Effect of Postmenopausal Hormone Therapy on Glucose and Insulin Concentrations

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OBJECTIVE — To characterize the long-term impact of four hormone therapy regimens on insulin and glucose concentrations measured during a standard oral glucose tolerance test.

RESEARCH DESIGN AND METHODS — The Postmenopausal Estrogen/Progestin Intervention Study was a 3-year placebo-controlled randomized trial to assess effects of four hormone regimens on cardiovascular risk factors. This efficacy analysis describes glucose and insulin concentrations from 788 adherent women at baseline and at 1 and 3 years' postrandomization.

RESULTS — When compared with women taking placebo, those taking conjugated equine estrogen (CEE) at 0.625 mg/day with or without a progestational agent had mean fasting insulin levels that were 16.1% lower, mean fasting glucose levels 2.2 mg/dl lower, and mean 2-h glucose levels 6.4 mg/dl higher (each nominal $P < 0.05$). No significant differences were apparent between women taking CEE only versus the three progestin regimens: medroxyprogesterone acetate (MPA) at 2.5 mg daily (continuous MPA), MPA at 10 mg on days 1–12 (cyclical MPA), and micronized progesterone (MP) (cyclical) at 200 mg on days 1–12. The impact of hormone therapy on insulin and glucose depended on baseline levels of fasting insulin and 1-h glucose ($P < 0.05$). However, the treatment effects on carbohydrate metabolism appeared to be consistent across participant subgroups formed by lifestyle, clinical, and demographic characteristics.

CONCLUSIONS — Oral hormone therapy involving 0.625 mg/day of CEE may modestly decrease fasting levels of insulin and glucose. Postchallenge glucose concentrations are increased, however, which may indicate delayed glucose clearance.

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Impaired glucose tolerance and type 2 diabetes are prevalent health problems among postmenopausal women (1). Despite some evidence of possible diabetogenic effects, postmenopausal hormone therapy is increasingly prescribed for its beneficial effects on bone loss and cardiovascular risk factors (2). The clinical impact of this practice is difficult to characterize. Even though many reports have examined the effect of postmenopausal hormone therapy on glucose, insulin, and insulin resistance, there remain considerable differences in interpretations and conclusions (3–13). It may be that variations in treatment effects are attributable to differences in baseline characteristics among individuals and/or differences in preparations, doses, or routes of administration.

The Postmenopausal Estrogen/Progestin Interventions (PEPI) trial assessed the impact of four regimens of hormone therapy, relative to each other and placebo, on cardiovascular disease risk factors. These included concentrations of plasma glucose and serum insulin at fasting and at 1- and 2-h postchallenge during a standard oral glucose tolerance test (OGTT). PEPI was the first randomized trial with sufficient size and diversity to allow a broad characterization of the impact on these analytes of conjugated equine estrogen (CEE) therapy, with and without progestins. It also allowed the consistency of this impact to be explored across subgroups defined by risk factor profiles.

The initial PEPI report (14) indicated that assignment to hormone therapy, relative to placebo, resulted in *1*) mean decreases in fasting insulin of 7–20% that did not reach statistical significance, 2) mean decreases in fasting glucose of 2–3% that were statistically significant, 3) little change in mean 2-h insulin, and 4) mean increases in 2-h glucose of 2–7% that reached nominal statistical significance. These conclusions were based on statistical analyses prespecified during the design of PEPI. Comparisons were performed separately on each outcome measure, controlled for the ten pairwise comparisons among the five treatment arms, and included all randomized women regardless of adherence to assigned medication. This approach, while consistent with good clinical trials practice (15), in which the aim is to provide a focused and conservative test of the principal study hypotheses, does not generally provide the best estimates of drug effects.

This current report provides a more complete description of the impact PEPI

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Abbreviations: CEE, conjugated equine estrogen; MP, micronized progesterone; MPA, medroxyprogesterone acetate; OGTT, oral glucose tolerance test; PEPI, Postmenopausal Estrogen/Progestin Interventions.

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

hormone regimens had on fasting and postchallenge glucose and insulin. We make several extensions of the PEPI analyses: *1*) unlike the primary intention-to-treat analysis, this efficacy analysis focuses on the response of women who were adherent to their assigned treatment; 2) we provide estimates of treatment effects attributable to estrogen, opposition with progestins, type of progestin, and dosing schedule of progestin; 3) we present results from multivariate analyses that address simultaneously fasting and postchallenge insulin and glucose measures and allow us to characterize consistently the impact of therapy on the relative balance of insulin and glucose levels across time; and *4*) we examine the impact of several patient characteristics (pretreatment levels of insulin and glucose, age, body weight, smoking, alcohol intake, physical activity, and prior hormone use) on the magnitude of treatment effects. The prior observation that the magnitude of treatment effects may be critically related to the dose (5) supports our decision to limit our analyses to the subset of women who were adherent to their assigned medication.

