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Effects of Tibolone and Conjugated Equine Estrogens With or Without Medroxyprogesterone Acetate on Body Composition and Fasting Carbohydrate Measures in Surgically Postmenopausal Monkeys

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The effects of tibolone on body weight, body composition, and fasting carbohydrate measures in surgically postmenopausal cynomolgus monkeys were compared to those of conjugated equine estrogens (CEE) with and without medroxyprogesterone acetate (MPA). Monkeys were fed a moderately atherogenic diet with either no hormones (control n = 29), CEE (0.042 mg/kg, n = 27), CEE + MPA (0.167 mg/kg, n = 29), low-dose tibolone (LoTib, 0.05 mg/kg, n = 30), or high-dose tibolone (HiTib, 0.20 mg/kg, n = 31) daily for 2 years. Body weight (BW) was measured throughout the study, and dual-energy x-ray absorptiometry (DEXA) scans of the abdominal region (lumbar vertebrae 1 through 5) were performed at the end of the trial to assess abdominal body composition. Fasting carbohydrate measures (glucose, insulin, C-peptide, and fructosamine) were determined at baseline and after 2 years of treatment. Compared to controls, BW significantly increased and abdominal soft tissue mass was greater (analysis of variance [ANOVA], $P < .001$, $P = 0.003$, respectively) in all but the CEE-treated group ($P = .78$, $P = .94$, respectively). HiTib-treated monkeys had greater abdominal lean mass compared to controls ($P = .008$), while there was no significant treatment effect on abdominal fat mass (analysis of covariance [ANCOVA], $P = .29$). Fasting insulin concentrations and fasting insulin/glucose ratios were greater in CEE + MPA– ($P = .002$, $P = .03$, respectively) and HiTib-treated monkeys ($P = .03$, $P = .02$, respectively) compared to controls. There was a strong trend for a treatment effect on fasting blood glucose concentration (ANCOVA, $P = .06$) with CEE + MPA–treated animals having the greatest values, despite no difference in fructosamine concentration (ANCOVA, $P = .57$). Using these fasting measures, the homeostasis model assessment (HOMA-IR) revealed significant insulin resistance with CEE + MPA treatment compared to controls ($P = .008$), while the quantitative insulin sensitivity check index (QUICKI) showed significantly impaired insulin sensitivity in all hormone replacement therapy (HRT) groups (all P values < .03), except CEE ($P = .12$). In conclusion, HRT with CEE + MPA or tibolone results in greater BW, abdominal soft tissue, and insulin resistance (CEE + MPA and HiTib) compared to control-treated monkeys.

MENOPAUSE is associated with an increase in traditional cardiovascular disease (CVD) risk factors such as increased low-density lipoprotein cholesterol and decreased high-density lipoprotein cholesterol, as well as increased body weight (BW) and specifically, abdominal body fat.[1,2] There is also an increase in insulin resistance, as greater than 44% of otherwise healthy postmenopausal women were found to be insulin-resistant in the Atherosclerosis Risk in Communities (ARIC) trial,[3] similar to results from a smaller study.[4] These alterations in carbohydrate metabolism associated with menopause may further increase risk for CVD and diabetes. The physiological mechanisms responsible for changes in insulin sensitivity associated with sex hormone levels are not known, but changes in BW and body composition may be underlying causes. The effects of hormone replacement therapy (HRT) on BW/body composition are unclear. Previous studies have shown that long-term (6 months to 2 years) estrogen replacement therapy (ERT) with estradiol or conjugated equine estrogens (CEE) administered to surgically postmenopausal monkeys[5,6] or rats[7] prevents the increase in BW and abdominal fat associated with ovariectomy. ERT has also been shown to decrease weight gain in postmenopausal women.[3,8-10] Combined HRT decreases weight gain in postmenopausal women compared to placebo, but not as much as estrogen treatment

