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Addition of medroxyprogesterone acetate to conjugated equine estrogens results in insulin resistance in adipose tissue

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Abstract

The purpose of this study was to determine if the insulin resistance we have previously reported in surgically postmenopausal primates treated with combined hormone therapy (HT) is due in part to effects on adipose tissue. Eighty-seven ovariectomized monkeys were fed a moderately atherogenic diet (0.28 mg cholesterol per kilocalorie [0.07 mg/kJ]) and randomized to receive no hormones (control, n = 29), estrogen therapy (ET, conjugated equine estrogens, 0.625 mg/d human equivalent; n = 29), or HT (ET + medroxyprogesterone acetate, 2.5 mg/d human equivalent; n = 29) in the diet for 2 years. Fasting glycemic measures were made at baseline and at the end of treatment. Circulating adiponectin measures, insulin tolerance tests, glucose tolerance tests, and isolated adipocyte glucose uptake assays were performed at the end of the trial. Hormone therapy–treated animals were insulin resistant, as determined by greater fasting insulin concentrations ($P = .008$), greater homeostasis model assessment of insulin resistance (HOMA-R) value ($P = .005$) and slower glucose disposal after insulin administration (K_{ITT}; $P = .02$) when compared with controls. Subcutaneous adipocytes from HT-treated monkeys had a greater ED_{50} for insulin ($P = .04$) and lower maximal glucose uptake per cell ($P < .001$) compared with controls, suggesting impaired adipocyte insulin sensitivity. Adipocytes were smaller ($P = .001$) and adiponectin concentrations were greatest in the ET group ($P = .02$), with no difference between controls and HT-treated monkeys. In conclusion, estrogen therapy resulted in smaller adipocyte size and greater adiponectin concentrations than control or HT. Hormone therapy resulted in impaired insulin sensitivity and adipocyte glucose uptake compared with controls, whereas there was no difference between ET and controls. Because no adverse effects were found with ET alone, it is likely that the progestin, medroxyprogesterone acetate, resulted in the negative effects of the combined HT regimen on whole-body insulin sensitivity, which were mediated, in part, by reductions in adipose tissue responses to insulin.

1. Introduction

Menopause results in estrogen and progesterone deficiency and is associated with insulin resistance and increases in cardiovascular disease (CVD) risk factors such as increased low-density lipoprotein cholesterol, decreased high-density lipoprotein cholesterol, and increased body fat. Approximately 44% of otherwise healthy postmenopausal women are insulin resistant [1,2]. More importantly, hyperinsulinemia, an indicator of insulin resistance, is independently associated with coronary artery disease [3]. Estrogen therapy (ET) favorably affects many CVD risk factors, including plasma lipids, body composition, and fasting glucose and insulin concentrations [4]. In women with diabetes mellitus, ET improves fasting glucose levels [5] and decreases insulin requirements [6,7], suggesting improved insulin sensitivity. In contrast, the addition of a synthetic progestin, such as medroxyprogesterone acetate (MPA), which is commonly prescribed with an ET regimen (hormone therapy [HT]), typically impairs glucose metabolism in both humans [2] and monkeys [8].

The physiologic mechanisms responsible for changes in insulin sensitivity with sex hormones is unclear, but effects on the main insulin-responsive tissues such as adipose, liver, and muscle are likely. Several studies suggest that women gain weight and abdominal fat at a more accelerated rate during menopause, and central deposition of fat is an

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independent predictor of CVD [9,10]. Treatment of monkeys with ET has been shown to prevent weight/fat gain associated with menopause, whereas addition of MPA attenuates the benefits of estrogen on body composition [8]. A previous report on the current trial showed that monkeys treated with HT had greater abdominal soft tissue mass (including fat mass) compared with control or ET-treated monkeys [11]. Similarly, the Postmenopausal Estrogen/Progestin Interventions Trial showed that women treated with HT (most regimens containing MPA) tended to gain more weight and to have greater increases in hip and waist girth compared with those assigned to ET, but all treatment groups gained less than controls [12].

Estrogen treatment in ovariectomized rats results in smaller adipocytes, whereas progesterone treatment results in larger, more insulin resistant fat cells [13,14]. Although compared with muscle, adipose tissue accounts for a relatively small percentage of insulin-stimulated glucose disposal [15], fat tissue influences whole-body insulin sensitivity via release of numerous adipocytokines, such as adiponectin, which may affect both insulin sensitivity and vascular reactivity [16]. Thus, the objective of the current study was to determine if adipose tissue is partly responsible for the insulin resistance associated with HT.