RESEARCH DESIGN AND METHODS

— Detailed descriptions of the PEPI study design, recruitment practices, methods, and baseline characteristics appear elsewhere (16–19). Briefly, PEPI was a 3-year double-masked placebo-controlled randomized clinical trial to assess the relative impact of four hormone therapy regimens and a placebo regimen on a number of cardiovascular risk factors, including insulin and glucose concentrations during an OGTT. The active regimens were oral CEE therapy at 0.625 mg daily, alone or in combination with each of three regimens of progestational agents: medroxyprogesterone acetate (MPA) at 2.5 mg daily (continuous MPA); MPA at 10 mg on days 1–12 (cyclical MPA); and micronized progesterone (MP) (cyclical) at 200 mg on days 1–12. Placebo MP was prescribed for days 1–12 for all women not assigned to active MP; placebo MPA was prescribed during days 12–28 for women assigned to cyclical MPA, and daily for all other women not receiving continuous MPA. Seven clinical centers randomized 875 women into the trial. Key exclusion criteria included *1*) natural menopause before age 44, within 12 months of enrollment, or >10 years ago; 2) hysterectomy within 2 months; 3) BMI ≥40 kg/m^2; and *4*) a medical history contraindicating postmenopausal hormone therapy. All women were ambulatory and provided informed consent. Women who were currently taking insulin or had baseline fasting plasma glucose levels ≥7.77 mmol/l were ineligible.

Adherence was defined as having pill counts of at least 80% of expected during the 6 months before clinic visits. Of the 875 randomized women, 745 (85%) met this criterion for adherence and participated in glucose tolerance tests at 12 months; 648 (74%) were adherent at 36 months. While the primary intention-to-treat analysis incorporated nonadherent women, this secondary analysis describes the insulin and glucose concentrations only of women who were adherent to their assigned hormone regimen. Combined, 788 (90%) contributed at least one follow-up measure to our analyses. Adherence varied by treatment assignment ($P < 0.001$): the percentages of women who were adherent for both visits were 72% (placebo), 54% (CEE only), 81% (CEE + MPA cyclical), 82% (CEE + MPA continuous), and 77% (CEE + MP). Hysterectomy status was also associated with adherence ($P = 0.02$): 71% of women with a uterus were adherent, compared with 79% of women without a uterus. After controlling for these factors, adherence was not associated with age, prior hormone therapy, baseline levels of insulin and glucose, or any other factor considered in this report. The validity of pill counts for measuring adherence was confirmed by serum estrone levels (20).

A standard 75-g OGTT was performed between 7:00 and 11:00 A.M. after a 12-h fast at baseline and 12 and 36 months postrandomization. Women were instructed to take 5–10 min to consume the glucose drink. Venous blood for glucose was collected in EDTA tubes, placed on ice immediately, and centrifuged within 20 min. Blood for insulin was centrifuged after 45 min at room temperature. Core lab work was done at the Diabetes Research Training Center Immunoassay Laboratory, Indiana University School of Medicine. Plasma glucose was measured using a colorimetric glucose oxidase method, after Somogyi precipitation (21). The interassay coefficients of variation during PEPI analyses, based on Boerhringer Mannheim Diagnostics control pools, were 2.0 and 1.8% for target glucose values of 3.94 and 16.10 mmol/l, respectively. Serum insulin levels were assayed in duplicate using a minor modification of a double antibody method (22). Based on analyses of Bio-Rad control pools, interassay coefficients of variation were 29, 14, and 13% for insulin target values of 49, 297, and 646 pmol/l, respectively. OGTTs were not restricted to a particular day of the medication cycle; however, each participant's 36-month follow-up visit was scheduled, as closely as possible, to occur at the same day of the cycle as her 12-month visit.

Height, weight, and waist and hip girths were measured while participants wore light clothing and no shoes. Cigarette smoking (current/former/never), current alcohol intake (drinks per day), and history of hormone use (ever/never) data were collected by standardized questionnaires.

Statistical analysis

Comparisons of changes (from baseline) of fasting, 1-, and 2-h glucose and log-transformed insulin concentrations involved two-sided tests based on Laird-Ware models (23,24); statistical significance was assessed with Wald tests (25). To describe treatment effects, we performed staged comparisons according to the following sequence: first, an overall comparison was made between placebo versus active (all four active regimens combined) therapy; second, a comparison was made between unopposed and opposed (all three estrogen/progestin arms combined); third, a comparison between MP and MPA (2 arms combined) progestins; and fourth, a comparison between MPA continuous and MPA cyclical progestin regimens. Each comparison included differences (at both 12 and 36 months) as dependent variables. Data from every exam at which a woman met our criterion for adherence (≥80% pill count) were included. This approach, rather than restricting these analyses to the subset of women who attended and were adherent at both exams, was adopted because it is statistically more robust with respect to missing data assumptions (26,27). Multivariate comparisons were performed with the six analytes (fasting, 1-, and 2-h glucose and log-transformed insulin) comprising the dependent variable and with analyte by treatment interaction terms as predictors. Mean relative treatment effects were generated from these models and reported in units of milligrams per deciliter (glucose) and percent (insulin).