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alone.[9] In ovariectomized rats[11,12] and monkeys,[5] progestogen administration is associated with increased BW. Thus, it appears that combined HRT is not as beneficial as estrogen alone for preventing postmenopausal weight gain and subsequent insulin resistance, as the relationship between obesity and insulin resistance has been well established.[13] The effects of HRT/ERT on carbohydrate metabolism and insulin sensitivity are also unclear, but in general, estrogens have neutral[5,10,14] or beneficial effects on fasting glucose and insulin concentrations.[5,10,15,16] The addition of a progestogen to ERT typically dampens any estrogen-associated benefit on fasting carbohydrate measures or insulin sensitivity in both humans[3,4,10,17-19] and monkeys.[5,14] Some of this adverse effect may be due to increases in BW and/or changes in body composition associated with progestogen use. Tibolone (Org-OD-14, 7 α,17 α-17hydroxy-7-methyl-19-norpregn-5(10)-en-20-yn-3-one; LIVIAL, Organon, Oss, The Netherlands) is used to treat climacteric symptoms and prevent osteoporosis in postmenopausal women. It is rapidly converted into 3 active metabolites that bind receptors for estrogen (3α-OH-, 3β-OH-isomer), progesterone (Δ^4-isomer), as well as androgen (Δ^4-isomer).[20] As the progestogenic properties protect against endometrial proliferation, no additional progestogen is needed in women with a uterus.[21] The androgenic properties of tibolone may be responsible for improving certain climacteric symptoms, ie, hot flushes and libido; however, this androgenic activity may have adverse effects on BW/body composition and carbohydrate metabolism, as occurs in women taking oral contraceptives containing androgenic progestogens.[22-24] The purpose of the study reported here was to examine the effects of long-term treatment with tibolone or a commonly used ERT/HRT on BW, body composition, and carbohydrate metabolism in ovariectomized cynomolgus monkeys.

MATERIALS AND METHODS

Study Details

One hundred forty-six premenopausal cynomolgus monkeys (*Macaca fascicularis*), 6 to 8 years of age, were obtained through our collaborative association with the Institut Pertainian Bogor, Indonesia as part of a study to determine the effects of hormone treatment on the development of coronary artery atherosclerosis.[25] Monkeys were shipped to our institution in 3 cohorts (n = 87, 42, 17) within a 4-month period and housed in 2 separate animal buildings. Ten weeks prior to the experimental phase, all animals were ovariectomized and fed a moderately atherogenic diet containing 42% of calories from fat (predominantly saturated) and 0.28 mg cholesterol/kcal to induce atherosclerosis. Monkeys were then randomized, using a permuted block randomization scheme, into 1 of 5 treatment groups, receiving either no hormones (control, n = 29), CEE alone (Premarin; Wyeth-Ayerst, Philadelphia, PA) (0.042 mg/kg/d, human equivalent ~0.625 mg/d, n = 27), CEE + MPA (0.167 mg/kg/d, human equivalent ~2.5 mg/d continuous, n = 29), or low-dose tibolone (Org-OD-14; Organon, Oss, Netherlands, 0.05 mg/kg/d, human equivalent ~0.75 mg/d) (LoTib, n = 30), high-dose tibolone (0.20 mg/kg/d, human equivalent ~3.0 mg/d) (HiTib, n = 31). The hormones were administered in the diet and were given in a split dose for the 24-month treatment period. To confirm hormone delivery, CEE metabolites (estradiol, estrone sulfate) were assessed in serum collected from animals fasted for 18 hours. Tibolone metabolites (3α-OH-, 3β-OH-, and Δ^4-isomers) were measured in plasma obtained 1, 1.5, 2, and 4 hours after administration of the dose and reported separately.[25] BW was measured at baseline and once monthly throughout the treatment period. Abdominal body composition analyses were performed after 23 months of treatment. Soft tissue and lean mass were assessed by dual-energy x-ray absorptiometry scans (DEXA; Norland XR26, Norland Bone Densitometer Host Software Version 3.9.6b, Fort Atkinson, WI) of the abdominal region (lumbar vertebrae 1through 5) as previously published.[5] Soft tissue is composed of lean and fat mass, with fat mass calculated as the difference between soft tissue and lean mass. One technician performed all of the scans and selected the regions of interest for analysis. The coefficient of variation was 1.1% for soft tissue, 1.3% for lean tissue, and 8.4% for fat tissue.After 24 months, the monkeys were sedated with ketamine hydrochloride (15 mg/kg intramuscularly) (Ketaset, Fort Dodge Animal Health; Fort Dodge, IA) and euthanized with sodium pentabarbital (100 mg/kg intravenously; Butler, Columbus, OH), a method that is consistent with recommendations of the American Veterinary Medical Association Panel on Euthanasia. All procedures involving animals were conducted in compliance with state and federal laws of the US Department of Health and Human Services and guidelines established by the Wake Forest University Institutional Animal Care and Use Committee.