2. Methods

2.1. Animal studies

This study used 87 adult female cynomolgus monkeys (*Macaca fascicularis*) imported from Indonesia through a collaborative agreement with Institut Pertanian Bogor (Bogor, Indonesia) as part of a larger, separate study [17] examining the effects of hormone treatment on development of atherosclerosis. After quarantine, all animals were ovariectomized and fed a moderately atherogenic diet containing 42% of energy from fat and 0.28 mg cholesterol per kilocalorie (0.07 mg/kJ) for 6 months. Social groups of monkeys ($n \cong 5$) were randomized by using a permuted block randomization scheme into 1 of 5 treatment groups. Only 3 treatment groups are reported in the current study. The following treatments were given for 2 years: (1) no hormones (control, n = 29); (2) ET as conjugated equine estrogens (CEE, 0.042 mg/kg, 0.625 mg/d dose equivalent for women, n = 29); or (3) HT as CEE combined with MPA (0.167 mg/kg, 2.5 mg/d equivalent dose for women, n = 29). The hormones were administered within the diet. At the end of the trial, animals were sedated with ketamine (Ketaset, 15 mg/kg IM; Fort Dodge Laboratories, Fort Dodge, IA) and anesthetized with sodium pentabarbitol (60 mg/kg IV; Butler, Columbus, OH), after which time subcutaneous and visceral abdominal fat samples were immediately removed. 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 Animal Care and Use Committee.

2.2. Clinical chemistry measures

Animals were sedated with ketamine (15 mg/kg IM) for sample collection of baseline plasma (6 weeks after ovariectomy) before initiation of treatment for determination of fasting glucose (intra-assay coefficient of variation [CV], <5.0%; Glucose Diagnostic Kit, Sigma, St Louis, MO), insulin (intra-assay CV, 5.5%; Human Insulin Specific RIA Kit, Linco Research, St Charles, MO), and fructosamine concentrations (intra-assay CV, 4.8%; Fructosamine Diagnostic Kit, Sigma). The human insulin radioimmunoassay has <0.2% cross-reactivity with human proinsulin. Homeostasis model assessment of insulin resistance (HOMA-R) values were calculated from the product of glucose (mmol/L) and insulin (μUI/mL) divided by 22.5 and used as an indicator of insulin resistance [18]. After 21 months of hormone treatment, insulin sensitivity was assessed by an intravenous glucose tolerance test (IVGTT) in half of the study animals (n = 14 for control; n = 12 for ET; n = 14 or HT) and by an insulin tolerance test (ITT) in the other half (n = 15 for control; n = 17 for ET; n = 15 for HT) as previously described [8,19]. Because the order of treatment was randomly assigned, this should provide a random sample for the 2 tests. Posttreatment fasting glucose and fructosamine concentrations were measured with the Glucose Reagent Kit (intra-assay CV, <3.0%) and Unimate Fructosamine Kit (intra-assay, CV <3.0%) for the Cobas Fara II (Roche Diagnostic Systems; Somerville, NJ). Adiponectin concentrations in plasma were determined by enzyme-linked immunosorbent assay (Linco Research). Measurements for the GTT and ITT included the glucose area under the curve (AUC), calculated as the total area including all time points; the disappearance rates (*K* value) for glucose and insulin were calculated from the linear portion of the curve [19].

2.3. Isolation of and glucose uptake in adipocytes

Fresh subcutaneous and visceral fat (2.0 g each) biopsy specimens from the abdominal region were minced and digested in 6.0 mL of sterile digestion medium (1.3 mg/mL collagenase, 10 g/L Dulbecco's modified Eagle's medium-low glucose, 25 mmol/L HEPES, 4 mmol/L $NaHCO_3$, 4% bovine serum albumin [BSA] fatty acid free) for 45 minutes at 37°C to release adipocytes [20]. After digestion, they were filtered (250 μmol/L) to remove connective tissue and washed 3 times in wash buffer (116 mmol/L NaCl, 5 mmol/L KCl, 2 mmol/L $CaCl_2$, 1 mmol/L $MgSO_4 \cdot 7H_2O$, 1 mmol/L $NaH_2PO_4 \cdot H_2O$, 25 mmol/L HEPES, 2 mmol/L Na pyruvate, 0.4% BSA, pH 7.6). Viability of cells was confirmed by Trypan Blue exclusion, and aliquots of cell suspension were stored at −70°C for DNA quantitation.

Glucose uptake was assessed by modification of previously published methods [21]. Cells were concentrated and resuspended in glucose transport buffer (GTB, 116 mmol/L NaCl, 5 mmol/L KCl, 2 mmol/L $CaCl_2$, 1 mmol/L $MgSO_4 \cdot 7H_2O$, 1 mmol/L $NaH_2PO_4 \cdot H_2O$, 25 mmol/L HEPES,

Table 1
Effect of hormone replacement therapy on fasting carbohydrate measures in ovariectomized cynomolgus monkeys after 2 years of hormone treatment

	Control, n = 29	ET, n = 29	HT, n = 29	ANCOVA *P*
Fasting glucose (mmol/L)	3.55 (−0.12, +0.12)	3.71 (−0.13, +0.13)	4.04 (−0.14,+0.14)	.08
Fasting insulin (pmol/L)	179 (−15.8, +17.9)	239 (−21.5, +23.7)	259* (−23.7,+25.8)	.02
HOMA-R value	4.05 (−0.88, +1.1)	5.43 (−0.9, +1.1)	6.78* (−0.86,+1.2)	.005
Fructosamine (μmol/L)	177.8 (−4.6, +4.6)	173.5 (−4.6, +4.6)	176.3 (−4.6,+4.7)	.87

Data were originally log transformed and are presented here as the retransformed mean (−SEM, +SEM).
*$P \leq .05$, significantly different from control group.