The consistency of these effects across subgroups (race, hysterectomy status, alcohol intake, and smoking status) and continuous risk factors (baseline insulin and glucose, age, BMI, and waist-to-hip ratio) was explored by examining interaction terms. Nominally significant interactions were portrayed using univariable regression.

Table 1—*Baseline concentrations of insulin and glucose in 788 adherent PEPI participants*

Treatment assignment	Insulin (pmol/l)			Glucose (mmol/l)		
	Fasting	1-h	2-h	Fasting	1-h	2-h
Placebo	36.7 ± 28.4 (149)	497.2 ± 209.5 (148)	332.2 ± 258.3 (149)	5.38 ± 0.53 (149)	8.52 ± 2.36 (148)	6.24 ± 1.85 (149)
CEE only	34.2 ± 28.5 (145)	470.9 ± 357.1 (144)	298.9 ± 262.9 (145)	5.40 ± 0.59 (145)	8.42 ± 2.50 (144)	6.43 ± 2.27 (144)
CEE + cyclical MPA	33.5 ± 26.1 (160)	509.1 ± 308.1 (158)	316.3 ± 247.9 (160)	5.35 ± 0.46 (160)	8.29 ± 2.45 (158)	6.09 ± 1.68 (160)
CEE + continuous MPA	36.8 ± 27.7 (159)	455.7 ± 278.9 (158)	304.8 ± 234.8 (159)	5.41 ± 0.59 (159)	8.37 ± 2.61 (158)	6.42 ± 2.34 (159)
CEE + continuous MP	35.3 ± 27.8 (165)	494.9 ± 272.0 (165)	316.4 ± 249.7 (163)	5.41 ± 0.56 (165)	8.24 ± 2.47 (165)	6.28 ± 1.98 (163)

Data are means ± SD (*n*).

RESULTS

Baseline characteristics

The average age (± SD) of the 788 women described in this report was 56.0 ± 4.3 years. Of these subjects, 68% had not had a hysterectomy, and 55% reported prior use of postmenopausal hormone therapy, which by protocol had been discontinued for at least 3 months. There were 4% who identified themselves as African-Americans, 5% as Hispanic, 2% as Asian, 1% as Native American, and 89% as Caucasian. Of these women, 50% had never smoked cigarettes regularly, 37% were former smokers, and 14% were current smokers. Reported alcohol intake was relatively low: 33% reported no alcohol intake during the prior year, and only 17% reported averaging one or more drinks per day. The average BMI of these women was 26.1 ± 4.5 kg/m^2; the average ratio of their waist-to-hip circumferences was 0.792 ± 0.069. Table 1 lists baseline mean insulin and glucose levels for the 788 adherent women by random treatment assignment. No differences by treatment assignments for the means or percentages of any of these baseline characteristics reached statistical significance.

Impact of hormone therapy on insulin and glucose concentrations

Table 2 presents the mean changes from baseline in glucose and insulin observed among PEPI women who were adherent to their assigned study medications. Included are standard errors of these means and the numbers of women attending each exam (n_{12} and n_{36}). Across all treatment groups, mean insulin levels decreased at 36 months. This decrease, which may be attributable to secular trends or other sources of drift (14), was consistent across all five arms of the study ($P = 0.78$), which supports the averaging of relative treatment effects across the 12- and 36-month examinations.

Separate analyses of fasting, 1-, and 2-h concentrations are summarized in Table 3. Mean relative treatment effects are presented as sequential analyses that address, in turn, differences associated with any therapy compared with placebo, with unopposed estrogen compared with opposed estrogen, with opposition with MPA compared with opposition with MP, and with cyclic MPA opposition compared with continuous MPA opposition. Effects whose 95% CI did not include zero are highlighted. Active therapy appeared to reduce mean fasting insulin (by 16.1%) and glucose (by 0.122 mmol/l) and increase mean 2-h glucose (by 0.355 mmol/l) compared with placebo. No differences reached nominal statistical significance between unopposed versus opposed estrogen, or between regimens involving