Clinical Chemistry Measures and Insulin Resistance/Sensitivity

Indicators of fasting carbohydrate metabolism were assessed at baseline (6 weeks after ovariectomy) and after 21 months of hormone treatment. Animals were sedated with ketamine hydrochloride (15 mg/kg intramuscularly), and blood was collected by venipuncture. Samples were placed on ice, spun for 30 minutes at 1,500 $\times$ *g*, and plasma stored at −70°C. Baseline glucose and fructosamine were analyzed by enzymatic (Glucose Diagnostic Kit; Sigma, St Louis, MO) and colorimetric (Fructosamine Diagnostic Kit, Sigma) methods. Post-treatment glucose and fructosamine concentrations were measured using the Glucose Reagent and Unimate Fructosamine Kit for the Cobas Fara II (Roche Diagnostic Systems, Somerville, NJ). Insulin and C-peptide were measured by commercially available radioimmunoassays (Human Insulin Specific RIA, Human C-Peptide RIA; Linco Research, St Charles, MO). The human insulin RIA had less than 0.2% cross-reactivity with human proinsulin. The homeostasis model assessment (HOMA-IR = [IU/mL insulin · mmol/L glucose]/22.5)[26] was used to determine insulin resistance, and the quantitative insulin-sensitivity check index (QUICKI, Insulin Sensitivity = 1/[log(fasting insulin) + log(fasting glucose)])[27] was used to assess insulin sensitivity.

Statistical Considerations

BMDP Statistical Software (Release 7.0, Los Angeles, CA) was used for all statistical calculations. Each variable was tested for normality and equality of variance between groups. Log transformations were performed for variables that violated these tests (fasting insulin, abdominal fat tissue, abdominal soft tissue). Data are reported as the retransformed mean and SEM. Repeated-measures analysis of variance (ANOVA) and analysis of covariance (ANCOVA), adjusting for baseline measures, were used to detect significant treatment effects. Since baseline body composition scans were not performed, post-treatment abdominal body composition measures were covaried by baseline BW, which significantly predicted the outcome measure. Fasting plasma carbohydrate measures were covaried by baseline values for each variable. If a significant ANOVA or ANCOVA *P* value was detected, then Bonferroni-adjusted *t* tests were used for post hoc comparisons (each treatment group *v* control group, 4 comparisons). Correlations were assessed using the Pearson correlation (*r*). Because animals were imported in 3 cohorts (shipments) and housed in 2 animal buildings, stepwise regression analysis was used to determine if there was a significant effect of cohort or building on baseline and outcome mea-

