D+E} = .06$§
Exercise	0.30	0.27 to 0.33	0.29	0.26 to 0.32	−0.01	−4.7	$P_C = .08$‡ $P_D < .001$§ $P_{D+E} < .001$§
Diet + exercise	0.32	0.29 to 0.35	0.23	0.21 to 0.26	−0.08	−26.0	$P_C < .001$‡ $P_D = .06$§ $P_E < .001$§

(continued on following page)

Table 3. Change in Sex Hormones by Study Group, Geometric Means, and 95% CI (continued)

Biomarker	Baseline		12 Months				
	Mean	95% CI	Mean	95% CI	Δ*	%Δ	P†
Free testosterone, pg/mL							
Control	4.9	4.4 to 5.6	5.1	4.6 to 5.7	0.13	2.6	
Diet	5.1	4.7 to 5.6	4.6	4.2 to 5.1	−0.51	−10.0	$P_C < .001$‡ $P_E = .02$§ $P_{D+E} = .02$§
Exercise	5.1	4.7 to 5.5	4.9	4.5 to 5.3	−0.23	−4.5	$P_C = .20$‡ $P_D = .02$§ $P_{D+E} < .001$§
Diet + exercise	5.3	4.9 to 5.7	4.5	4.1 to 4.8	−0.82	−15.6	$P_C < .001$‡ $P_D = .02$§ $P_E < .001$§

Abbreviations: SHBG, sex hormone–binding globulin; Δ, change.
*Change at 12 months from baseline.
†*P* values for comparing the change from baseline to 12 months between groups. There are six between-group comparisons ($P < .05/6$; .008 is the critical value).
‡P_C, *P* values for comparing the changes between control group and three intervention groups.
§P_D or P_E, *P* values for comparing the changes between exercise group and diet group; P_{D+E}, *P* values for comparing the changes between diet + exercise group and two other intervention groups (exercise group or diet group).
‖Free estradiol, not calculated for individuals with estradiol below the level of detection (N = 420; n = 79 for control; n = 115 for diet; n = 110 for exercise; n = 116 for diet + exercise groups).

blood values, and 17 patients were excluded from analyses for sex hormone concentrations outside acceptable postmenopausal ranges: follicle-stimulating hormone less than 23.0 IU/L (n = 1), estrogen use (n = 1), serum estradiol ≥ 42 pg/mL (n = 13), total testosterone ≥ 100 ng/dL (n = 1), or SHBG ≥ 180 nmol/L (n = 1). Therefore, 421 women were included in the analyses.

Baseline Data

Groups were balanced on main demographic factors at baseline (Table 1). The mean age of participants was 58 years. The majority were non-Hispanic white (85%) and well-educated (65.4% college graduate or above). Participants had a mean BMI of 30.9 kg/m^2, 47.2% body fat, and VO_2max of 1.90 L/min (indicating mean poor aerobic fitness).

Weight and Body Composition Changes

Weight and body composition results were previously reported.[35] Mean weight changes at 12 months were as follows: diet, −10.8% ($P < .001$); exercise, −3.3% ($P = .02$); diet + exercise, −11.9% ($P < .001$); and controls, −0.6% (Table 2).

Main Hormone Effects

Estrone significantly decreased with diet (−9.6%, $P = .001$) and diet + exercise (−11.1%, $P < .001$), and to a smaller extent with exercise (−5.5%, $P = .01$), versus controls (+8.1%; Table 3). Estradiol decreased significantly with diet (−16.2%, $P < .001$) and diet + exercise (−20.3%, $P < .001$) and minimally with exercise (−4.9%, $P = .10$), versus controls (+4.9%). Total testosterone decreased non-significantly with diet + exercise, (−5.9%, $P = .02$). No changes in androstenedione were noted in any of the groups. SHBG increased with diet (+22.4%) and diet + exercise (+25.8%; both $P < .001$ *v* controls), with no change with exercise (−0.7%, $P = .41$) versus controls (−2.7%). Free estradiol decreased with diet (−21.4%) and diet + exercise (−26.0%) versus controls (+6.3%, both $P < .001$); little change was observed with exercise (−4.7%, $P = .08$). Free testosterone decreased with diet (−10.0%, $P < .001$) and diet + exercise (−15.6%, $P < .001$) versus controls.