Table 2—*Mean changes from baseline by treatment assignment*

Analyte	Month	Placebo	CEE only	CEE + continuous MPA	CEE + cyclical MPA	CEE + cyclical MP
n_{12}	—	141	138	152	155	159
n_{36}	—	127	97	145	140	139
Insulin (%)						
Fasting	12	7.5 ± 8.2	−6.3 ± 7.9	−18.0 ± 6.3	0.5 ± 7.8	−14.8 ± 6.4
	36	15.3 ± 9.0	−5.6 ± 8.6	−9.3 ± 6.2	10.7 ± 8.3	−8.5 ± 5.8
1-h	12	−2.3 ± 5.2	0.6 ± 6.0	1.8 ± 4.8	−7.5 ± 4.5	−7.8 ± 3.7
	36	−9.4 ± 6.2	−19.5 ± 5.7	−15.1 ± 4.3	−19.1 ± 5.7	−19.6 ± 4.0
2-h	12	0.8 ± 5.8	4.1 ± 6.8	10.0 ± 6.6	11.9 ± 6.3	−1.4 ± 5.5
	36	−9.3 ± 6.2	−1.2 ± 7.4	−4.6 ± 5.7	−2.0 ± 6.2	−13.9 ± 5.3
Glucose (mmol/l)						
Fasting	12	−0.012 ± 0.037	−0.155 ± 0.033	−0.094 ± 0.029	−0.108 ± 0.037	−0.105 ± 0.033
	36	−0.061 ± 0.048	−0.189 ± 0.058	−0.186 ± 0.031	−0.237 ± 0.039	−0.163 ± 0.045
1-h	12	−0.346 ± 0.147	0.080 ± 0.164	0.061 ± 0.145	−0.093 ± 0.159	−0.132 ± 0.153
	36	−0.342 ± 0.175	0.150 ± 0.189	0.167 ± 0.144	0.001 ± 0.182	−0.229 ± 0.184
2-h	12	−0.176 ± 0.147	0.106 ± 0.142	0.294 ± 0.122	0.356 ± 0.117	0.146 ± 0.112
	36	0.097 ± 0.156	0.359 ± 0.188	0.538 ± 0.123	0.568 ± 0.144	0.269 ± 0.147

Data are means ± SEM.

Table 3—*Effect of hormone therapy on insulin and glucose concentrations*

Change from baseline	Active therapy versus placebo	Unopposed estrogen versus opposed estrogen	MPA versus MP	Cyclic versus continuous MPA
Insulin (%)				
Fasting	−16.1* (−27.1 to −3.4)	0.8 (−13.2 to 17.0)	−6.3 (−19.2 to 8.6)	22.5* (1.0 to 45.5)
1-h	−5.7 (−14.9 to 4.5)	−2.3 (−12.3 to 8.9)	−4.1 (−13.8 to 6.8)	−6.1 (−17.2 to 6.4)
2-h	3.1 (−8.2 to 15.8)	−0.3 (−11.8 to 12.7)	−10.3 (−20.6 to 1.4)	2.9 (−10.7 to 18.6)
Glucose (mmol/l)				
Fasting	−0.122* (−0.189 to −0.050)	0.028 (−0.044 to 0.105)	0.017 (−0.056 to 0.094)	−0.039 (−0.122 to 0.050)
1-h	0.300 (−0.011 to 0.605)	−0.128 (−0.450 to 0.194)	−0.239 (−0.561 to 0.078)	−0.172 (−0.544 to 0.200)
2-h	0.355* (0.094 to 0.611)	0.150 (−0.118 to 0.419)	−0.228 (−0.500 to 0.044)	0.044 (−0.272 to 0.361)

Data are means (95% CI). The table presents average (12- and 36-month) effects on changes from baseline. Analyses are restricted to women who were adherent to assigned medications (based on pill counts) for the 6 months before the visit.*CI does not include 0.

MPA versus MP. Mean fasting insulin levels were 22.5% greater for cyclic MPA than for continuous MPA.

Figure 1, from multivariate analyses, describes the overall patterns of treatment effects on glucose and insulin (relative to placebo) across analytes and OGTT times. Tables 2 and 3 present estimates of mean effects and relative effects; these are graphed in Fig. 1*A* and *B* for each active arm. As suggested by univariate results, all active treatments were fairly comparable. These active therapies, relative to placebo, appeared to lower mean fasting glucose and insulin levels. While mean 1-h insulin levels among the active arms were slightly less than placebo, mean 1-h glucose levels were elevated. At 2 h, mean insulin levels among active arms appeared to edge toward or above placebo levels, while glucose levels remained elevated. A formal statistical test to examine these multivariate patterns found significant differences between placebo and combined active treatment groups (nominal $P < 0.0001$). No significant differences were evident from multivariate analyses that were attributable to progestin opposition ($P = 0.68$), between MPA and MP ($P = 0.48$), or between cyclical and continuous MPA ($P = 0.18$).