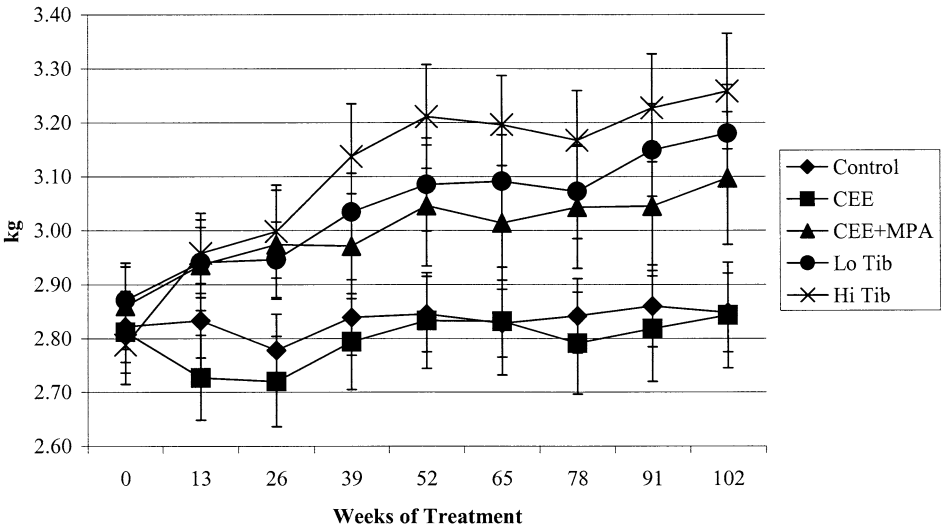

Fig 1. BW (mean ± SEM) in ovariectomized cynomolgus monkeys treated with various HRT regimens for 2 years. Control, n = 29; CEE, n = 27, CEE + MPA, n = 29; LoTib, n = 30; HiTib, n = 31. CEE, conjugated equine estrogens, ~0.625 mg/d equivalent; MPA, medroxyprogesterone acetate given continuously as ~2.5 mg/d equivalent; LoTib, low-dose tibolone, ~0.05 mg/d equivalent; HiTib, high-dose tibolone, ~0.20 mg/d equivalent.

sures. Importation cohort was identified as a significant factor influencing several outcomes (BW, fasting insulin, fasting glucose); therefore, it was included as a covariate for all variables.

RESULTS

Body Weight and Body Composition Analysis

There was a significant treatment effect on BW (Fig 1) (ANOVA, $P < .001$) and abdominal soft tissue (Table 1) (ANCOVA, $P = .003$). Post hoc comparison revealed that monkeys treated with CEE + MPA, LoTib, or HiTib gained more weight ($P = .005$, $P = .001$, $P < .001$, respectively) and had greater abdominal soft tissue mass compared to controls ($P = .02$, $P = .04$, $P = .002$, respectively), whereas the CEE-treated animals had the same BW ($P = .87$) and abdominal soft tissue as controls ($P = .80$). The greater abdominal soft tissue mass in tibolone-treated animals was due to more lean mass compared to controls ($P = .008$ for HiTib; $P = .002$ for LoTib), whereas CEE + MPA-treated animals had greater abdominal soft tissue mass due to a tendency for more fat mass (ANCOVA, $P = .29$) with no change in lean mass compared to controls (Table 1).

Fasting Carbohydrate Measures

CEE + MPA and HiTib treatment was associated with greater fasting insulin concentrations ($P = .008$, $P = .03$) and insulin/glucose ratios ($P = .03$, $P = .02$) compared to controls (Table 2). There was a strong trend for a treatment effect on fasting glucose concentrations (ANCOVA, $P = .06$), with CEE + MPA-treated animals having the greatest values. There was no treatment effect on fasting C-peptide (ANCOVA, $P = .32$), or fructosamine concentrations (ANCOVA, $P = .57$) (Table 2). Using fasting plasma glucose and insulin concentrations, the HOMA-IR calculation showed that compared to controls, treatment with CEE + MPA was associated with greater insulin resistance ($P = .008$), while the QUICKI calculation revealed impaired insulin sensitivity with CEE + MPA, LoTib, and HiTib treatment compared to controls ($P = .002$, $P = .03$, $P = .01$, respectively). CEE-treated animals were equivalent to controls in all plasma measures of carbohydrate metabolism. Significant correlations with HOMA-IR (all P values $\leq .05$) included BW ($r = 0.26$), soft mass ($r = 0.25$), fat mass ($r = 0.29$), and fructosamine levels ($r = 0.23$).

DISCUSSION

The objectives of the current study were to compare the effects of various HRT regimens on BW, body composition, and carbohydrate metabolism in surgically postmenopausal monkeys. We found that administration of CEE + MPA, LoTib, or HiTib was associated with increased BW and greater abdominal soft tissue compared to controls. The increase in soft tissue was due to significantly greater abdominal lean mass in monkeys treated with tibolone and nonsignificantly greater fat

Table 1. Effect of HRT Regimens on Body Weight and Abdominal Body Composition of Ovariectomized Cynomolgus Monkeys After 2 Years of Treatment

	Control (n = 29)	CEE (n = 27)	CEE + MPA (n = 28)	LoTib (n = 30)	HiTib (n = 31)	ANCOVA *P* Value
BW (kg)*	2.84 ± 0.04	2.83 ± 0.04	2.99 ± 0.04†	3.01 ± 0.04†	3.19 ± 0.04†	<.001
Soft tissue (g)	440.1 ± 14.9	454.4 ± 15.7	494.3 ± 15.9†	494.6 ± 14.9†	514.2 ± 14.4†	.003
Lean mass (g)	334.0 ± 7.5	324.7 ± 7.9	342.7 ± 8.0	356.2 ± 7.5	367.0 ± 7.3†	.001
Fat mass (g)	106.1 ± 15.8	129.7 ± 16.7	151.6 ± 16.9	138.5 ± 15.8	147.2 ± 15.3	.29

NOTE. Data are presented as the mean ± SEM, covaried by baseline BW and cohort of animals.

