

# Conjugated Estrogens and Bazedoxifene Improve $\beta$ Cell Function in Obese Menopausal Women

Dragana Lovre,<sup>1,2</sup> Erin Peacock,<sup>3</sup> Bonnie Katalenich,<sup>4</sup> Cynthia Moreau,<sup>4</sup> Beibei Xu,<sup>1</sup> Chandra Tate,<sup>1</sup> Kristina M. Utzschneider,<sup>5</sup> Jean-François Gautier,<sup>6,7</sup> Vivian Fonseca,<sup>1,2</sup> and Franck Mauvais-Jarvis<sup>1,2</sup>

<sup>1</sup>Section of Endocrinology and Metabolism, Tulane University Health Sciences Center, New Orleans, Louisiana 70112; <sup>2</sup>Section of Endocrinology, Southeast Louisiana Veterans Healthcare System, New Orleans, Louisiana 70119; <sup>3</sup>Department of Medicine, Tulane University Health Sciences Center, New Orleans, Louisiana 70112; <sup>4</sup>Clinical Translational Unit, School of Medicine, Tulane University, New Orleans, Louisiana 70112; <sup>5</sup>Division of Metabolism, Endocrinology and Nutrition, VA Puget Sound Health Care System and University of Washington, Seattle, Washington 98108; <sup>6</sup>Department of Diabetes and Endocrinology, Assistance Publique-Hôpitaux de Paris, Lariboisière Hospital, University Paris-Diderot Paris-7, 75475 Paris, France; and <sup>7</sup>INSERM UMRS 1138, Cordeliers Research Center, 75006 Paris, France

ORCID numbers: 0000-0002-2607-8768 (D. Lovre); 0000-0002-0874-0754 (F. Mauvais-Jarvis).

**Context:** Studies suggest that menopausal hormone therapy (MHT) prevents type 2 diabetes (T2D). The combination of conjugated estrogens (CE) with the selective estrogen receptor modulator bazedoxifene (BZA) is an MHT that improves obesity and T2D in preclinical models of menopausal metabolic syndrome. The effect of CE/BZA on adiposity and glucose homeostasis in obese postmenopausal women is unknown.

**Objective:** To investigate the effect of CE/BZA on body composition, glucose homeostasis, and markers of inflammation in obese postmenopausal women.

**Research Design, Intervention, and Participants:** Randomized, double-blind, placebo-controlled pilot trial of 12 obese menopausal women assigned to 12-week treatment with CE 0.45 mg/BZA 20 mg (n = 7) or placebo (n = 5). At baseline and after 12 weeks, we assessed body composition (dual-energy X-ray absorptiometry), glucose homeostasis (IV glucose tolerance test), and inflammation biomarkers.

**Results:** Women treated with CE/BZA exhibited increased  $\beta$  cell function using homeostatic model assessment-B [median (interquartile range) CE/BZA vs placebo: 18.5 (−0.9 to 320.6)  $\mu\text{U}/\text{mM}$  vs −25.5 (−39.9 to −0.1)  $\mu\text{U}/\text{mM}$ ; P = 0.045], and decreased basal glucose concentrations ( $G_b$ ) [−5.2 (−9.2 to −1.7) mg/dL vs 2.7 (0.9 to 4.9) mg/dL; P = 0.029]. Insulin sensitivity was higher in the placebo arm [1.35 (1.12 to 1.82) ( $\mu\text{U}/\text{mL}$ )  $\text{min}^{-1}$  vs −0.24 (−1.50 to 0.19) ( $\mu\text{U}/\text{mL}$ )  $\text{min}^{-1}$ ; P = 0.029]. No changes between treatment groups were observed for the acute insulin response to glucose (AIRg), the disposition index (DI), body composition, and inflammatory biomarkers.

**Conclusions:** A 12-week treatment of obese postmenopausal women with CE/BZA improves fasting  $\beta$  cell function and glucose concentrations without change in AIRg, HOMA-IR, DI, body composition, or markers of inflammation.