Impact of baseline levels of insulin and glucose on treatment effects

The pattern of treatment effects attributable to hormone therapy, in multivariate analyses, varied by baseline levels of fasting insulin ($P = 0.01$) and 1-h glucose ($P = 0.006$); no significant differences in patterns were detected among women grouped by baseline levels of 1-h insulin ($P = 0.57$), 2-h insulin ($P = 0.45$), fasting glucose ($P = 0.73$), or 2-h glucose ($P = 0.26$). To portray these multivariable results more succinctly, regression analyses were used to examine the relative treatment effects of hormone therapy on fasting glucose across baseline levels of fasting insulin or 1-h glucose. Figure 2 depicts these fitted relationships, each of which reached nominal levels of statistical significance: log-transformed fasting insulin (nominal $P = 0.0009$) and 1-h glucose (nominal $P = 0.01$). Reductions in fasting glucose attributable to hormone therapy were most apparent among women with the highest baseline levels of fasting insulin and 1-h glucose. No other univariable relationships between treatment effects and

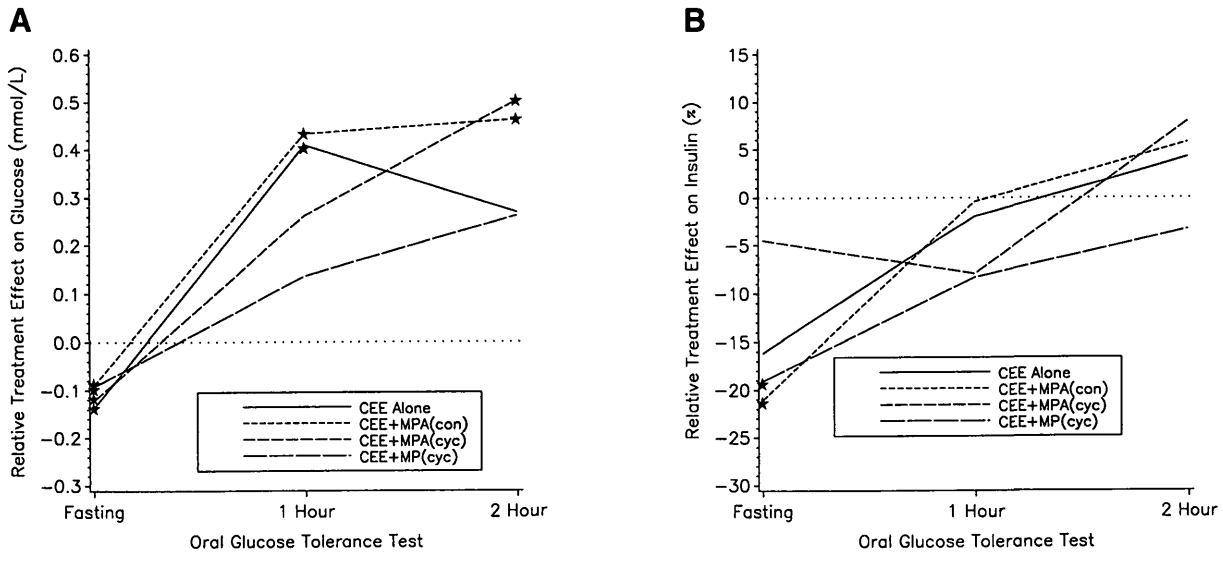

Figure 1—*Relative impact of hormone therapy on glucose (A) and insulin (B), based on multivariate analyses. Graphed are mean differences in changes from baseline between the placebo group and each active arm. ★, relative treatment differences exceeding nominal levels of statistical significance (P < 0.05).*