*Mean BW during treatment.

†Significantly different from control after Bonferroni adjustment (post hoc *P* value ≤ .05 for treatment *v* control, 4 comparisons.

Table 2. Effect of Various HRT Regimens on Fasting Plasma Measures of Ovariectomized Cynomolgus Monkeys After 2 Years of Treatment

	Control (n = 29)	CEE (n = 27)	CEE + MPA (n = 29)	LoTib (n = 30)	HiTib (n = 31)	ANCOVA *P* Value
Glucose (mg/dL)	65.7 ± 2.3	68.1 ± 2.4	75.6 ± 2.4	70.4 ± 2.3	68.7 ± 2.2	.06
Insulin (IU/mL)	27.7 ± 4.6	36.8 ± 4.9	49.0 ± 4.9*	43.5 ± 4.6	45.0 ± 4.4*	.02
Insulin/glucose ratio	0.42 ± 0.06	0.54 ± 0.06	0.64 ± 0.06*	0.58 ± 0.06	0.64 ± 0.05*	.03
C-peptide (ng/mL)	0.47 ± 0.05	0.55 ± 0.05	0.61 ± 0.06	0.62 ± 0.06	0.54 ± 0.05	.32
Fructosamine (μmol/L)	178 ± 5	177 ± 5	177 ± 5	177 ± 5	187 ± 5	.57
HOMA-IR	4.84 ± 1.0	6.5 ± 1.1	9.6 ± 1.1*	8.4 ± 1.0	8.2 ± 1.0	.02
QUICKI	0.361 ± 0.006	0.342 ± 0.006	0.329 ± 0.006*	0.338 ± 0.006*	0.337 ± 0.006*	.01

NOTE. Data are presented as the mean ± SEM, covaried by baseline values and cohort of animals.
*Significantly different from control after Bonferroni adjustment (post hoc *P* value ≤.05 for treatment *v* control, 4 comparisons.

mass in CEE + MPA–treated monkeys (Table 1). Treatment with CEE + MPA and HiTib resulted in greater fasting insulin concentrations and insulin/glucose ratio compared to controls (Table 2). All HRT-treated monkeys except those receiving unopposed CEE became less insulin sensitive (by QUICKI calculation); CEE + MPA–treated animals became more insulin-resistant compared to controls (by HOMA-IR). The increased BW and greater abdominal soft tissue mass associated with CEE + MPA treatment in the current study is consistent with previous studies[5] and is likely due to an MPA-related phenomenon, since, similar to previous trials,[14] animals treated with CEE did not experience weight gain or have greater abdominal soft tissue compared to controls. LoTib- and HiTib-treated monkeys also gained weight compared to controls, consistent with results of one short-term study in women,[28] but different from others.[29,30] As in previous studies, estrogen treatment protected against weight and fat gain,[5,6] but unlike previous studies in monkeys,[5] we did not find that BW increased over time with ovariectomy (Fig 1) and have no clear explanation as to why this occurred. It is possible that there may have been differences in the 3 shipments (cohorts) of imported animals, predisposing them to respond differently to the diet and to hormone intervention, as 1 cohort of animals did not gain as much weight as the other 2 cohorts. The confounding effect of importation cohort on outcome variables was statistically corrected by covarying by cohort; however, this manipulation may not fully correct for all the affected outcome variables. Treatment with CEE + MPA, LoTib, or HiTib resulted in greater abdominal soft mass compared to controls; however, the composition of the abdominal soft tissue was different among these 3 treatment groups. There was significantly more lean tissue in HiTib-treated monkeys (9.0% greater than control) and a tendency for greater fat mass (38.7% greater than control). A similar pattern was seen with LoTib (6.6% greater lean tissue and 30.5% greater fat tissue than control). The effect of tibolone on abdominal lean mass was likely due to the androgenic activity of the Δ^4-isomer metabolite of tibolone, consistent with previous studies where tibolone treatment increased lean body mass in postmenopausal women.[31] CEE + MPA–treated animals had no difference in abdominal lean tissue compared to controls (2.6% greater lean tissue than control) but a tendency for greater abdominal fat mass (42.9% greater fat tissue than control). Previous trials with CEE + MPA–treated animals also report significantly increased soft tissue mass, accounted for by increased fat mass with no effect of treatment on lean mass.[5]

