Effects of Postmenopausal Hormone Replacement Therapy on Central Abdominal Fat, Glycemic Control, Lipid Metabolism, and Vascular Factors in Type 2 Diabetes

A prospective study

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OBJECTIVE — To examine the effects of hormone replacement therapy (HRT) on lipid metabolism, glycemic control, total body and central abdominal fat, blood pressure (BP), and arterial pulse wave velocity (APWV) in overweight postmenopausal females with type 2 diabetes.

RESEARCH DESIGN AND METHODS — This was a 12-month prospective study of 14 subjects (mean ± SD age 57.5 ± 5.6 years, BMI 29.5 ± 4.8 kg/m^2) randomized to 6 months of observation or HRT before crossover. HRT consisted of 2 months of conjugated equine estrogen (CEE) 0.625 mg daily, followed by 4 months CEE and medroxyprogesterone 5 mg daily. Measures included anthropometry, fasting glucose, insulin, HbA_{1c}, total and HDL cholesterol, triglycerides, apolipoprotein B, LDL particle size, nonesterified fatty acids (NEFA), sex hormone–binding globulin, resting energy expenditure (REE), total and central abdominal fat (by dual-energy X-ray absorptiometry), resting BP, APWV (by applanation tonometry), physical activity, well-being, and sexual function.

RESULTS — Six months of HRT resulted in significant reductions in waist-to-hip ratio (−0.03 ± 0.01 vs. 0.01 ± 0.009, $P = 0.007$), HbA_{1c} (−0.34 ± 0.24 vs. 0.6 ± 0.4%, $P = 0.04$), total cholesterol (−0.6 ± 0.1 vs. 0.2 ± 0.2 mmol/l, $P = 0.001$), central abdominal fat (−175 ± 51 vs. −24 ± 56 g, $P = 0.05$), and improved physical functioning ($P = 0.05$), compared with observation. There was a minor increase in REE with HRT (33 ± 23 vs. −38 ± 23 kJ/day, $P = 0.04$). Total fat mass, fasting glucose, insulin, triglyceride, apolipoprotein B, NEFA, resting BP, APWV, and physical activity were unchanged.

CONCLUSIONS — Postmenopausal HRT in these overweight women with type 2 diabetes was associated with a reduction in central adiposity and improvement in lipid metabolism and glycemic control without deterioration in weight status or cardiovascular parameters measured. Whether HRT-induced improvements in these cardiovascular risk factors result in lower long-term cardiovascular morbidity and mortality, as observed in nondiabetic women, awaits further study.

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Central abdominal obesity relates strongly to insulin resistance (1,2) and predicts the development of type 2 diabetes (3) and cardiovascular disease (4) in women. The menopause is associated with increases in central abdominal adiposity (5,6) and hormone replacement therapy (HRT) attenuates this gain in nondiabetic women (7). However, prospective studies with accurate body composition assessment in diabetic women are lacking. We have previously reported lower total and central abdominal fat in HRT users in a large population of Caucasian women where body composition was measured directly using dual-energy X-ray absorptiometry (DEXA) (8). A co-twin case control analysis in monozygotic twin pairs performed as part of this study (to control for genetic and other environmental factors) found that within each twin pair the HRT-using twin had lower central abdominal fat (8). The reduced cardiovascular risk observed with HRT use in nondiabetic women (9–12) may, in part, be explained by lower central abdominal fat.

Type 2 diabetes is a strong risk factor for cardiovascular disease. In fact, the relative risk of heart disease in women with diabetes may be higher than that for men with diabetes (13). The mechanisms accounting for this may include concomitant disturbances in carbohydrate and lipid metabolism, vascular dysfunction, hypertension, altered hemostasis, and generalized and/or central abdominal obesity.

Despite suggestions that women with type 2 diabetes are a special risk group that may benefit from HRT-associated cardio-

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Abbreviations: ANOVA, analysis of variance; APWV, arterial pulse wave velocity; BP, blood pressure; CEE, conjugated equine estrogen; CV, coefficient of variation; DEXA, dual-energy X-ray absorptiometry; HRT, hormone replacement therapy; NEFA, nonesterified fatty acids; REE, resting energy expenditure; SF-36, Short Form 36; SHBG, sex hormone–binding globulin.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

protection (14,15), the prevalence of HRT use in such women is less than half that in nondiabetic women (16,17). In addition, few studies have specifically evaluated the effects of HRT in this high-risk group. Two studies found lipid effects quantitatively similar to those in nondiabetic women: decreased LDL and increased HDL cholesterol (18,19) and, of clinical relevance in type 2 diabetes, improvements in glycemic control (19,20) and fibrinolysis (20). These studies are limited, however, by relatively short intervention (6–12 weeks only), no or minimal progestin use, and lack of detailed evaluation of body composition, energy expenditure, or vascular function.

The postmenopausal woman with type 2 diabetes represents a considerable clinical challenge in risk factor management and primary and secondary cardiac prevention. Therefore, the objectives of this study were to examine the early and longer-term effects of hormone replacement on possible risk factors for cardiovascular disease: lipid metabolism, glycemic control, blood pressure, vascular distensibility, and total and central abdominal adiposity.