2 mmol/L Na pyruvate, 1% BSA fatty acid free, pH 7.6) at a ratio of 1 g cells per 12 mL GTB. Cell suspension (120 μL) was incubated 30 minutes in 80 μL GTB containing 0 to 43 nmol/L insulin (Novolin R; Novo Nordisk, Clayton, NC). The glucose analogue, phloretin (12 nmol/L, Sigma), was used as a negative control because it inhibits glucose transport. Cells were incubated in [2-^{3}H]deoxy-D-glucose (0.062 μCi mmol^{-1} L^{-1}; ICN Radiochemicals, Costa Mesa, CA) for 10 minutes and then placed in a 500-μl microcentrifuge tube containing 200 μL silicon oil (L-45, OSi Specialties, Danbury, CT). The suspension was centrifuged for 30 seconds (16,000*g*) to separate cells (above oil interface) from unincorporated glucose (below oil interface). The top half of the tubes were counted (Beckman Coulter LS6500 Multi-Purpose Scintillation Counter; Fullerton, CA), and counts were divided by phloretin controls to correct for unincorporated glucose trapped in silicon oil (intra-assay CV, 15.9%). Half-maximal glucose uptake (ED_{50} for insulin) was calculated by nonlinear regression analysis. Glucose uptake counts were normalized by cell number (nanograms DNA in 120 μL cell suspension per 6 pg DNA per cell). DNA was quantitated by using Hoechst 33258 fluorescent dye (Eastman Kodak, Rochester, NY) in a minifluorometer (Hoefer, San Francisco, CA).

Fat cell size was assessed histomorphometrically as the cell diameter of 100 viable adipocytes per animal for each fat depot. Images were captured using a Sony Mavica digital camera mounted on a Nikon Labophot-2 microscope and analyzed with Scion Image for Windows (Beta 3b for Windows; Scion, Frederick, MD).

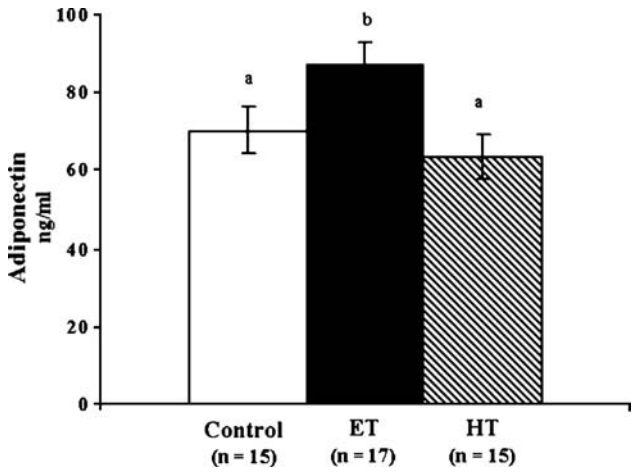

Fig. 1. Mean ± SEM for adiponectin concentrations in ovariectomized monkeys treated for 2 years with no hormones (control), ET (CEE 0.625 mg/d human equivalent), or HT (ET + MPA 2.5 mg/d human equivalent). ANOVA, $P = .02$. Unlike letters denote significantly different values by post hoc analysis.

2.4. Statistical considerations

BMDP Statistical Software (Release 7.0, Los Angeles, CA) was used for all statistical calculations. Log transformations were performed for variables that violated assumptions for parametric tests (fasting glucose, fasting insulin, fructosamine, all GTT calculations, all adipocyte measures), and reported means and SEM were retransformed into original units. Analysis of variance (ANOVA) was used to detect whether treatment group was a significant factor determining adiponectin, cell diameter, glucose uptake, and calculated ED_{50}. Where baseline pretreatment values were available measures (glucose, insulin, fructosamine, and HOMA-R values), they were included in the ANOVA model as a covariate (analysis of covariance [ANCOVA]) in detecting whether treatment group was a significant factor determining fasting carbohydrate. Duncan multiple range test was used for post hoc comparisons for variables with significant ANOVA/ANCOVA *P* values ($\leq .05$). Pearson *r* was used to determine correlations between insulin sensitivity and various outcomes.

To assess adipocyte 2-deoxy-D-glucose uptake in response to different concentrations of insulin, nonlinear regression analysis was performed with Sigma Plot version 4.0 (San Rafael, CA). The hyperbolic curve-fitting equation ($f = y_0 + ax/(b + x)$, where $y_0 = y$ intercept, a = maximum, $b = ED_{50}$, estimated dose of insulin required for 50% glucose uptake) was used to derive for each animal the ED_{50} and maximal glucose uptake per cell. Curves that did not conform to the constraints of the model were excluded from the analysis, including 9% of the curves from the control group, 15% from the ET group, and 15% from the HT group.