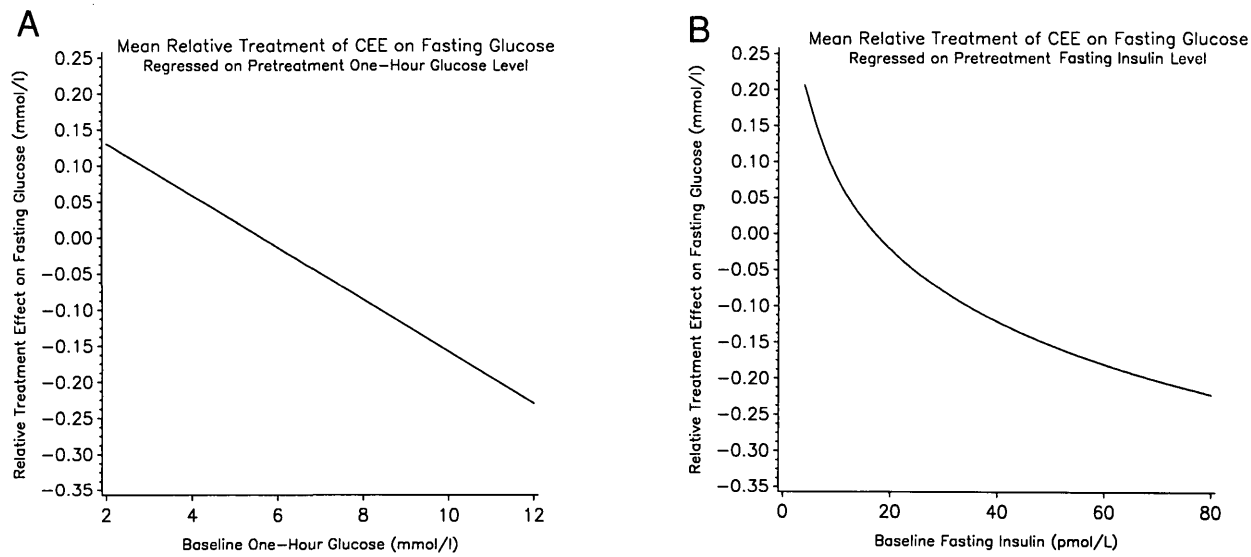

Figure 2—*Predicted relative treatment effects of hormone therapy on fasting glucose based on regression on baseline levels of 1-h glucose* (A) *and fasting insulin* (B).

baseline levels of insulin or glucose reached nominal statistical significance.

Consistency of treatment effects across subgroups defined by other baseline characteristics

Each of the baseline characteristics discussed above were included in multivariate analyses to assess the consistency of the pattern of treatment effects on insulin and glucose concentrations; no interactions reached statistical significance: age (P = 0.53), race (grouped as African-American, Hispanic, and other; P = 0.23), hysterectomy status (P = 0.62), reported alcohol intake (grouped as none, <1 drink per day, and ≥1 drink per day; P = 0.85), smoking status (grouped as never, former, and current; P = 0.99), BMI (P = 0.69), and waist-to-hip ratio (P = 0.73).

CONCLUSIONS — These analyses on the subset of adherent women support the conclusions of the protocol-defined analyses of the PEPI trial: oral CEE therapy (0.625 mg/day) may lead to modest decreases in levels of fasting glucose and insulin and a modest increase in 2-h glucose. The additional analyses indicate that this hormone therapy may also lead to increased 1-h glucose levels. These effects appear to be maintained across 3 years of hormone administration; however, their magnitude is not great, and their clinical significance is not clear.

Insulin and glucose concentrations

The transition to postmenopausal status is marked by a decrease in insulin secretion and a coordinated decrease in hepatic insulin clearance (28). In the short term, this balance may have little impact on response to glucose challenge; however, the prevalence of insulin resistance increases with age and has been reported to be present in 44% of untreated postmenopausal women (5). It is natural to speculate that pharmacological estrogen therapy may have potential for preventing this increased prevalence. Some evidence exists that estrogen therapy may improve insulin sensitivity (13,29–31). However, the effect may critically depend on dose: oral CEE therapy at 0.625 mg/day has been reported to improve insulin sensitivity by 25%, whereas a dose of 1.25 mg/day may decrease insulin sensitivity by an equal amount.

PEPI did not directly assess insulin resistance. Its findings of modest treatment effects on glucose and insulin levels may be most useful in shedding light on the impact of estrogen on glucose secretion and insulin secretion and clearance. Estrogen therapy has been variously reported to suppress hepatic glucose production (32,33), to hamper the pancreatic response to rising glucose levels thereby delaying release of insulin (10,34,35), and to increase insulin clearance (12,29,30,32). In PEPI, treatment with 0.625 mg/day oral CEE appeared to reduce fasting glucose and insulin concentrations but to raise postchallenge glucose. Lower fasting glucose levels may reflect reduced hepatic glucose output. After fasting, the insulin-sensitive disposal of glucose by insulin-sensitive tissues is minimal; the prevailing glucose concentration is modulated by non–insulin sensitive tissues (primarily the central nervous system) and by glucose production in the liver and (with longer-term fasting) kidney. These treatment effects appeared to be most pronounced among women who may have been predisposed to the development of carbohydrate intolerance, i.e., who had relatively elevated levels of pretreatment fasting insulin and 1-h postprandial glucose. These PEPI findings are consistent with a report that estradiol therapy may improve glucose homeostasis among postmenopausal women with type 2 diabetes (33).

The apparently delayed postchallenge glucose clearance observed in PEPI may reflect a CEE-induced limitation of insulin secretion or, more likely, enhanced hepatic clearance of insulin with consequent decreased glucose disposal by peripheral tissues. No significant relationships were observed between these postprandial effects and pretreatment insulin and glucose levels.

Impact of progestins

In contrast to other reports (9,34,36), PEPI found that the impact on insulin and glucose levels of adding three progestin regimens to oppose estrogen therapy was small. Neither of the two MPA regimens or the MP regimen differed significantly from estrogen alone (or among each other) in multivariate analyses. This suggests that, to the degree separate progestin effects may exist, they may be agent-, dose-, and/or model-

specific and were not evident among the regimens evaluated by PEPI.

Consistency of treatment effects

The impact of estrogen therapy on insulin and glucose levels was fairly consistent across subgroups defined by several known risk factors for insulin resistance and diabetes. PEPI is the first clinical trial with sufficient size and diversity to allow the comparison of effects across such subgroups; however, the numbers of Hispanic and African-American participants were relatively small, so that differences associated with ethnicity may not be ruled out.