RESEARCH DESIGN AND METHODS

— Subjects (n = 14) were recruited through a teaching hospital diabetes outpatient clinic or media advertising. Menopause was defined as at least 12 months' amenorrhea. Treatment regimens included diet and exercise alone (n = 4), additional metformin (n = 2), sulfonylureas (n = 3), and combined sulfonylurea/metformin (n = 5). Seven subjects had treated hypertension and received ACE inhibitors (n = 6) or prazosin (n = 1). Exclusion criteria included menopause duration of >10 years, HRT in the preceding 2 years, documented cardiac disease, weight loss >3 kg in the preceding 6 months, postural drop in blood pressure (BP) >30 mmHg, symptoms of autonomic neuropathy, fasting triglycerides >4.0 mmol/l, vitamin supplementation, or any severe concomitant illness.

Subjects were randomized to either 6 months of observation or 6 months of open-label HRT. During the observation period, subjects were studied at 0 and 6 months. During the HRT arm, subjects received conjugated equine estrogen (CEE) 0.625 mg daily for 2 months, followed by 4 months of CEE 0.625 mg and medroxyprogesterone 5 mg daily, and were studied before commencing estrogen, after 2 months of estrogen alone, and after 4 months of combined therapy. Subjects crossed over at 6 months. All subjects completed the HRT arm of the study. Two did not complete the observation period: one subject died of an acute myocardial infarction; the other subject did not attend the final follow-up visit. Compliance with HRT was confirmed by tablet count. Subjects were asked to maintain their usual dietary intake and physical activity level and no advice was given regarding either.

A single investigator (K.S.) performed the following anthropometric measurements: weight to the nearest 0.1 kg with the subject in light street clothing; height to the nearest 0.01 m using a stadiometer with the subject barefoot; waist circumference to the nearest 0.01 m at the narrowest point between the ribcage and anterior superior iliac crest; and hip circumference at the widest point between the anterior superior iliac crest and greater trochanter. BMI (weight · height^{-1} · height^{-1} [kg/m^2]) and waist-to-hip ratio were calculated.

Body composition was measured by DEXA (Lunar DPXL, Madison, WI) at the beginning and end of each study arm. Total body fat and fat-free mass were measured directly using the standard software calculation. Central abdominal fat mass was measured by scan reanalysis by a trained technician, who was blinded to whether subjects were in the intervention or observation arm, as described in detail elsewhere (21,22).

All blood specimens were collected between 0800 and 0900 following a 10-h overnight fast. Total cholesterol was measured by adaptation of the cholesterol oxidase/peroxidase method; HDL cholesterol was separated using the phosphotungstic acid/Mg^{2+} method and measured by the oxidase/peroxidase method; triglycerides by the lipase/glycerol kinase method (Boehringer Mannheim, Mannheim, Germany); glucose by the hexokinase method (Boehringer Mannheim); HbA_{1c} by high-performance liquid chromatography (Pharmacia, Uppsala, Sweden); sex hormone–binding globulin (SHBG) by immunoradiometric assay (Orion Diagnostica, Espoo, Finland) (female reference range: 15–140 mmol/l); insulin by an in-house double-antibody radioimmunoassay (intra-assay coefficient of variation [CV] = 6%; interassay CV = 7% at 5mU/l); nonesterified fatty acids by acyl-CoA oxidase–based calorimetric kit (Wako, Osaka, Japan); apolipoprotein B by immunonephelometry (Behring nephelometer) with commercially available antibodies (Behring; Behringwerke, Marburg, Germany). The intra-assay CV for the nephelometric determinations was 4%. LDL was estimated using the Friedewald equation (LDL = total cholesterol – HDL – triglycerides/2.2).

The Stokes' diameter (nm) of LDL cholesterol particles was determined by gel electrophoresis with 3–13% gradient gels (Gradipore, Sydney, Australia). For each gel, a complete set of plasma samples from each subject was brought to room temperature from storage at −70° C. Prior testing of stored plasma after one freeze/thaw cycle showed minimal change to LDL Stokes' diameter in both our laboratory and elsewhere (23). Plasma (30 µl) was mixed with a 40% solution of sucrose and 0.01% bromophenol blue (10 µl) and loaded (10 µl) onto an inner lane of the gel. Reference proteins (thyroglobulin radius 8.5 nm) (Pharmacia high-molecular-weight proteins) and a lane of calibrated latex beads (radius 19 nm) (Duke Scientific, Palo Alto, CA) were included at each end. Electrophoresis was performed as described previously (24) except that fresh buffer was used for each electrophoretic run. Gels were cut to isolate the standards from plasma samples and fixed in 10% sulphosalicylic acid. The gel containing plasma was stained for lipid by overnight incubation in Sudan black B (0.1% in 60% ethanol), washed, and rehydrated in water. Reference markers were stained for protein. Gels were reassembled, scanned (633 nm) with an LKB Ultroscan XL laser densitometer (LBK-Produkter AB, Bromma, Sweden), and Stokes' diameter estimated from a log linear regression equation generated from two sets of standards that migrated either side of LDL. The between-run CV of an internal standard was 4.3%.