3. Results

3.1. Clinical chemistry measures

HT-treated animals had greater fasting insulin concentrations ($P = .02$) and a greater HOMA-R value ($P = .005$) (Table 1). There was no treatment effect on plasma fructosamine ($P = .87$) or glucose concentrations ($P = .08$). Adiponectin concentrations were 24% higher in the ET

Table 2
Effect of HT on glucose tolerance and insulin sensitivity

	Control, n = 14	ET, n = 12	HT, n = 14	ANOVA *P*
GTT				
Glucose AUC	8025 ± 420	8155 ± 552	8156 ± 372	.97
Insulin AUC	5598 (−745, +859)	6887 (−902, +1038)	5623 (−804, +938)	.55
Insulin-glucose ratio AUC	0.71 (−0.09, +0.10)	0.87 (−0.15, +0.18)	0.70 (−0.10, +0.12)	.58
1-h post-GTT glucose (mmol/L)	2.2 ± 0.140	2.40 ± 0.26	3.03 ± 0.41	.12
K_{GTT}	4.96 ± 0.405	4.99 ± 0.43	4.57 ± 0.26	.65
Insulin tolerance test				
K_{ITT}	9.95 ± 0.610	9.75 ± 0.61	8.01 ± 0.35[a]	.04

Intravenous glucose and insulin tolerance tests were administered to ovariectomized cynomolgus monkeys after 2 years of hormone treatment. Data are presented as mean ± SEM or the retransformed mean (−SEM, +SEM) for variables requiring log transformation (insulin AUC, insulin-glucose ratio AUC).
[a] Significantly different from control group.

group compared with control (P = .04), whereas concentrations in the HT-treated monkeys were not different from that of the control group (Fig. 1).

Insulin sensitivity determined by ITT was affected by treatment (P = .04; Table 2) such that the K_{ITT} of HT-treated animals was impaired (8.01% ± 0.35%/min) compared with both controls (9.95% ± 0.61%/min) and ET-treated monkeys (9.74% ± 0.61%/min). There was no significant treatment effect on any measure of glucose tolerance, but a trend (P = .12) for greater postchallenge glucose concentrations with HT was observed (Table 2).

3.2. Adipocyte size and glucose uptake

Compared with controls, ET-treated animals had smaller subcutaneous (P = .001) and visceral (P = .001) adipocytes (Fig. 2). Insulin sensitivity of subcutaneous adipocytes, assessed by ex vivo incubation with insulin, was significantly lower, as indicated by a greater ED_{50} for HT-treated animals compared with controls or ET-treated monkeys (P = .02; Fig. 3A) but no difference in visceral adipocyte ED_{50} (P = .43; Fig. 3B). Maximal glucose uptake was significantly different in subcutaneous adipocytes where the maximal glucose uptake in HT-treated animals was less than in the other treatment groups (P = .001; Fig. 4A). A similar trend was seen in the visceral fat cells; however, this was not significantly different (P = .37; Fig. 4B).

3.3. Body weight and composition

As previously reported, over the 2-year treatment period, HT-treated monkeys gained weight, whereas ET-treated and control animals did not [11]. These body weight changes were reflected in posttreatment body compositional differences, such that HT-treated monkeys had 43% greater abdominal fat mass compared with controls.

3.4. Correlations

With all treatment groups combined, insulin sensitivity (K_{ITT}) was associated with subcutaneous adipocyte insulin sensitivity (r = −0.49, P = .03), fasting glucose concentrations (r = −0.29, P = .05), and GTT glucose AUC (r = −0.62, P < .0001). Insulin resistance (HOMA-R value) was significantly associated with subcutaneous adipocyte size (r = 0.37, P = .003) with a strong significant association within the HT group (r = 0.75). Associations were also seen between HOMA-R value and total body fat (r = 0.3, P = .006), visceral adipocyte ED_{50} (r = 0.41, P = .009), adiponectin concentrations (r = −0.27, P = .013), and visceral adipocyte size (r = 0.28, P = .024).

4. Discussion

In this study, treatment with HT impaired whole-body insulin metabolism as evidenced by 49% greater fasting

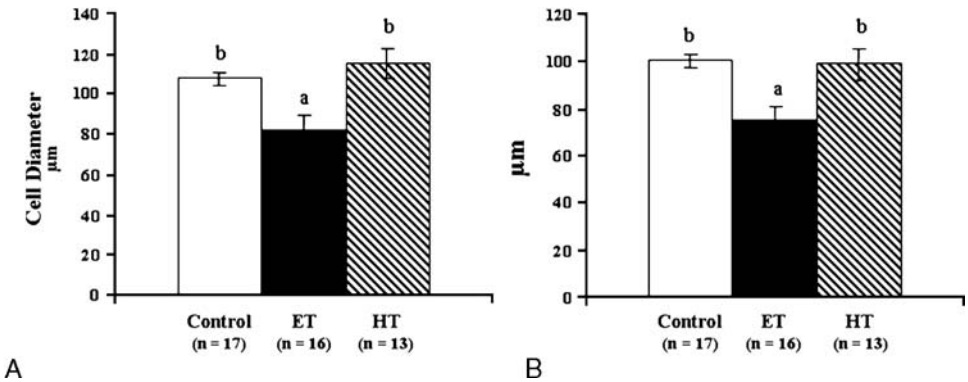

Fig. 2. Mean ± SEM for cell diameter (micrometers) of isolated subcutaneous (A) or visceral (B) adipocytes from ovariectomized monkeys treated for 2 years with hormone replacement therapy containing control, ET (CEE 0.625 mg/d human equivalent), or HT (ET + MPA 2.5 mg/d human equivalent). ANOVA: P = .001 (A); P = .001 (B). Unlike letters denote significantly different values by post hoc analysis.