No comparisons of diet with diet + exercise groups reached Bonferroni-corrected statistical significance (Table 3). Compared with exercise, diet + exercise produced significantly greater reductions in estradiol, free estradiol, and free testosterone and a larger increase in SHBG (all $P < .001$).

There were greater reductions in fasting insulin[44] and C-reactive protein with diet and diet + exercise (both $P < .001$), compared with control, with no changes in the exercise group (Table 4). A significant reduction in leptin was noted in all intervention groups compared with controls, whereas an increase in adiponectin was only noted in the diet and diet + exercise groups (both $P < .001$).

Subgroup Analyses

Reductions in estrone, estradiol, free estradiol, and free testosterone and increases in SHBG were larger with greater degrees of weight loss (Fig 2 and Data Supplement). Weight loss more than 10% in the diet, but not diet + exercise, group produced markedly greater reductions in estrone (Fig 2A), estradiol (Fig 2B), free estradiol (Fig 2E), and free testosterone (Fig 2F) and greater increase in SHBG (Fig 2D) compared with lower amounts of weight loss. We observed similar results when hormone changes were assessed by fat loss (Data Supplement).

Intervention Adherence Effects

Higher attendance at diet sessions was variably associated with greater reductions in some of the hormones (Data Supplement). Measures of exercise adherence, conversely, were unrelated to hormone changes (Data Supplement).

Adverse Outcomes

Compared with controls (24%), musculoskeletal injuries were reported in 34%, 18%, and 19% of patients , respectively, in the exercise, diet + exercise, and diet groups (all $P > .10$). Total bone

Table 4. Change in Insulin, CRP, Leptin, and Adiponectin by Study Group, Geometric Mean, and 95% CI

Biomarker	Baseline		12 Months		Δ*	%Δ	P†
	Mean	95% CI	Mean	95% CI			
Insulin,‡ μU/mL							
Control	11.99	10.83 to 13.28	11.55	10.38 to 12.85	−0.44	−3.7	
Diet	11.00	9.89 to 12.23	8.12	7.31 to 9.02	−2.87	−26.1	$P_C < .001$§ $P_E < .01$‖ $P_{D+E} = .87$‖
Exercise	10.94	9.97 to 12.0	10.05	9.14 to 11.04	−0.89	−8.2	$P_C = .26$§ $P_D < .01$‖ $P_{D+E} < .001$‖
Diet + exercise	10.67	9.65 to 11.81	7.85	7.07 to 8.71	−2.83	−26.5	$P_C < .001$§ $P_D = .87$‖ $P_E < .001$‖
High-sensitivity CRP,‡ mg/L							
Control	1.89	1.50 to 2.39	1.89	1.48 to 2.42	0.00	−0.2	
Diet	2.60	2.18 to 3.11	1.53	1.28 to 1.82	−1.07	−41.2	$P_C < .001$§ $P_E < .001$‖ $P_{D+E} = .47$‖
Exercise	2.50	2.10 to 2.99	2.23	1.79 to 2.78	−0.27	−10.9	$P_C = .37$§ $P_D < .001$‖ $P_{D+E} < .001$‖
Diet + exercise	2.12	1.79 to 2.52	1.23	0.99 to 1.53	−0.89	−42.1	$P_C < .001$§ $P_D = .47$‖ $P_E < .001$‖
Leptin,¶ ng/mL							
Control	24.85	22.94 to 26.92	24.64	22.23 to 27.30	−0.21	−0.9	
Diet	23.07	21.41 to 24.86	15.71	14.18 to 17.40	−7.37	−31.9	$P_C < .001$§ $P_E < .001$‖ $P_{D+E} = .001$‖
Exercise	23.50	21.73 to 25.41	20.34	18.52 to 22.33	−3.16	−13.4	$P_C < .01$§ $P_D < .001$‖ $P_{D+E} < .001$‖
Diet + exercise	23.75	22.02 to 25.63	13.77	12.40 to 15.30	−9.98	−42.0	$P_C < .001$§ $P_D = .001$‖ $P_E < .001$‖
Adiponectin,¶ μg/mL							
Control	12.78	11.70 to 13.96	12.44	11.36 to 13.64	−0.34	−2.6	
Diet	12.36	11.37 to 13.44	13.46	12.41 to 14.60	1.10	8.9	$P_C < .001$§ $P_E < .001$‖ $P_{D+E} = .34$‖
Exercise	12.46	11.47 to 13.54	12.09	11.03 to 13.25	−0.38	−3.0	$P_C = .86$§ $P_D < .001$‖ $P_{D+E} < .01$‖
Diet + exercise	12.76	11.72 to 13.88	14.00	12.89 to 15.20	1.24	9.7	$P_C < .001$§ $P_D = .34$‖ $P_E < .01$‖