Resting energy expenditure (REE) was measured in the fasted state by indirect calorimetry (Deltatrac Metabolic Monitor; Datex Instrumentarium, Helsinki, Finland). The subject rested in the recumbent position for at least 20 min before any measurements. Readings were taken for 30 min and all measurements during the first 10 min were excluded from calculations. The respiratory quotient was defined as the ratio of carbon dioxide production to oxygen consumption.

BP control was measured by a mercury sphygmomanometer with the subject supine after a 5-min rest. Arterial pulse wave velocity (APWV), an index of arterial distensibility, was measured along the aorto-femoral segment by paired pressure waveforms

Table 1—*Effects of CEE alone or combined with medroxyprogesterone on characteristics in overweight female subjects with type 2 diabetes*

	HRT			Observation	
	Baseline	2 months (CEE)	6 months (CEE + MP)	Baseline	6 months
Weight (kg)	78.6 ± 3.3	78.7 ± 3.4	78.0 ± 3.0	78.3 ± 3.3	76.6 ± 3.3
BMI (kg/m²)	29.7 ± 1.3	29.7 ± 1.3	29.5 ± 1.1	29.5 ± 1.3	29.1 ± 1.3
Waist (m)	0.95 ± 0.03	0.95 ± 0.03	0.93 ± 0.02*†	0.94 ± 0.03	0.92 ± 0.03
Waist-to-hip ratio	0.86 ± 0.02	0.85 ± 0.01	0.83 ± 0.01*†	0.84 ± 0.01	0.85 ± 0.02
Total fat mass (kg)	34.50 ± 2.19	—	33.82 ± 2.02	34.19 ± 2.24	33.05 ± 2.15
Total fat (%)	45.5 ± 1.4	—	45.0 ± 1.3	45.0 ± 1.4	44.7 ± 1.6
Trunk fat (kg)	16.75 ± 0.82	—	16.22 ± 0.74	16.74 ± 0.78	16.3 ± 0.95
Trunk fat (%)	44.3 ± 0.9	—	43.8 ± 1.0	44.3 ± 1.0	43.8 ± 1.2
Central fat mass (kg)	2.38 ± 0.14	—	2.20 ± 0.09*	2.35 ± 0.12	2.25 ± 0.14
Central fat (%)	45.2 ± 1.0	—	43.5 ± 1.0*	45.2 ± 1.2	44.4 ± 1.4
Fat-free mass (kg)	40.85 ± 1.55	—	40.98 ± 1.55	41.16 ± 1.6	40.37 ± 1.78
Systolic BP (mmHg)	148 ± 4	147 ± 6	143 ± 4	157 ± 7	141 ± 5
Diastolic BP (mmHg)	85 ± 2	84 ± 2	82 ± 1	84 ± 3	81 ± 2
APWV (m/s)	10.32 ± 0.33	10.02 ± 0.56	10.31 ± 0.43	10.19 ± 0.38	10.35 ± 0.37
Activity score (metabolic units)	159.4 ± 8.7	171.9 ± 12.9	161.1 ± 11.24	163.6 ± 13.8	162.9 ± 9.4

Data are means ± SEM. Comparisons are by repeated-measures ANOVA. MP, medroxyprogesterone. *$P < 0.01$, HRT 0 vs. 6 months; †$P < 0.05$, HRT 2 vs. 6 months.

obtained by applanation tonometry, as described elsewhere (25).

Physical activity levels were measured using a standardized, validated questionnaire (26) that quantified time spent in performing different activities at home, work, and in leisure during the previous week. Activities were classified into four categories (sedentary, mild, moderate, and heavy exertion) and an activity score in metabolic units (METS) was determined for each subject using a standardized formula (26).

The perceived level of well-being was assessed using a standardized, validated questionnaire, the Short Form 36 (SF-36) (27), which assesses a number of variables, including physical functioning, general health, bodily pain, vitality, social functioning, and mental health.

Sexual function was evaluated by a standardized questionnaire examining the level of sexual desire, vaginal lubrication, frequency of intercourse, discomfort during intercourse, difficulty in arousal or achieving orgasm, frequency of orgasm, and dislike of sexual intercourse.

The effect of HRT was examined by repeated-measures analysis of variance (ANOVA). Changes occurring during observation and HRT were also compared by paired Student's *t* test. To ensure that negative results were not a result of sample size or skewed data distribution, analyses were confirmed by nonparametric tests. The relationships between changes in parameters were determined using simple linear regression analysis.

RESULTS — Baseline characteristics are given in Tables 1 and 2. The mean (± SD) age was 57.5 ± 5.6 years and the majority of subjects were overweight or obese (BMI 29.7 ± 4.8 kg/m², range 23.1–39.2), with body fat 45.0 ± 5.3% (range 37.0–53.7%). The mean duration of menopause was 4.4 ± 3.3 years (1–10); the duration of documented type 2 diabetes was 5 ± 5 years (0.5–16).