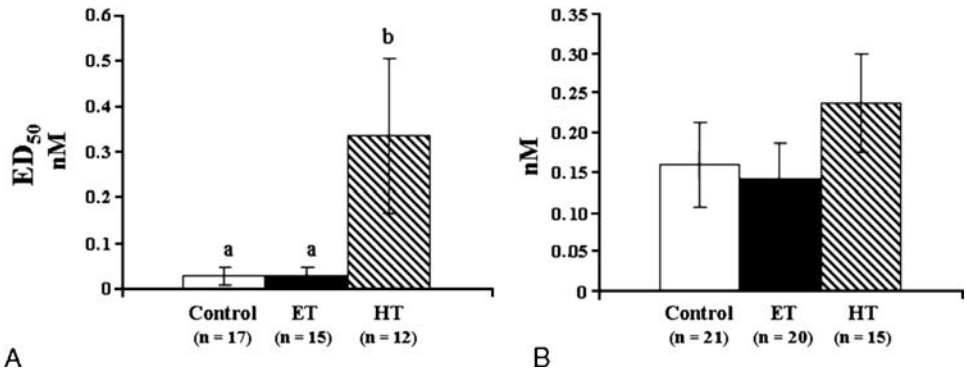

Fig. 3. Effect of various hormone replacement therapies on subcutaneous (A) or visceral (B) adipocyte insulin sensitivity in ovariectomized cynomolgus monkeys treated for 2 years with control, ET (CEE 0.625 mg/d human equivalent), or HT (ET + MPA 2.5 mg/d human equivalent). Isolated adipocytes were suspended in glucose transport buffer, incubated in various concentrations of insulin (0 to 43 nmol/L) followed by [2-^{3}H]deoxy-D-glucose (0.062 μCi mmol^{-1} L^{-1}). Data are the mean ± SEM for the estimated dose of insulin (nanomoles per liter) to stimulate 50% maximal glucose uptake (ED_{50}). ANOVA: P = .02 (A); P = .43 (B). Unlike letters denote significantly different values by post hoc analysis.

insulin concentrations, 68% greater HOMA-R value, and 19% slower K_{ITT} compared with controls. Subcutaneous adipocytes reflected these differences in whole-body insulin metabolism in that, compared with controls, there was impaired insulin sensitivity (Fig. 3) and lower maximal glucose uptake in adipocytes from animals treated with HT (Fig. 4). ET was associated with smaller adipocytes and greater adiponectin concentrations, whereas HT resulted in adipocytes of the same size and similar adiponectin concentrations as controls. Significant correlations between calculations of whole-body insulin sensitivity and insulin resistance with total body fat, adipocyte insulin sensitivity, and adiponectin concentrations suggest that adipose tissue insulin sensitivity is associated with peripheral insulin sensitivity.

The relative impairment of insulin sensitivity with HT reported in the current study agrees with previous trials where administration of CEE + MPA impaired insulin sensitivity in ovariectomized monkeys [22], premenopausal women [23], and postmenopausal women [24] when compared with treatment with CEE alone. The greater fasting insulin concentrations and HOMA-R values with HT compared with controls is similar to previous reports showing that treatment with CEE + MPA or MPA alone results in greater fasting and postchallenge plasma glucose and insulin concentrations [8,22,25].

HOMA-R values were higher in this nonhuman primate population than those reported in people [18], being the result of greater insulin concentrations that were determined by using an older assay that had cross reactivity with proinsulin. Newer assays for insulin in this species have been consistent with human values [26].

Estrogen therapy resulted in relatively smaller subcutaneous and visceral adipocytes (Fig. 2), consistent with our prior report [8]; however, the size of adipocytes did not correlate with adipocyte glucose uptake. Because the ET-treated animals had smaller adipocytes with no net difference in total body fat, this suggests a greater number of small fat cells, consistent with a previous report of