Abbreviations: AIRg, acute insulin response to glucose; AUC, area under the curve; BZA, bazedoxifene; BMI, body mass index; CE, conjugated estrogens; CRP, C-reactive protein; DI, disposition index; DXA, dual-energy X-ray absorptiometry; FDA, Food and Drug Administration; FGF-21, fibroblast growth factor 21; FIC, fasting insulin clearance; G0, glucose at time zero;  $G_b$ , basal glucose concentration; GEZI, glucose effectiveness at zero insulin; GSIC, glucose stimulated insulin clearance; HDL, high-density lipoprotein; HOMA, homeostatic model assessment;  $I_b$ , basal insulin concentration; IQR, interquartile range; IR, insulin resistance; IVGTT, IV glucose tolerance test; LCN2, lipocalin 2; LDL, low-density lipoprotein; LMP, last menstrual period; MHT, menopause hormone therapy; P2, removal rate of insulin from the interstitial space; P3, the movement of circulating insulin to the interstitial space; PAI-1, plasminogen activator inhibitor-1; RBP4, retinol binding protein 4;  $S_G$ , glucose effectiveness;  $S_I$ , insulin sensitivity; SERM, selective estrogen receptor modulator; T2D, type 2 diabetes; TBARS, thiobarbituric acid reactive substances; TC, total cholesterol; WC, waist circumference; WHR, waist-to-hip ratio.

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**Freeform/Key Words:** menopause, estrogens, bazedoxifene,  $\beta$  cell function, diabetes

Observational studies and large randomized controlled trials suggest that menopausal hormone therapy (MHT) reduces adiposity, improves insulin resistance (IR), and delays the incidence of type 2 diabetes (T2D) [1–6]. However, using general estrogen therapy to prevent diabetes in women is neither recommended nor approved by the Food and Drug Administration (FDA). Therefore, treatments that provide the beneficial effects of estrogens on glucose homeostasis without adverse effects are needed. Selective estrogen receptor modulators (SERMs) are compounds that exert tissue-selective estrogen receptor agonist or antagonist activity. For example, bazedoxifene (BZA) is a SERM that exhibits estrogen agonist activity in bone but estrogen antagonist activity in breast and uterus. The combination of conjugated estrogens (CE) with BZA is approved by the FDA for treatment of postmenopausal vasomotor symptoms and prevention of osteoporosis [7, 8]. The combination CE and BZA (CE/BZA) provides the benefits of estrogen therapy such as reducing hot flashes and vulvar–vaginal atrophy, preventing menopausal osteoporosis while simultaneously protecting the endometrium and breast from estrogen stimulation and without the need of a progestin [9–15]. Using a mouse model of postmenopausal metabolic syndrome, we reported that CE/BZA prevents estrogen deficiency-induced obesity, T2D, and nonalcoholic fatty liver disease as efficiently as CE alone [16]. We found that CE/BZA increased fat oxidation and energy expenditure, thus preventing ectopic fat accumulation in liver and skeletal muscle and improving IR and glucose intolerance. In addition, in female diabetic mouse models of insulin deficiency, CE/BZA prevents  $\beta$  cell failure and delays diabetes [17]. The current randomized, double-blind, placebo-controlled pilot trial was designed to assess the effect of a 12-week treatment with CE/BZA vs placebo on body composition, glucose homeostasis, and markers of inflammation in obese postmenopausal women.

## 1. Participants and Methods

### A. Study Population

Participants were overweight or obese postmenopausal women 50 to 60 years of age ( $n = 12$ ), symptomatic (hot flashes, vaginal dryness) or asymptomatic, with fasting glucose  $<125$  mg/dL, and a normal mammogram within the past 12 months. Ten women had spontaneous menopause and 2 had surgical menopause. Key exclusion criteria were recent weight changes, current use of drugs that promote weight changes, MHT use within 3 months, contraindications to estrogens (history of thromboembolic disorder, coronary artery or cerebrovascular disease, clotting disorder, chronic liver disease, history of breast or uterine cancer, or unexplained vaginal bleeding). Menopause was defined as either (i) women with intact uterus and last menstrual period  $>1$  year but  $<5$  years or (ii) women with partial or complete hysterectomy with menopausal symptoms for  $>1$  year and  $<5$  years. Anthropometric data were measured at baseline and after 4 weeks, 8 weeks, and 12 weeks of treatment. Although 18 women were enrolled in the study, 4 withdrew from the study and 2 did not return to their planned study visit, resulting in 12 women completing the study. All volunteers gave their informed written consent to participate in the study. The Tulane University's Institutional Review Board reviewed and approved the protocols. Investigational New Drug exemption was granted by the FDA to use CE/BZA in women with history of hysterectomy.