Metabolic, body composition, and cardiovascular parameters

HRT induced significant reductions in waist circumference, waist-to-hip ratio, HbA_{1c}, total and LDL cholesterol, and central abdominal fat (Tables 1 and 2). Decrements in HbA_{1c} from baseline were significant after 2 months of CEE alone, but not after combined therapy (Table 2). NEFA levels increased with CEE alone and returned to baseline values after combined therapy (Table 2). SHBG levels increased significantly after 2 months of CEE and remained elevated after 4 months of combined therapy. There was a significant increase in fasting insulin levels after 4 months of combined therapy (Table 2). BMI, total and truncal fat, fasting glucose, resting energy expenditure, HDL cholesterol, triglycerides, LDL particle size, apolipoprotein B, and physical activity level were unchanged (Tables 1 and 2). There was no deterioration in systolic or diastolic BP control during HRT (CEE alone or combined therapy) (Table 1). APWV did not change during HRT or observation (Table 1). There were no significant changes in any measured parameter during the 6-month observation phase except for a minor decrease in respiratory quotient (Tables 1 and 2).

Using simple linear regression analysis, decreases in BMI during HRT related significantly to changes in waist circumference ($r^2 = 0.48$, $P = 0.006$) and total cholesterol ($r^2 = 0.42$, $P = 0.01$). Changes in physical activity level did not relate to the improvements in HbA_{1c} ($P = 0.53$) or central adiposity ($P = 0.78$).

The changes in parameters between the observation and HRT (6–0 months) were compared. Reductions in HbA_{1c} were found during the HRT phase compared with increases during observation (−0.34 ± 0.24 vs. 0.6 ± 0.4%, respectively, $P = 0.04$). Similar results were found for total cholesterol (−0.6 ± 0.1 vs. 0.2 ± 0.2 mmol/l, $P = 0.001$), LDL cholesterol (−0.8 ± 0.2 vs. 0.2 ± 0.2 mmol/l, $P = 0.002$), REE (33 ± 23 vs. −38 ± 23 kJ/day, $P = 0.04$), and waist-to-hip ratio (−0.03 ± 0.01 vs. 0.01 ± 0.01, $P = 0.007$). The decrease in central abdominal fat was greater during the HRT phase than during observation (−175 ± 51 vs. −24 ± 56 g, respectively, $P = 0.05$). SHBG increased during HRT and decreased during observation (42.08 ± 5.60 vs. −10.28 ± 5.07 mmol/l, respectively, $P < 0.0001$).

Well-being and sexual functioning

The mean scores for the eight aspects of well-being tested by the SF-36 are presented in Table 3. The perceived level of

Table 2—*Effects of CEE alone or combined with medroxyprogesterone on metabolic and lipid characteristics in overweight female subjects with type 2 diabetes*

	HRT			Observation	
	Baseline	2 months (CEE)	6 months (CEE + MP)	Baseline	6 months
HbA_{1c} (%)	8.6 ± 0.5	8.0 ± 0.4*	8.2 ± 0.4	8.3 ± 0.5	9.0 ± 0.5
Fructosamine	316 ± 20	310 ± 22	310 ± 15	313 ± 16	323 ± 19
Glucose (mmol/l)	10.7 ± 1.3	9.4 ± 0.8	9.7 ± 0.9	10.5 ± 0.8	11.2 ± 1.5
Insulin (mIU/l)	17.8 ± 2.3	15.8 ± 1.9	18.5 ± 2.5†	18.3 ± 2.4	18.0 ± 2.8
Total cholesterol (mmol/l)	5.4 ± 0.2	4.8 ± 0.2*	4.8 ± 0.2‡	5.2 ± 0.3	5.2 ± 0.3
LDL cholesterol (mmol/l)	3.5 ± 0.2	2.8 ± 0.3*	2.6 ± 0.2§	3.2 ± 0.3	3.3 ± 0.3
HDL cholesterol (mmol/l)	1.2 ± 0.07	1.4 ± 0.1	1.3 ± 0.08	1.3 ± 0.1	1.2 ± 0.1
Triglycerides (mmol/l)	1.55 ± 0.15	1.76 ± 0.14	1.9 ± 0.16	1.54 ± 0.15	1.64 ± 0.16
Apolipoprotein B	1.03 ± 0.05	0.98 ± 0.06	1.0 ± 0.07	1.0 ± 0.5	1.06 ± 0.05
LDL particle size (nm)	28.7 ± 0.7	28.8 ± 0.7	28.8 ± 0.6	28.8 ± 0.6	28.6 ± 0.6
NEFA	0.66 ± 0.07	0.82 ± 0.07*	0.65 ± 0.07	0.53 ± 0.05	0.64 ± 0.08
SHBG	24.69 ± 3.21	80.46 ± 8.02*	66.77 ± 7.06†§	38.39 ± 7.34	26.11 ± 3.70
REE (kcal/day)	1,492 ± 53	1,513 ± 51	1,525 ± 48	1,491 ± 50	1,446 ± 54
Respiratory quotient	0.79 ± 0.01	0.79 ± 0.01	0.80 ± 0.01	0.81 ± 0.01	0.79 ± 0.01‖

Data are means ± SEM. Comparisons are by repeated-measures ANOVA. MP, medroxyprogesterone. *$P < 0.05$, HRT 2 vs. 0 months; †$P < 0.05$, HRT 6 vs. 2 months; ‡$P < 0.01$, HRT 6 vs. 0 months; §$P < 0.001$, HRT 6 vs. 0 months; ‖$P < 0.05$, observation 6 vs. 0 months.

physical limitation was greater in this cohort of overweight women with type 2 diabetes compared with published normative data for women of a similar age-group (27).