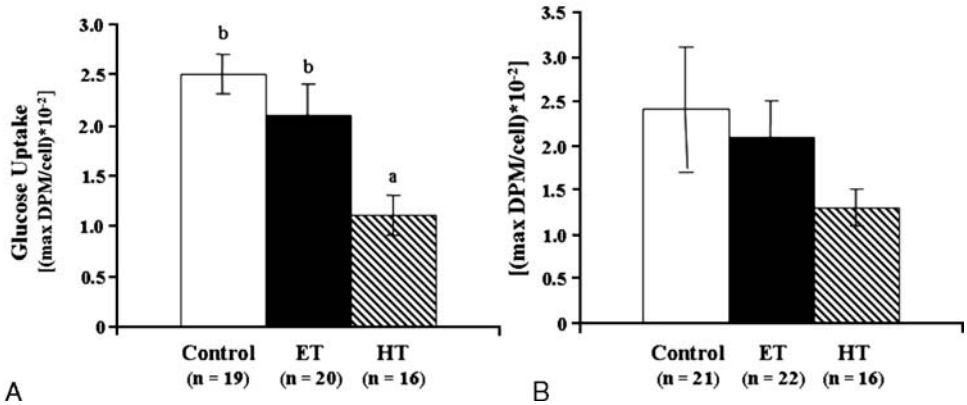

Fig. 4. Effect of various hormone replacement therapies on glucose uptake in isolated subcutaneous (A) or visceral (B) adipocytes from ovariectomized cynomolgus monkeys treated for 2 years with control, ET (CEE 0.625 mg/d human equivalent), or HT (ET + MPA 2.5 mg/d human equivalent). Isolated adipocytes were suspended in glucose transport buffer, incubated in various concentrations of insulin (0 to 43 nmol/L) followed by [2-^{3}H]deoxy-D-glucose (0.062 μCi mmol^{-1} L^{-1}). Data are the mean ± SEM for the calculated maximal glucose uptake per adipocyte (maximal DPM per cell × 10^{-2}). ANOVA: P = .001 (A); P = .37 (B). Unlike letters denote significantly different values by post hoc analysis.

estrogen-inducing preadipocyte proliferation [27]. This may be similar to the mechanism whereby thiazolidinediones improve insulin sensitivity in rats via stimulating proliferation of small adipocytes and thus shifting the fat distribution from the visceral to the subcutaneous depot [28]. It is unclear why improvements in fat cell size and adiponectin concentration with ET were not associated with improved insulin sensitivity. A potential limitation of this study is that more sensitive methodologies, such as glucose clamps or frequently sampled IVGTT, were not used to assess in vivo insulin sensitivity.

Hormone therapy resulted in relatively impaired glucose uptake (greater ED_{50}) and less maximal glucose uptake in subcutaneous adipocytes, consistent with previous reports that progesterone alone [13] or in combination with estradiol [29] decreases insulin sensitivity of fat cells. The impairment in adipocyte insulin sensitivity may have occurred secondary to changes in body composition because treatment with HT increased body weight in the current study in monkeys [11], and it has been shown that fat cells from obese and overweight humans are less responsive to insulin [30,31]. The correlation between body fat and insulin resistance by HOMA supports this possibility; however, the lack of baseline body fat measurement and adipocyte characterization prevents a complete assessment of monkeys before ovariectomy and initiation of hormone treatment.

Concordant with this, we show that fat mass is associated with adiponectin and that estrogen replacement causes adipocytes to be smaller; the addition of MPA abrogated differences in adiponectin concentrations. Visceral fat accumulation occurring during menopause is characterized by larger cells with greater potential for lipolysis [32,33]. This results in more circulating free fatty acids which are characteristic of an insulin-resistant state. Subcutaneous fat production of adiponectin is less influential in the circulating concentrations [34]. It is interesting to note that in this study adiponectin concentrations were associated not only with whole-body insulin sensitivity as measured by ITT, but also with visceral adipocyte insulin sensitivity.

Despite the adverse effects on whole-body and adipocyte insulin sensitivity reported here with HT, 2 large clinical trials, the Heart and Estrogen-Progestin Replacement Study and Women's Health Initiative Hormone Trial, reported a 20% to 35% decreased incidence of diabetes in postmenopausal women treated with CEE + MPA [35,36]. The beneficial effects seen in the clinical trials are likely due to the estrogenic components of HT, because studies have shown improvements in insulin sensitivity with estrogens [24], and others, including the present study, have shown adverse effects with the further addition of MPA [2]. The mechanism for the decreased incidence in diabetes does not appear to be related to changes in body weight [35]. It is possible that potential adverse effects of MPA on skeletal muscle (the primary tissue responsible for glucose clearance seen with the ITT) and fat may be countered by beneficial effects on the liver or pancreas. In women, estrogens have shown to improve hepatic insulin clearance rate [24] and suppress hepatic glucose production [6]. There is also evidence for increased insulin secretion from islets incubated with estrogens and progestogens [37,38]. Other mechanisms for protection against diabetes may include antioxidant effects against beta-cell damage [39], anti-inflammatory actions [40], or by activation of peroxisome proliferator–activated receptors [41]. We anticipate that results from the estrogen-only arm of the Women's Health Initiative Hormone Trial may support this study in finding further reduction in measures of insulin resistance and incident diabetes compared with the HT [36].