### B. Randomization, Intervention, and Study Calendar

An independent biostatistician provided a random number table to the unblinded pharmacist for randomization. The random number table was generated based on the total number of subjects to be enrolled in a 1:1 ratio for placebo vs CE/BZA. The unblinded pharmacist used the random number table in sequential order for randomization assignment as subjects were enrolled. The research team remained blinded to the randomization assignment throughout the study. Treatments were CE 0.45 mg/BZA 20 mg, vs placebo. Drug was supplied by Pfizer Inc. Study design and visit schedule are shown in [Figure 1](#).

### C. Measurement of Body Composition

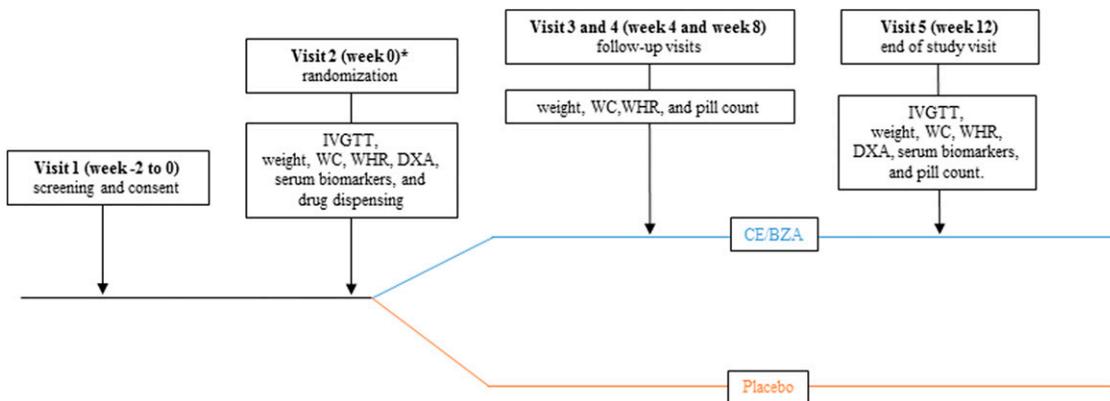
Bone density, body composition, adipose, and lean mass indices were measured with a dual-energy X-ray absorptiometry (DXA) apparatus (Hologic Inc., Bedford, MA) by trained and certified research staff. The machine was calibrated daily, and software and hardware were kept up to date during the data collection process.

### D. IV Glucose Tolerance Test

All women underwent an IV glucose tolerance test (IVGTT) at baseline and 12 weeks after treatment. After a 12-hour fast, blood was sampled from the antecubital vein at -15, -5, -1, 0, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 20, 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, and 180 minutes for measurement of serum glucose, insulin, and C-peptide concentrations (33 blood samples for glucose and insulin measurements over 3.5 hours and 16 samples for C-peptide measurement over the first 35 minutes). A bolus of dextrose was given at  $11.4 \text{ g/m}^2$  body surface area over 1 minute into the antecubital vein (at time zero) followed by a 30-second insulin infusion given at  $0.03 \text{ U/kg}$  body weight (at 20 minutes postdextrose injection). Note that for technical reasons, the IVGTT serum was processed in only 11 of 12 subjects (CE/BZA  $n = 6$ ).

### E. Laboratory Measurements

Plasma glucose was analyzed using an automated glucose analyzer (Quest Diagnostics Laboratory, Irving, TX). Insulin and C-peptide concentrations were quantified by ELISA (EMD Millipore Corporation, Billerica, MA) [18, 19]. Serum markers of IR and inflammation were determined using commercially available ELISA kits: fibroblast growth factor 21 (FGF-21) (R&D Systems, Inc., Minneapolis, MN) [20], leptin, plasminogen activator inhibitor-1 (PAI-1), retinol binding protein 4 (RBP4), and lipocalin 2 (LCN2) (Boster Biological Technology, Pleasanton, CA) [21–24], adiponectin (Crystal Chem, Elk Grove Village, IL) [25], and C-reactive protein (CRP) (Thermo Fisher Scientific Inc., Carlsbad, CA) [26], Gla-type and