Scores improved significantly with HRT for several aspects of well-being: physical functioning vitality, mental health and general health perceptions (Table 3). Compared with changes during observation, HRT was associated with significant increases in vitality and energy scores (10 ± 5 vs. 3 ± 4, $P = 0.05$). Changes in well-being scores did not relate to changes in physical activity scores nor to changes in metabolic or body composition parameters measured (data not shown).

Nine subjects (64%) were sexually inactive. Four subjects (28%) reported increased sexual desire, two (14%) increased vaginal lubrication, and three (21%) less discomfort during sexual intercourse during HRT intervention.

CONCLUSIONS — Epidemiological studies of nondiabetic women have associated postmenopausal HRT use with substantial reductions in cardiovascular morbidity and mortality (9–12). Effects of HRT on known cardiovascular risks factors account for the large proportion of this reduction: reduced total cholesterol and increased HDL cholesterol (28–32), improved vascular or endothelial function (33), and fibrinolysis (34–36). In women with established ischemic heart disease, HRT reduces long-term mortality (37). In intervention studies, HRT potentiates endothelium-dependent and -independent vasodilation (38), prolongs time to exercise-induced ischemia in women with proven coronary disease (39), and improves outcomes following coronary angioplasty (40) and coronary artery bypass grafting (41).

Whether women with type 2 diabetes, with a markedly increased risk for cardiac disease, receive the same or lesser degree of cardioprotection or risk reduction with HRT is not yet known.

This is the first study to assess a large range of immediate and longer-term effects of combined HRT in women with type 2 diabetes. Other studies are limited by their cross-sectional nature (42) or by a short

Table 3—*Effects of CEE alone or combined with medroxyprogesterone on aspects of well-being in overweight female subjects with type 2 diabetes*

	HRT			Observation	
	Baseline	2 months (CEE)	6 months (CEE + MP)	Baseline	6 months
Physical functioning	73 ± 28	68 ± 31	78 ± 19*	73 ± 28	76 ± 26
Social functioning	77 ± 29	81 ± 23	86 ± 16	74 ± 34	79 ± 28
Role limitations					
Physical	60 ± 46	56 ± 47	81 ± 32*†	73 ± 44	67 ± 42
Emotional	75 ± 40	67 ± 45	89 ± 30*	86 ± 33	75 ± 40
Mental health	74 ± 19	77 ± 17	84 ± 13†	78 ± 18	77 ± 17
Vitality/energy	56 ± 27	57 ± 25	68 ± 18*‡	62 ± 29	60 ± 27
Body pain	71 ± 28	79 ± 23	85 ± 19†	81 ± 22	83 ± 10
General health perceptions	68 ± 24	65 ± 24	72 ± 20*	64 ± 24	71 ± 23

Data are scores for well-being measured by SF-36 (27), based on a maximum score of 100 for each aspect of well-being. A total of 12 subjects completed the observation period, and 14 completed HRT. MP, medroxyprogesterone. *$P < 0.05$, HRT 6 vs. 2 months; †$P < 0.05$, ‡$P < 0.01$, HRT 6 vs. 0 months.

duration of therapy and lack of progestin therapy (18–20). Cross-sectional data comparing lipids in women with and without diabetes suggest that HRT may have a lesser effect on HDL and accentuate hypertriglyceridemia (42) in diabetes. Two short-term (6–12 weeks) intervention studies have found quantitative improvements in lipids (18,19). In the 6-month intervention arm of our 12-month crossover study, we found slightly greater decrements in total cholesterol than found in other studies: 0.6 mmol/l by 24 weeks, compared with 0.3 mmol/l at 6 weeks (18) and 0.5 mmol/l by 12 weeks (19). The increment in HDL cholesterol in our study was similar to that reported elsewhere (18,19). These differences may be due to differences in HRT regimens: the latter studies either used 17β-estradiol alone for 6 weeks (18) or added norethisterone in the last 16 days of 12 weeks (19). In contrast, our study used CEE alone for 8 weeks and in combination with medroxyprogesterone for an additional 4 months. Our results were similar to those of the Prospective Estrogen Progestin Investigation Trial, which found the HDL increment to be lower with combination therapy than with CEE alone (31). Whether use of lower medroxyprogesterone doses (such as 2.5 mg) allows maintenance of the initial gain in HDL is not known. Nevertheless, our study and others (18,19) confirm that HRT induces HDL increments of a magnitude similar to those observed in nondiabetic women (28,31). The lack of statistical significance of the HDL increment at 6 months may be due, in part, to small sample size, but may also be due to diabetes per se attenuating HRT effects on hepatic lipid metabolism. No change in LDL particle size was found after 6 months of HRT, as with short-term estradiol intervention (18).