In summary, HT in postmenopausal monkeys impaired whole-body insulin sensitivity, and this negative effect was likely mediated by the progestin, MPA, as ET alone did not affect this outcome. Lack of an MPA-only treated group does not allow us to make definitive conclusions about progestin effects; however, our previous study [22] found decreased insulin sensitivity with MPA alone, similar to combined CEE + MPA. Hormone therapy was also associated with impaired glucose uptake in response to ex vivo incubation of isolated subcutaneous fat cells with insulin. In a previous study, we showed that skeletal muscle from these same monkeys treated with HT expressed less glucose transporter 4 compared with ET- or control-treated animals [42]. Thus, the negative effects of HT on insulin sensitivity appear to involve both adipose and muscle tissue. Estrogen therapy resulted in smaller fat cell size and increased adiponectin concentrations. Although these beneficial effects of ET were not associated with improved insulin sensitivity in this study, the increases in adiponectin may be associated with improved vascular reactivity [16] and the decrease in CVD risk observed with ET [4].

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References

[1] Nabulsi AA, Folsom AR, White A, Patsch W, Heiss G, Wu KK, et al. Association of hormone-replacement therapy with various cardiovascular risk factors in postmenopausal women. N Engl J Med 1993;328:1069-75.

[2] Lindheim SR, Buchanan TA, Duffy DM, Vijod MA, Kojima T, Stanczyk FZ, et al. Comparison of estimates of insulin sensitivity in pre- and postmenopausal women using the insulin tolerance test and frequently sampled intravenous glucose tolerance test. J Soc Gynecol Investig 1994;1:150-4.
[3] Baltali M, Korkmaz ME, Kiziltan HT, Muderris IH, Ozin B, Anarat R. Association between postprandial hyperinsulinemia and coronary artery disease among non-diabetic women: a case control study. Int J Cardiol 2003;88:215-21.
[4] Grodstein F, Stampfer MJ, Manson JE, Colditz GA, Willett WC, Rosner B, et al. Postmenopausal estrogen and progestin use and the risk of cardiovascular disease. N Engl J Med 1996;335:453-61.
[5] Friday KE, Dong C, Fontenot RU. Conjugated equine estrogen improves glycemic control and blood lipoproteins in postmenopausal women with type 2 diabetes. J Clin Endocrinol Metab 2001;86:48-52.
[6] Brussaard HE, Leuven JAG, Frolich M, Kluft C, Krans HMJ. Short-term oestrogen replacement therapy improves insulin resistance, lipids and fibrinolysis in postmenopausal women with NIDDM. Diabetologia 1997;40:843-9.
[7] Barrett-Connor E, Laakso M. Ischemic heart disease risk in postmenopausal women: effects of estrogen use on glucose and insulin levels. Arteriosclerosis 1990;10:531-4.
[8] 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. Metab Clin Exp 1996;45:1254-62.
[9] Tchernof A, Poehlman ET. Effects of the menopause transition on body fatness and body fat distribution. Obes Res 1998;6:246-54.
[10] Lapidus L, Bengtsson C, Larsson B. Distribution of adipose tissue and risk of cardiovascular disease and death: a 12 year follow up of participants in the population study of women in Gothenburg, Sweden. Br Med J 1984;288:1401-4.
[11] Shadoan MK, Anthony MS, Rankin SE, Clarkson TB, Wagner JD. Effects of tibolone and conjugated equine estrogens with or without medroxyprogesterone acetate on body composition and fasting carbohydrate measures in surgically postmenopausal monkeys. Metab Clin Exp 2003;52:1085-91.
[12] Espeland MA, Stefanick ML, Kritz-Silverstein D, Fineberg SE, Waclawiw MA, James MK, et al. Effect of postmenopausal hormone therapy on body weight and waist and hip girths. J Clin Endocrinol Metab 1997;82:1549-56.
[13] Sutter-Dub MT, Kaaya A, Sfaxi A, Sodoyez-Goffaux F, Sodoyez JC, Sutter BC. Progesterone and synthetic steroids produce insulin resistance at the post-receptor level in adipocytes of female rats. Steroids 1988;52:583-608.
[14] Steingrimsdottir L, Brasel J, Greenwood MR. Hormonal modulation of adipose tissue lipoprotein lipase may alter food intake in rats. Am J Physiol 1980;239:E162-7.
[15] Ferrannini E, Bjorkman O, Reichard GAJ, Pilo A, Olsson M, Wahren J, et al. The disposal of an oral glucose load in healthy subjects. A quantitative study. Diabetes 1985;34:580-8.
[16] Ouchi N, Ohishi M, Kihara S, Funahashi T, Nakamura T, Nagaretani H, et al. Association of hypoadiponectinemia with impaired vasoreactivity. Hypertension 2003;42:231-4.
[17] Clarkson TB, Anthony MS, Wagner JD. A comparison of tibolone and conjugated equine estrogens effects on coronary artery atherosclerosis and bone density of postmenopausal monkeys. J Clin Endocrinol Metab 2001;86:5396-404.
[18] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9.