Abbreviations: CRP, C-reactive protein; Δ, change.
*Change at 12 months from baseline.
†*P* values for comparing the change from baseline to 12 months between groups.
‡For insulin and CRP, N = 406 (control, n = 79; diet, n = 105; exercise, n = 106; diet + exercise, n = 108).
§P_C, *P* values for comparing the changes between control group and three intervention groups.
‖P_D or P_E, *P* values for comparing the changes between exercise group and diet group; P_{D+E}, *P* values for comparing the changes between diet + exercise group and two other intervention groups (exercise group or diet group).
¶For adiponectin and leptin, N = 399 (control, n = 79; diet, n = 105; exercise, n = 106; diet + exercise, n = 109).

mineral density was reduced in all intervention groups: diet, −1.2% ($P < .001$); exercise, −0.8% ($P = .03$); and diet + exercise, −1.7% ($P < .001$) versus no change in controls. Hot flash number or severity did not change differently in the four arms.

DISCUSSION

A 12-month reduced-calorie weight loss, with or without exercise, produced large and statistically significant reductions in postmenopausal serum estrone, estradiol, free estradiol, and free testosterone and increases in SHBG. The weight loss interventions also significantly reduced insulin, C-reactive protein, and leptin and increased adiponectin. Exercise had little effect on sex hormones or the other potential breast cancer biomarkers.

Elevated blood estrogen and testosterone concentrations have consistently been associated with increased breast cancer risk, with doubling or greater effect on risk in women in the highest versus lowest quartiles or quintiles in prospective cohort studies.[5,7,52,53] In one of these, Woolcott et al[7] measured sex hormones in the same laboratory as for the present study and found that a doubling of free estradiol raised breast cancer risk by a factor of 2.26. Therefore, the