In this study there was a nonsignificant rise in triglycerides of a similar magnitude to other studies in women with diabetes (19). The clinical significance of the estrogen-induced triglyceride increase is not definite and is suggested to be of lesser atherogenic potential, especially if accompanied by rises in HDL levels (32). Nevertheless, prudent clinical practice would include initial triglyceride measurement in diabetic women. Severe hypertriglyceridemia would preclude HRT use; however, those with preexisting hypertriglyceridemia could receive triglyceride-lowering therapy before the commencement of HRT with careful monitoring of triglycerides thereafter.

Importantly, our study found that 6 months of combined estrogen/progestin therapy induced significant improvements in glycemic control with an overall reduction in HbA_{1c}, in contrast to deterioration during observation, consistent with other studies (19,20). This improvement did not relate to change in weight, fat mass, or physical activity level.

No deterioration in resting BP was found, as in other studies (19). Our study also found HRT did not alter pulse wave velocity, an aspect of vascular distensibility and a marker of vascular stiffness, consistent with previous short-term studies in nondiabetic women (43).

There has been much controversy regarding weight change and HRT, despite several prospective studies reporting no excess of weight gain with HRT (31,44,45) (albeit relying on surrogate measures of adiposity such as BMI). Few studies have evaluated the effects of hormone replacement using direct measures of body composition. BMI is unable to quantitate changes in fat mass when accompanied by concomitant alterations in lean tissue. Prospective studies of the menopause have found increments in fat mass and decrements in lean tissue (directly measured by DEXA) with no significant change in weight (5). Poehlman's study (5) also demonstrated increased central abdominal fat during the menopause (using waist-to-hip ratio) and a 2-year controlled study reported HRT-induced attenuation of the increase in central abdominal fat (7). In our study, central abdominal fat decreased with 6 months of HRT with no change in total body fat mass, an important finding in this overweight and obese population, particularly given the reluctance to prescribe HRT in the overweight, and patient and clinician beliefs that HRT causes weight gain (46). In the absence of any change in total fat mass, our findings suggest HRT induces a redistribution of adipose tissue from the central abdominal region to estrogen-responsive peripheral sites, such as the gluteofemoral region (47). Lipid deposition in this region is facilitated by estrogen-stimulated upregulation of local lipoprotein lipase activity (48). The partitioning of lipid away from the central abdominal region (characterized by high rates of lipolysis and fatty acid release) to the gluteofemoral region (with low rates of lipolysis and fatty acid turnover) may explain the improvement in glycemic control observed in this and other prospective studies of HRT in women with diabetes (19,20). Reduced hepatic glucose production with estrogen in normotriglyceridemic subjects (using euglycemic-hyperinsulinemic clamp) is also reported (20). Therefore, estrogen-induced alterations in lipid metabolism may explain improvements in glycemic control: partitioning fatty acids away from the central abdominal depot (and thus out of the circulation) reduces circulating fatty acid effects on insulin action (49,50).

The metabolic improvements could be due to effects of HRT on physical activity level; to our knowledge, this is the first study to measure the effects of HRT on physical activity levels and well-being in subjects with diabetes. While we found no effect on reported physical activity level, there were improvements in physical functioning, vitality, mental health, and general health perceptions, which could have altered physical activity level, undetected by the physical activity questionnaire used.

Whether menopause duration modulates the influence of HRT on reversing menopause effects is not known but could be determined by stratification of study cohorts by menopause duration, possible in larger prospective studies. In studies with a wide range of menopause duration, such as our own, a potential bias is introduced by age-effects on metabolic and body composition variables that may not respond to HRT.

In conclusion, there are no short- or longer-term adverse effects of HRT on factors associated with cardiovascular risk or metabolic control in overweight and obese women with type 2 diabetes. Six months of CEE combined with 4 months of medroxy-progesterone induces improvements in lipid metabolism, glycemic control, and some aspects of perceived well-being and reduces central abdominal fat mass without deterioration in total fat mass. This longer-term study indicates that progestin therapy does not obviate the benefits of estrogen and suggests that women with diabetes can expect improvements in cardiovascular risk factors with HRT. The effects of long-term HRT on the excess cardiovascular morbidity and mortality suffered by this high-risk (and often excluded) group requires long-term study as in nondiabetic women.