[19] Gee M, Zhang L, Rankin SE, Collins J, Kauffman RF, Wagner JD. Rosiglitazone treatment improves insulin regulation and dyslipidemia in type 2 diabetic cynomolgus monkeys. Metabolism 2004;53:1121-5.
[20] Rodbell M. Metabolism of isolated fat cells. J Biol Chem 1964;239:375-80.
[21] Pederson O, Gliemann J. Hexose transport in human adipocytes: factors influencing the response to insulin and kinetics of methylglucose and glucose transport. Diabetologia 1981;20:630-5.
[22] Cefalu WT, Wagner JD, Bell-Farrow AD, Wang ZQ, Adams MR, Toffolo G, et al. The effect of hormonal replacement therapy on insulin sensitivity in surgically postmenopausal cynomolgus monkeys (*Macaca fascicularis*). Am J Obstet Gynecol 1994;171:440-5.
[23] Kim C, Seidel KW, Begier EA, Kwok YS. Diabetes and depot medroxyprogesterone contraception in Navajo women. Arch Intern Med 2001;161:1766-71.
[24] Lindheim SR, Duffy DM, Kojima T, Vijod MA, Stanczyk FZ, Lobo RA. The route of administration influences the effect of estrogen on insulin sensitivity in postmenopausal women. Fertil Steril 1994;62:1176-80.
[25] 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 1995;273:199-208.
[26] Wager JD, Kavanagh K, Ward GM, Auerbach BJ, Harwood HJ, Kaplan JR. Old World nonhuman primate models of type 2 diabetes mellitus. ILAR J 2006;47:259-71.
[27] Anderson LA, McTernan PG, Barnett AH, Kumar S. The effects of androgens and estrogens on preadipocyte proliferation in human adipose tissue: influence of gender and site. J Clin Endocrinol Metab 2001;86:5045-51.
[28] Okuno A, Tamemoto H, Tobe K, Ueki K, Mori Y, Iwamoto K, et al. Troglitazone increases the number of small adipocytes without the change of white adipose tissue mass in obese Zucker rats. J Clin Invest 1998;101:1354-61.
[29] Kumagai S, Holmang A, Bjorntorp P. The effects of oestrogen and progesterone on insulin sensitivity in female rats. Acta Physiol Scand 1993;149:91-7.
[30] Stolic M, Russell A, Hutley L, Fielding G, Hay J, MacDonald G, et al. Glucose uptake and insulin action in human adipose tissue—influence of BMI, anatomical depot and body fat distribution. Int J Obes Relat Metab Disord 2002;26:17-23.
[31] Johnson JA, Fried SK, Pi-Sunyer FX, Albu JB. Impaired insulin action in subcutaneous adipocytes from women with visceral obesity. Am J Physiol Endocrinol Metab 2001;280:E40-9.
[32] Tchernof A, Desmeules A, Richard C, Laberge P, Daris M, Mailloux J, et al. Ovarian hormone status and abdominal visceral adipose tissue metabolism. J Clin Endocrinol Metab 2004;89:3425-30.
[33] Ferrara CM, Lynch NA, Nicklas BJ, Ryan AS, Berman DM. Differences in adipose tissue metabolism between postmenopausal and perimenopausal women. J Clin Endocrinol Metab 2002;87:4166-70.
[34] Garaulet M, Viguerie N, Porubsky S, Klimcakova E, Clement K, Langin D, et al. Adiponectin gene expression and plasma values in obese women during very-low-calorie diet. Relationship with cardiovascular risk factors and insulin resistance. J Clin Endocrinol Metab 2004;89:756-60.
[35] Kanaya AM, Herrington D, Vittinghoff E, Lin F, Grady D, Bittner V, et al. Glycemic effects of postmenopausal hormone therapy: the Heart and Estrogen/Progestin Replacement Study. A randomized, double-blind, placebo-controlled trial. Ann Intern Med 2003;138:1-9.
[36] Margolis KL, Bonds DE, Rodabough RJ, Tinker L, Phillips LS, Allen C, et al. Effect of oestrogen plus progestin on the incidence of diabetes in postmenopausal women: results from the Women's Health Initiative Hormone Trial. Diabetologia 2004;47:1175-87.
[37] Etchegoyen GS, Borelli MI, Rossi JP, Gagliardino JJ. Effect of 2-hydroxyoestradiol on insulin secretion in normal rat pancreatic islets. Diabetes Metab 1998;24:428-33.
[38] Sorenson RL, Brelje TC, Roth C. Effects of steroid and lactogenic hormones on islets of Langerhans: a new hypothesis for the role of pregnancy steroids in the adaptation of islets to pregnancy. Endocrinology 1993;133:2227-34.

[39] Eckhoff DE, Eckstein C, Smyth CA, Vilatoba M, Bilbao G, Rahemtulla FG, et al. Enhanced isolated pancreatic islet recovery and functionality in rats by 17beta-estradiol treatment of brain death donors. Surgery 2004;136:336-45.

[40] Thomas TN, Rhodin JA, Clark L, Garces A, Bryant M. A comparison of the anti-inflammatory activities of conjugated estrogens and 17-beta estradiol. Inflamm Res 2003;52:452-60.

[41] Keller H, Givel F, Perroud M, Wahli W. Signaling cross-talk between peroxisome proliferator–activated receptor/retinoid X receptor and estrogen receptor through estrogen response elements. Mol Endocrinol 1995;9:794-804.

[42] Shadoan MK, Zhang L, Wagner JD. Effects of hormone therapy on insulin signaling proteins in skeletal muscle of cynomolgus monkeys. Steroids 2004;69:313-8.