Statistical issues

Multivariate analyses, rather than series of univariate analyses of analytes at each time point or summary measures, such as areas under the curves or ratios, provided empirically based comparisons.

Limiting analyses to adherent women raises statistical concerns. When results from analyses based on subsets of adhering participants differ from those based on the entire cohort, care should be taken in drawing conclusions (37,38). Intention-to-treat analyses may provide the best gauge of clinical effectiveness, i.e., the likely impact of a prescription practice on a population, but may present a flawed description of efficacy and mechanisms. Analyses limited to adherers may provide good estimates of drug-induced effects; these, however, may be biased because of selection factors related to tolerance and compliance. Our analyses indicated that adherence was related to hysterectomy status. Unmeasured factors may have played even larger roles. Statistical methodology to remove these biases involve many assumptions and do not extend to multiple outcome measures and confounders (39,40). The sensitivity of these methods to model-based assumptions has not been broadly explored. Thus, we are not able to address fully the degree to which any differences between results from our intention-to-treat and adherers-only analyses are attributable to compliance versus confounders.

Summary

Oral postmenopausal CEE therapy at 0.625 mg/day, for the relatively healthy PEPI cohort, slightly decreased fasting insulin and glucose concentrations and increased postchallenge glucose concentrations. Treatment effects on fasting glucose appeared to be most beneficial among women with elevated baseline concentrations of fasting insulin or 1-h glucose. Opposition of this CEE regimen with MPA or MP, at the doses in PEPI, had little impact on these effects. The overall estrogen effect was consistent across subgroups of women defined by a number of risk factors for insulin resistance and diabetes and was stable across 3 years of treatment.

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References

1. Harris MI, Hadden WC, Knowler WC, Bennett PH: Prevalence of diabetes and impaired glucose tolerance and plasma glucose levels in U.S. population aged 20–74 yr. *Diabetes* 36:523–534, 1987
2. Stevenson JC: The metabolic and cardiovascular consequences of HRT. *Br J Clin Pract* 49:87–90, 1995
3. Barrett-Connor E, Laakso M: Ischemic heart disease in postmenopausal women: effects of estrogen use on glucose and insulin levels. *Arteriosclerosis* 10:531–534, 1990
4. Nabulsi AA, Folsom A, 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: 1069–1975, 1993
5. Lindheim SR, Presser SC, Ditkoff EC, Vijod BS, Stanczyk FZ, Lobo RA: A possible bimodal effect of estrogen on insulin sensitivity in postmenopausal women and the attenuating effect of added progestin. *Fertil Steril* 60:664–667, 1993
6. Spellacy WN: Carbohydrate metabolism during treatment with estrogen, progestogen, and low-dose oral contraceptives. *Am J Obstet Gynecol* 142:732–734, 1982
7. O'Sullivan AJ, Ho KKY: A comparison of the effects of oral and transdermal estrogen replacement on insulin sensitivity in postmenopausal women. *J Clin Endocrinol Metab* 80:1783–1788, 1985
8. Thom M, Chakravarti S, Oram DH, Studd JWW: Effect of hormone replacement therapy on glucose and insulin tolerance in postmenopausal women. *Brit J Obstet Gynecol* 84:776–784, 1977
9. Wagner JD, Martino MA, Jayo MJ, Anthony MS, Clarkson TB, Cefalu WT: The effects of hormone replacement therapy on carbohydrate metabolism and cardiovascular risk factors in surgically postmenopausal cynomolgus monkeys. *Metabolism* 45:1–10, 1996
10. Larsson-Cohn U, Wallentin L: Metabolic and hormonal effects of post-menopausal oestrogen replacement treatment. *Acta Endocrinol* 86:583–596, 1977
11. Baskin DG, Gierke EP, Wilcox BJ, Matsumoto AM, Schwartz MW: Food intake and estradiol effects on insulin binding in brain and liver. *Physiolog Behav* 53:757–762, 1993
12. Godsland IF, Gangar K, Walton C, Cust MP, Whitehead MI, Wynn V, Stevenson JC: Insulin resistance, secretion, and elimination in postmenopausal women receiving oral or transdermal hormone replacement therapy. *Metabolism* 42:846–853, 1993
13. Wilcox JG, Hwang JG, Hodis HN, Sevanian A, Stanczyk FZ, Loba RA: Cardioprotective effects of individual conjugated equine estrogens through their possible modulation of insulin resistance and oxidation of low-density lipoprotein. *Fertil Steril* 67:57–62, 1997
14. The Writing Group for the PEPI Trial: Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women: the Postmenopausal Estrogen/Progestin Interventions (PEPI) trial. *JAMA* 273:199–208, 1995
15. Friedman LM, Furberg CD, DeMets DL: *Fundamentals of Clinical Trials*. 2nd ed. Littleton, MA, PSG Publishing, 1985
16. Espeland MA, Bush TL, Mebane-Sims I, Stefanick ML, Johnson S, Sherwin R, Waclawiw M: Rationale, design and conduct of the PEPI trial. *Control Clin Trials* 16:3S–19S, 1995
17. Wood PD, Kessler G, Lippel K, Stefanick ML, Wasilauskas CH, Wells HB: Physical and laboratory measurements in the PEPI trial. *Control Clin Trials* 16:36S–53S, 1995
18. Miller VT, Byington RL, Espeland MA, Langer R, Marcus R, Shumaker S, Stern MP: Baseline characteristics of the PEPI participants. *Control Clin Trials* 16:54S–65S, 1995
19. Johnson S, Mebane-Sims I, Hogan PE, Stoy DB. Recruitment of postmenopausal women in the PEPI trial. *Control Clin Trials* 16:20S–35S, 1995
20. Barrett-Connor E, Slone S, Greendale G, Kritz-Silverstein D, Espeland M, Johnson SR, Waclawiw M, Fineberg S: The Postmenopausal Estrogen/ Progestin Interventions Study: primary outcomes in adherent