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References

1. Abate N, Garg A, Peshock RM, Stray-Gundersen J, Grundy SM: Relationships of generalized and regional adiposity to insulin sensitivity in men. *J Clin Invest* 96:88–98, 1995
2. Carey DG, Jenkins AB, Campbell LV, Freund J, Chisholm DJ: Abdominal fat and insulin resistance in normal and overweight women: direct measures reveal a strong relationship in subjects at both low and high risk of NIDDM. *Diabetes* 45:633–638, 1996
3. Lundgren H, Bengtsson C, Blohme G, Lapidus L, Sjöström L: Adiposity and adipose tissue distribution in relation to incidence of diabetes in women: results from a prospective population study in Gothenburg, Sweden. *Int J Obesity* 13:413–423, 1989
4. Lapidus L, Bengtsson C, Larson B, Pennert K, Rybo E, Sjöstrom L: Distribution of adipose tissue and risk of cardiovascular disease: a 12 year follow-up of participants in the population study of women in Gothenburg, Sweden. *Br Med J* 289:1261–1263, 1984
5. Poehlman ET, Toth MJ, Gardner AW: Changes in energy balance and body composition at menopause: a controlled longitudinal study. *Ann Intern Med* 123:673–675, 1995
6. Svendsen OL, Hassager C, Christiansen C: Age- and menopause-associated variations in body composition and fat distribution in healthy women as measured by dual-energy x-ray absorptiometry. *Metabolism* 44:369–373, 1995
7. Haarbo J, Marslew U, Gotfredsen A, Christiansen C: Postmenopausal hormone replacement therapy prevents central distribution of body fat after the menopause. *Metabolism* 40:1323–1326, 1991
8. Samaras K, Kelly PJ, Spector TD, Chiano MN, Campbell LV: Tobacco smoking and oestrogen replacement are associated with lower total and central fat in monozygotic twins. *Int J Obes* 22:149–156, 1998
9. Stampfer MJ, Willett WC, Colditz GA, Rosner B, Speizer FE, Hennekens CH: A prospective study of postmenopausal estrogen therapy and coronary heart disease. *N Engl J Med* 313:1044–1049, 1985
10. Wilson PWF, Garrison RJ, Castelli WP: Postmenopausal estrogen use, cigarette smoking, and cardiovascular mortality in women over 50: the Framingham Study. *N Engl J Med* 313:1038–1043, 1985
11. Grodstein F, Stampfer MJ, Manson JE, Colditz GA, Willett WC, Rosner B, Speizer FE, Hennekens CH: Postmenopausal estrogen and progestin use and the risk of cardiovascular disease. *N Engl J Med* 335: 453–461, 1996
12. Criqui MH, Suarez L, Barrett-Connor E, McPhillips J, Wingard DL, Garland C: Postmenopausal estrogen use and mortality: results from a prospective study in a defined homogeneous community. *Am J Epidemiol* 128:606–614, 1988
13. Barrett-Connor E, Cohn BB, Wingard DL, Edelstein SL: Why is diabetes mellitus a stronger risk factor for fatal ischemic heart disease in women than in men? *JAMA* 265:627–631, 1991
14. Sattar N, Jaap AJ, MacCuish AC: Hormone replacement therapy and cardiovascular risk on post-menopausal women with NIDDM. *Diabet Med* 13:782–788, 1996
15. Dunne FP: Should women with diabetes mellitus receive hormone replacement therapy? *Int J Clin Pract* 51:299–303, 1997
16. Feher MD, Isaacs AJ: Is hormone replacement therapy prescribed for postmenopausal diabetic women? *Brit J Clin Pract* 50:431–432, 1996
17. Moorhead T, Hannaford P, Warskyj M: Prevalence and characteristics associated with hormone replacement therapy in Britain. *Br J Obstet Gynaecol* 104:290–297, 1997
18. Brussard HE, Gevers Leuven JA, Kluft C, Krans MJ, van Duyvenvoorde W, Buytenhek R, van der Laarse A, Princen HMG: Effect of 17β-estradiol on plasma lipids and LDL oxidation in postmenopausal women with type II diabetes mellitus. *Arterioscler* 17:324–330, 1997
19. Andersson B, Mattsson L-A, Hahn L, Mårin P, Lapidus L, Holm G, Bengtsson B-A, Björntorp P: Estrogen replacement therapy decreases hyperandrogenicity and improves glucose homeostasis and plasma lipids in postmenopausal women with noninsulin-dependent diabetes. *J Clin Endocrinol Metab* 82:638–643, 1997
20. Brussard HE, Gevers Leuven JA, Frölich M, Kluft C, Krans HMJ: Short-term oestrogen replacement improves insulin resistance, lipids and fibrinolysis in postmenopausal women with NIDDM. *Diabetologia* 40: 843–849, 1997
21. Samaras K, Spector TD, Nguyen TV, Baan K, Campbell LV, Kelly PJ: Independent genetic factors determine the amount and distribution of fat in women after the menopause. *J Clin Endocrinol Metab* 82: 781–785, 1997
22. Campbell LV, Carey DG, Chisholm DJ: Measuring central fat: a bet each way? *Diabetes Care* 19:1033–1034, 1996
23. McNamara JR, Schaefer EJ: Automated enzymatic standardized lipid analyses for plasma and lipoprotein fractions. *Chim Clin Acta* 166:1–8, 1987
24. Blanche PJ, Gong EM, Forte TL, Nichols AV: Characterisation of human high density lipoproteins by gradient gel electrophoresis. *Biochim Biophys Acta* 665:408–419, 1981
25. Hayward CS, Knight DC, Wren BR, Kelly RP: The effect of hormone replacement therapy on noninvasive cardiovascular hemodynamics. *J Hypertens*15:987–993, 1997
26. Wicks J: *Guide to Exercise*. Canberra, Australia, National Heart Foundation of Australia, 1983
27. Jenkinson C, Coulter A, Wright L: Short Form 36 (SF 36) health survey questionnaire: normative data for adults of working age *BMJ* 306:1437–1444, 1993
28. Walsh BW, Schiff I, Rosner B, Greenberg L, Ravnikar V, Sacks FM: Effects of postmenopausal estrogen replacement on the concentrations and metabolism of plasma lipoproteins. *N Engl J Med* 325:1196–1204, 1991
29. Nabulsi AA, Folsom AR, White A, Patsch W, Heiss G, Wu KK, Szklo M: Association of hormone-replacement therapy with various cardiovascular risk factors in postmenopausal women. *N Engl J Med* 328: 1069–1075, 1993
30. Barrett-Connor E, Wingard DL, Criqui MH: Postmenopausal estrogen use and heart disease risk factors in the 1980s: Rancho Bernardo, Calif, revisited. *JAMA* 261: 2095–2100, 1989
31. The Writing Group for the PEPI Trial: Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women. *JAMA* 273:199–208, 1995
32. Darling GM, Johns JA, McCloud PI, Davis SR: Estrogen and progestin compared with simvastatin for hypercholesterolemia in postmenopausal women. *N Engl J Med* 337:595–601, 1997
33. Gangar KF, Reid BA, Crook D, Hillard TC, Whitehead M: Oestrogens and atherosclerotic vascular disease: local vascular factors. *Bailliére's Clin Endocrinol Metab* 7:47–59, 1993
34. Salomaa V, Rasi V, Pekkanen J, Vahtera E, Jauhianen M, Vartiainen E, Ehnholm C, Tuomilehto J, Myllylä G: Association of hormone replacement therapy with hemostatic and other cardiovascular risk factors: the FINRISK Hemostasis Study. *Arterioscler Thromb Vasc Biol* 15:1549–1555, 1995
35. Koh KK, Mincemoyer R, Bui MH, Csako G, Pucino F, Guetta V, Waclwiw M, Cannon RO: Effects of hormone-replacement therapy on fibrinolysis in postmenopausal women. *N Engl J Med* 336:683–690, 1997
36. Fröhlich M, Schunkert H, Hense H-W, Tropitzsch A, Hendricks P, Döring A, Riegger GAJ , Koenig W: Effect of hormone replacement therapies on fibrinogen and plasma viscosity in postmenopausal women. *Br J Haematol* 100:577–581, 1998
37. Sullivan JM, Vander Zwaag R, Hughes JP,