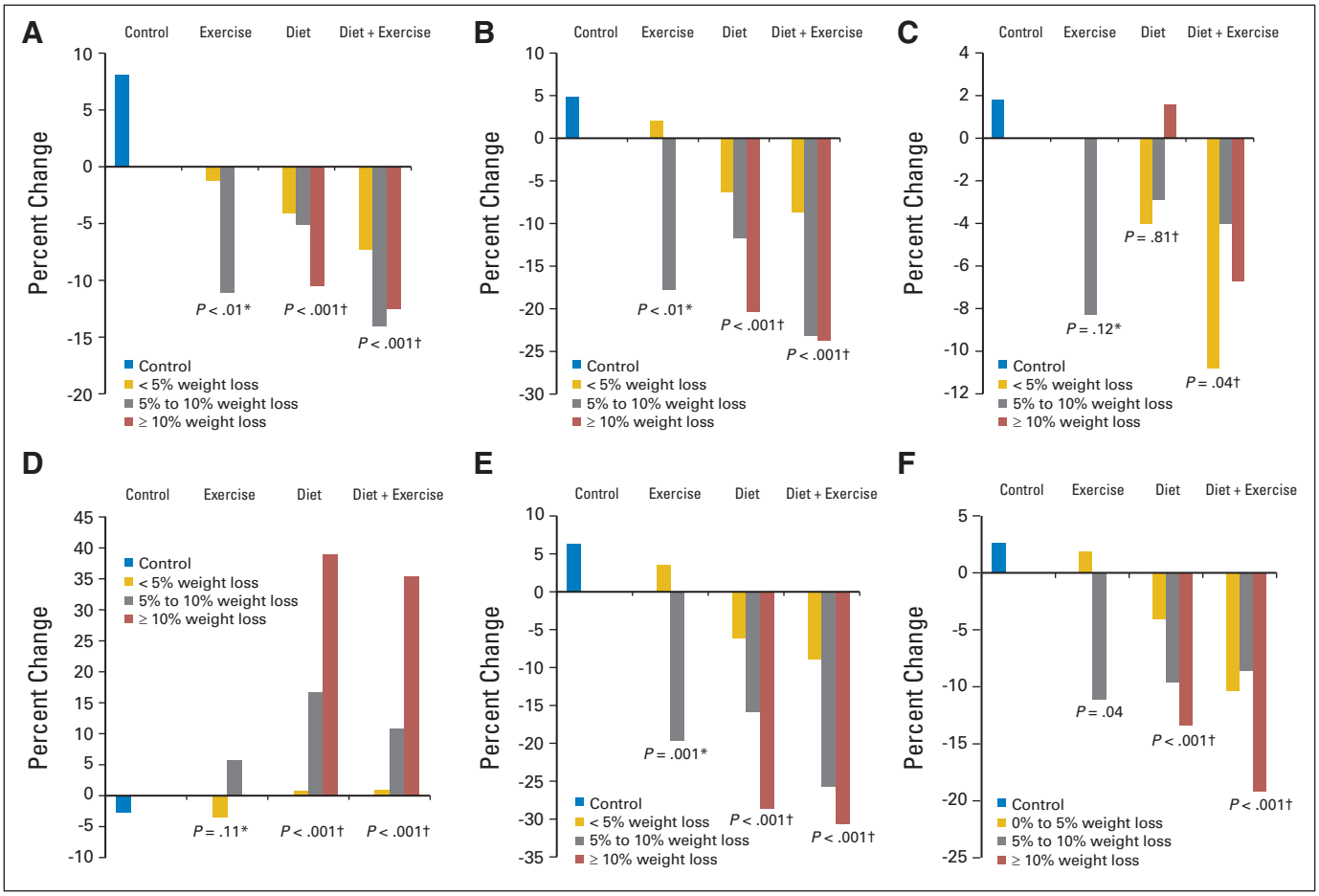

Fig 2. Change in hormone levels by weight loss category. (A) Estrone; (B) estradiol; (C) total testosterone; (D) sex hormone–binding globulin; (E) free estradiol; (F) free testosterone. (*) Testing for a trend in change from baseline to 12 months in hormones from controls through weight loss < 5% and ≥ 5% (for exercise only). For exercise: n = 80 for control, n = 72 for weight loss < 5%, n = 27 for weight loss ≥ 5%. (†) Testing for a trend in change from baseline to 12 months in hormones from controls through weight loss ≥ 10%. For diet: n = 80 for control, n = 28 for weight loss < 5%, n = 27 for weight loss 5% to 10%, n = 46 for weight loss ≥ 10%. For diet + exercise: n = 80 for control, n = 18 for weight loss < 5%; n = 21 for weight loss 5% to 10%; n = 69 for weight loss ≥ 10%. Statistical significance *P* value < .05.

30% drop in free estradiol we observed with ≥ 5% of weight loss (achieved by 78% and 65% of the diet + exercise and diet groups, respectively) could be associated with a 22% decrease in breast cancer risk. Our participants' mean baseline estradiol concentration is in the top estradiol quartile range for the Woolcott study, and the diet and diet + exercise groups' mean 12-month values fall into the third highest quartile range. Because the odds ratio for breast cancer decreased from 6.4 to 2 in the fourth and third quartiles in that study, respectively, our weight loss intervention's decrease in mean estradiol could represent a ≥ 50% reduction in breast cancer risk. However, the results of these prospective cohort studies are based on a one-time sample, rather than serial samples. Therefore, our study suggests that a modest degree of weight loss could have a powerful effect on breast cancer risk; however, the impact of a reduction in sex hormones on breast cancer risk reduction is still unknown.