Previous studies in monkeys treated with CEE + MPA show, by computerized tomography (CT), preferential accumulation of subcutaneous rather than the more deleterious visceral fat depot.[32] Unlike DEXA, CT scans do not measure lean tissue mass, and because the androgenic metabolite of tibolone was likely to affect lean tissue mass, DEXA was the method of choice in the current study, despite its inability to distinguish subcutaneous and visceral fat. To reduce anesthesia time for the animals, body scans were limited to the abdominal region. Although a strong correlate with insulin resistance, abdominal body composition of women does not always parallel that of the entire body.[33] Despite this limitation, there is evidence that ponderosity and abdominal fat but not peripheral fat strongly correlate with impaired glucose tolerance in female cynomolgus monkeys[34]; therefore the abdominal region scans in our current study were likely sufficient to assess changes in whole body composition related to carbohydrate metabolism. Further, soft tissue and fat mass strongly correlated with BW in the current study ($r = 0.90$, $r = 0.81$, respectively; $P < .05$ for both correlations). While baseline BW was not different, baseline body scans would have further elucidated whether post-treatment differences in abdominal body composition were due to a change over time with HRT.

There was a strong trend for an HRT treatment effect on fasting glucose concentrations, despite no differences between groups in overall glycemic control (fructosamine concentrations, Table 2), suggesting a different treatment effect on the fasting versus the postprandial state. In particular, CEE + MPA–treated animals had a tendency for the greatest fasting glucose concentrations in the current study (15.1% greater than controls), consistent with previous findings in nonhuman primates.[5] The neutral effect of tibolone on blood glucose values in the current trial agrees with the majority of the earlier studies in women,[28,35-38] although there are some reports of tibolone increasing fasting glucose concentrations.[29,39]

Greater fasting insulin concentrations and a greater fasting insulin/glucose ratio occurred with CEE + MPA treatment, compared to controls, consistent with a previous trial where treatment with CEE + MPA increased fasting insulin concentrations compared to both controls and CEE-treated animals.[5] HiTib treatment was also associated with greater fasting insulin concentrations and a greater fasting insulin/glucose ratio, sim-

ilar to one study[40] but different from others.[41,42] The HOMA-IR analysis, despite its limitations regarding sensitivity of the calculation, revealed that CEE + MPA animals had greater insulin resistance. Insulin sensitivity by QUICKI calculation was impaired with CEE + MPA, LoTib, and HiTib treatment compared to control-treated animals. This is consistent with our previous report that treatment of ovariectomized monkeys with MPA alone or CEE + MPA equally reduces insulin sensitivity (as determined by minimal model analysis) by about 50% compared to both controls and CEE-treated animals.[14] The less pronounced impact of CEE + MPA on insulin sensitivity in the current study may be due to the methods of assessment, ie, QUICKI calculation versus minimal model analysis.

In the current trial, abdominal body fat was significantly associated with all measures of insulin resistance, suggesting that changes in body composition are associated with unfavorable changes in insulin sensitivity and carbohydrate-related CVD risk factors. A previous long-term study confirms changes in BW/body composition and insulin concentrations with long-term HRT in monkeys[5]; however, this and the current study were unable to determine if changes in insulin sensitivity were prior to or secondary to changes in body composition. Short-term studies (12 weeks) with HRT have documented changes in insulin sensitivity (by minimal model analysis or hyperinsulinemic euglycemic clamp) but not fasting insulin concentrations, prior to changes in BW.[14,43,44] This suggests a BW-independent mechanism is responsible for the rapid, subtle effects of HRT on insulin sensitivity, while alterations in body composition with long-term HRT administration may promote insulin resistance enough to be detected by increased fasting insulin or glucose concentrations. Because the current study was long-term, there was ample opportunity for treatment to affect body composition and, subsequently, insulin resistance. Mechanisms for this relationship likely include changes in adipocyte release of free fatty acids, tumor necrosis factor-α, adiponectin, resistin, and leptin. These factors directly impact insulin action and secretion and profoundly influence whole body insulin sensitivity.[45-48]

There are numerous observations that progestogens reduce insulin sensitivity in women[4,10,43,49-51] and various animal models,[52-55] and the mechanisms include impairment in post-insulin receptor signaling,[56] which, in the case of MPA, may be due to activation of the glucorticoid receptor.[57] Interestingly, animals treated with tibolone gained more weight than MPA-treated animals, but MPA was associated with greater insulin resistance, possibly attributed to the greater abdominal fat mass with CEE + MPA treatment versus the greater lean mass with tibolone treatment.