Maddock V, Kroetz FW, Ramanathan KB, Mirvis DM: Estrogen replacement and coronary artery disease. *Arch Intern Med* 150:2557–2562, 1990
38. Gilligan DM, Badar DM, Panza JA, Quyyumi AA, Cannon RO: Acute vascular effects of estrogen in postmenopausal women. *Circulation* 90:786–791, 1994
39. Rosano GM, Sarrell PM, Poole-Wilson PA, Collins P: Beneficial effect of oestrogen on exercise-induced myocardial ischaemia in women with coronary artery disease. *Lancet* 342:133–136, 1993
40. O'Keefe JH, Kim SC, Hall RR, Cochran VC, Lawhorn SL, McCallister BD: Estrogen therapy after coronary angioplasty in women. *J Am Coll Cardiol* 29:1–5, 1997
41. Sullivan JM, El-Zeky F, Vander Zwaag R, Ramanathan KB: Effect on survival of estrogen replacement therapy after coronary artery bypass grafting. *Am J Cardiol* 79: 847–850, 1997
42. Robinson JG, Folsom AR, Nabulsi AA, Watson R, Brancati FL, Cai J, for the Atherosclerosis Risk in Communities Study: Can postmenopausal hormone replacement improve plasma lipids in women with diabetes? *Diabetes Care* 19:480–485, 1996
43. Lehmann ED, Hopkins KD, Parker JR, Turay RC, Rymer J, Fogelman I, Gosling RG: Aortic distensibility in postmenopausal women receiving tibolone. *Br J Radiol* 67:701–705, 1994
44. Kritz-Silverstein D, Barrett-Connor E: Long-term postmenopausal hormone use, obesity and fat distribution in older women. *JAMA* 275:46–49, 1996
45. Khoo S-K, Coglan MJ, Wright GR, De Voss KN, Battistuta D: Hormone therapy in women in the menopause transition: randomised, double-blind, placebo-controlled trial of the effects on body weight, blood pressure, lipoprotein levels, antithrombin III activity and the endometrium. *Med J Aust* 168:216–220, 1989
46. Berman RE, Epstein RS, Lydick E: Risk factors associated with women's compliance with estrogen replacement therapy. *J Women's Health* 6:219–226, 1997
47. Rebuffe-Scrivé M, Enk L, Crona N, Lönnroth P, Abrahamson L, Smith U, Björntorp P: Fat cell metabolism in different regions in women: effect of menstrual cycle, pregnancy, lactation. *J Clin Invest* 75:1973–1976, 1985
48. Rebuffe-Scrivé M, Lönnroth P, Mårin P, Wesslau C, Björntorp P, Smith U: Regional adipose tissue metabolism in men and postmenopausal women. *Int J Obes* 11: 347–355, 1987
49. Jenkins AB, Storlein LH, Chisholm DJ, Kraegen EW: Effects of non-esterified fatty acid availability on tissue-specific glucose utilization in rats in vivo. *J Clin Invest* 82:293–299, 1988
50. Boden G: Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. *Diabetes* 45:3–10, 1997