Low-fat dietary interventions without weight loss have reported either no or small change in estrogens.[22-24,26,27,29,54] Two previous randomized controlled trials in postmenopausal women found modest reductions of 2% to 14% in estrogens after 1-year aerobic exercise interventions.[19,20] In one of these, those exercisers who reduced percent body fat by more than 2% (mean absolute value) experienced a 15% decline in estradiol.[19] A third 1-year randomized controlled trial found a significant lowering of testosterone in postmenopausal women randomly assigned to exercise who lost more than 2% body fat.[21] In the present study, weight loss ≥ 5% was associated with significantly greater reductions in estradiol, free estradiol, and free testosterone and significantly increased SHBG. Taken together, these findings suggest that weight loss is the key factor linking alterations in diet or exercise to sex hormone changes. The effect of weight loss on estrogens may occur through a reduction in adipose tissue aromatase levels.[10,55] In addition to being the first study to examine the effect of weight loss on sex hormones in postmenopausal women, the Nutrition and Exercise for Women Trial achieved greater adherence to a higher exercise goal and greater weight loss than the original Diabetes Prevention Program lifestyle intervention.[36]

The lack of effect of exercise alone does not agree with epidemiologic studies in which physical activity is associated with decreased risk of breast cancer.[3,56] Therefore, exercise could play a role in reducing risk of postmenopausal breast cancer though different biologic mechanisms than were examined in this study. Exercise may also play a role in reducing breast cancer risk by augmenting dietary weight loss[57] and maintenance,[58] which will be critical for long-term risk reduction.[1]

Strengths of our study include a large sample size and long duration with excellent adherence and low attrition. The weight loss intervention was a group-based modification of the Diabetes Prevention Program intervention,[36] which has publically available materials that have been tested in a variety of populations.[59-63] This suggests that successful replication without the high costs of individually delivered weight loss programs is feasible. The exercise intervention, which consisted primarily of brisk walking, should be easily adoptable by most women in a clinical or community setting. However, the effects of these dietary weight loss and exercise interventions on breast cancer incidence are unknown.

Our study had some limitations. We tested only one dietary weight loss and one exercise intervention and cannot speculate on effects of other weight loss or exercise modalities. The study population was primarily non-Hispanic whites, and intervention effects in women from other race or ethnic groups cannot be inferred. Furthermore, the trial did not test whether weight loss or exercise reduced incidence of breast cancer, which would require a trial with a much larger scope.[64]

Total bone mineral density declined in all intervention groups, although the clinical significance of the change in total bone density is not defined.[65] Future weight loss studies should consider the inclusion of resistance exercise to avoid loss of bone mass.[65-68] The exercise group reported more musculoskeletal injuries than the diet and diet + exercise groups, which suggests that weight loss protects women from exercise-induced injury.[69,70]

The results of this study could be relevant even to women who choose to use breast cancer chemoprevention. Tamoxifen may have a lower breast cancer risk reduction effect in obese than normal weight women.[71] Aromatase inhibitors, which have been found to reduce risk of new[72] or recurrent breast cancer, had lower effectiveness in obese versus normal-weight patients in some,[73] but not other,[72,74] trials. Conditions that are increased with some of these agents, such as deep vein thrombosis and endometrial cancer, may be increased to a greater extent in obese than normal-weight women.[71,75] Therefore, even for women who chose treatment with these agents, weight reduction if overweight or obese may be beneficial. Furthermore, although recent reports indicate that tamoxifen and raloxifene treatment confers long-term (10+ years) protection against breast cancer risk,[76-80] the long-term efficacy of aromatase inhibitors on breast cancer risk reduction has not yet been established.[72] Weight loss in overweight or obese women therefore represents an additional option for long-term breast cancer risk reduction.

In summary, a moderate degree of weight loss achieved through a reduced-calorie weight loss diet intervention reduced serum concentrations of estrogens, free testosterone, and other potential breast cancer biomarkers in overweight or obese postmenopausal women. These results have implications for the significant majority of postmenopausal women who are overweight or obese[81,82] and therefore at elevated risk for breast cancer incidence[2] and mortality.[83]

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: None **Consultant or Advisory Role:** Anne McTiernan, Merck (C), Metagenics (C), Novartis (C), Proctor & Gamble (C), ZymoGenetics (C) **Stock Ownership:** Anne McTiernan, Merck **Honoraria:** Anne McTiernan, Novartis, Wyeth **Research Funding:** Anne McTiernan, Wyeth **Expert Testimony:** None **Other Remuneration:** None

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