The neutral effect of CEE on carbohydrate metabolism in the current study agrees with previous short-term and long-term studies.[5,14] However, there appears to be a dose-dependent effect of CEE on insulin sensitivity in postmenopausal women, in that while 0.3 mg CEE/d improves, 0.625 mg/d is neutral, and 1.25 mg/d impairs insulin sensitivity.[10,58] Estradiol appears to improve[44] or have neutral effects on insulin sensitivity,[6] and thus may be a better estrogen choice for ERT, although comparisons of CEE and estradiol in the same study are limited and routes of hormone delivery vary within the available reports.[59-61] Thus, it is difficult to ascertain whether it is an estradiol benefit and/or the transdermal administration that confers benefit.

Studies in postmenopausal women treated with HRT are comparable, but not fully extendable, to findings in the current trial with ovariectomized monkeys. For example, during menopause women gain weight and abdominal fat,[62] and treatment with CEE only slows, rather than prevents this weight/fat gain.[9,63] Addition of MPA also protects against postmenopausal weight/fat gain in women, but not as robustly as does CEE alone.[15] Treatment with either CEE or CEE + MPA improves or has no effect on fasting glucose[15,61,64-73] and fasting insulin concentrations.[59,74] One large study suggested that women who received the greatest benefits of HRT on these carbohydrate measures had the highest fasting glucose concentrations at baseline,[15] consistent with beneficial effects in diabetic women and a decrease in occurrence of diabetes with HRT.[75] Addition of MPA tends to impair glucose tolerance in postmenopausal women,[15,64,65,67] but this is not always evident by changes in fasting glucose concentrations.[67-71] More sensitive indices of insulin sensitivity such as the insulin tolerance test or minimal model analysis reveal that moderate doses of CEE typically have neutral[59-61] or beneficial effects,[4,10,74,76,77] while addition of MPA typically worsens insulin sensitivity in women.[4,10,73]

Limitations of the current study include the lack of an intact (non-ovariectomized) group. This group would have been useful to determine if ovariectomy itself was associated with changes in body composition and plasma carbohydrate measures; however, this was not the specific aim of the study. The atherogenic diet selected for the current study was intended to mimic a typical western diet and to promote dyslipidemia and atherosclerosis.[25] This diet may have had potentially confounding effects on body composition and, subsequently, insulin sensitivity; however, all treatment groups were fed this same diet. As previously discussed, baseline scans to compare changes across time would have been useful as would have been the use of CT scans to distinguish subcutaneous from visceral fat with this technique, since abdominal body composition is not fully representative of the total body. There was a 2-month difference between the time of body composition scans (23 months) and assessment of fasting carbohydrate measures (21 months) due to technical logistics involved with the large number of animals in this study. It is unlikely that any changes happened in this relatively short period of time (compared to the 2-year duration of the study) that would affect correlations between these 2 measures.

As obesity and insulin resistance are associated with diabetes and CVD in humans, it would be expected that unfavorable changes in body composition and fasting insulin concentrations in the current trial would be detrimental. It has been reported previously by our group that monkeys with greater central obesity have more coronary artery atherosclerosis.[78] However, in the current trial, compared to controls, there was less atherosclerosis in monkeys treated with both CEE and CEE + MPA and no difference between controls and monkeys treated with LoTib or HiTib.[25] Thus, it appears that if in the current trial, the changes in body composition and fasting glucose and insulin concentrations are increasing risk for CVD, it is through another pathway such as those involving plaque stability and

thrombosis, rather than merely atherosclerosis extent. However, the negative effects of CEE + MPA and tibolone on body composition and carbohydrate metabolism remain significant risk factors for development of diabetes which may subsequently increase risk of CVD.

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