women. *Mauritas* 27:261–274, 1997
21. Trinder P: Determination of glucose levels in blood using glucose oxidase with an alternative oxygen acceptor. *Ann Clin Biochem* 6:24–27, 1969
22. Morgan CR, Lazarow A: Immunoassay of insulin: two antibody system: plasma levels of normal, subdiabetic and diabetic rats. *Diabetes* 12:115–126, 1963
23. Laird NM, Ware JH. Random-effects models for longitudinal data. *Biometrics* 38:963–974, 1982
24. Jennrich RI, Schluchter MD: Unbalanced repeated measures models with structured covariance matrices. *Biometrics* 42:805–820, 1986
25. SAS/STAT Software: *Changes and Enhancements, Release 6.06*. Cary, NC, SAS Institute, 1992 (SAS tech. rep. P-229)
26. Little RJA, Rubin DB: *Statistical Analysis With Missing Data*. New York, Wiley, 1987
27. Little RJA: Modeling the drop-out mechanism in repeated-measures studies. *J Am Stat Assoc* 90:1112–1121, 1995
28. Walton C, Godsland IF, Proudler AJ, Wynn V, Stevenson JC: The effects of menopause on insulin sensitivity, secretion and elimination in nonobese healthy women. *Euro J Clin Invest* 23:466–473, 1993
29. Kumagai S, Holmang A, Bjorntorp P: The effects of oestrogen and progesterone on insulin in female rats. *Acta Physiol Scand* 149:91–97, 1993
30. Lindheim SR, Duffy DM, Kojima TK, Vijod MA, Stanczyk FZ, Lobo RA: The route of administration influences the effect of estrogen on insulin sensitivity in postmenopausal women. *Fertil Steril* 62:1176–1180, 1994
31. Andersson B, Mattsson L-A, Hahn L, Marin P, Lapidus L, Holm G, Bengtsson B-A, Bjorntorp P: Estrogen replacement therapy decreases hyperandrogenicity and improves glucose homeostasis and plasma lipids in postmenopausal women with non-insulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 82:638–643, 1997
32. Cagnacci A, Soldani R, Carriero PH, Paoletti AM, Fiorette P, Melis GB: Effects of low doses of transdermal 17B-estradiol on carbohydrate metabolism in postmenopausal women. *J Clin Endocrinol Metab* 74:1396–1400, 1992
33. Matute M, Kalkhoff RK: Sex steroid influences on hepatic glucogenesis and glycogen formulation. *Endocrinology* 92:762–768, 1973
34. DeCleyn K, Buyaert P, Coppens M: Carbohydrate metabolism during hormone substitution therapy. *Mauritas* 11:235–242, 1989
35. Spellacy W, Buhi W, Birk S: The effects of norgestrel on carbohydrate and lipid metabolism over one year. *Am J Obstet Gynecol* 125:984–986, 1976
36. Tuttle S, Turkington V: Effects of medroxyprogesterone acetate on carbohydrate metabolism. *Obstet Gynecol* 43:685–692, 1974
37. Sackett DL, Gent M: Controversy in counting and attributing events in clinical trials, *N Engl J Med* 301:1410–1412, 1979
38. Armitage P: Controversies and achievements in clinical trials. *Control Clin Trials* 5:67–72, 1984
39. Efron B, Feldman D: Compliance as an explanatory variable in clinical trials (with discussion). *J Am Stat Assoc* 86:9–26, 1991
40. Rochon J: Supplementing the intent-to-treat analysis: accounting for covariates observed postrandomization in clinical trials. *J Am Stat Assoc* 90:292–300, 1995