**Figure 1.** Visit schedule. \*, Visit 1 and 2 can be combined if participant has had recent labs.

Glu-type Osteocalcin (Takara Bio Inc., Mountain View, CA) [27, 28], and Intact Osteocalcin (Alpco, Salem, NH) [29]. Quantitative determination of thiobarbituric acid reactive substances (TBARS) was done using a TBARS assay kit (ZeptoMetrix Corporation, Buffalo, NY). All tests were run in the same assay in a batch other than insulin and C-peptide. Because of large numbers of insulin and C-peptide samples, we those combining samples before treatment and 12 weeks after treatment in one assay. All samples were processed blindly to the clinical status of the participants.

### F. Data Analysis

Measures of insulin secretion, elimination, and sensitivity were derived by mathematical modeling analysis of the IVGTT glucose and insulin concentration profiles using the minimal model (MINMOD Millennium, Pasadena, CA, version 6.02) as previously described [30]. Four major independent parameters were derived from the analysis by the minimal model: the insulin sensitivity ( $S_I$ ) index, the acute insulin response to glucose (AIRg), the disposition index (DI), and glucose effectiveness ( $S_G$ ). Homeostatic model assessment (HOMA)-B and HOMA-IR were derived from the HOMA model as described by Matthews et al. [31]. HOMA- $\beta$  cell function [HOMA-B =  $20 \times$  fasting insulin/(fasting glucose/18–3.5)] and HOMA-IR [HOMA-IR = fasting glucose  $\times$  fasting insulin/405]. HOMA2- $\beta$  and HOMA2-IR (using fasting C-peptide and glucose) were calculated with a HOMA2 calculator software (version 2.2.3) released by the Diabetes Trials Unit, University of Oxford (see [www.dtu.ox.ac.uk/homacalculator/download.php](http://www.dtu.ox.ac.uk/homacalculator/download.php)). The measurement of insulin secretion, fasting insulin clearance (FIC), and glucose-stimulated insulin clearance (GSIC) over time zero to 20 minutes of the IVGTT were calculated by C-peptide area under the curve ( $AUC_{20}$ ), molar ratio of fasting C-peptide to insulin, and molar ratio of C-peptide to insulin  $AUC_{20}$ , respectively. Fasting insulin, glucose, and C-peptide were calculated as an average of three values drawn 5 minutes apart, at –15, –5, and –1 minute.

### F-1. Statistical analyses

Medians and interquartile ranges (IQRs) of baseline characteristics were calculated; differences in baseline characteristics between groups were tested using Wilcoxon rank sum tests. Differences in outcomes between baseline and 12 weeks were calculated; medians and IQRs of baseline, 12-week, and 12-week minus baseline outcome data are presented. Shapiro-Wilk tests were used to test for normality of all outcome variables. Because the null hypothesis of normality was rejected for some outcomes, Wilcoxon rank sum tests were used to test for differences between groups for all outcomes. Wilcoxon rank sum tests were also used to test for differences in baseline hormones by baseline metabolic parameters. All analyses were conducted using Stata 14.2 (StataCorp, College Station, TX).

## 2. Results

Twelve women received medication and completed the 12-week study between October 2015 and March 2018. There were no differences between the two groups at baseline (Table 1). Treatment with CE/BZA did not produce any change in body composition as assessed by anthropometric measures [weight, body mass index (BMI), waist circumference, and waist-to-hip ratio] or DXA compared with placebo (Table 2) [32]. No change in total fat mass or visceral fat mass was observed. As previously reported, women assigned to CE/BZA exhibited a trend toward increase in bone mineral density ( $P = 0.067$ ),  $t$  score and  $z$  score [33] compared with the placebo arm [32]. Women assigned to CE/BZA exhibited a decrease in  $G_b$  and an increase in  $\beta$  cell function assessed by HOMA-B, which utilizes insulin, associated with a trend toward increase in  $\beta$  cell function assessed by HOMA2-B ( $P = 0.068$ ), which utilizes C-peptide (Table 3; Fig. 2). There was no difference between the two groups in IR assessed by HOMA-IR or HOMA2-IR. Glucose homeostasis was further assessed using an

**Table 1. Baseline Characteristics**

	Treatment			<i>P</i> <sup>a</sup>
	Overall	Placebo (n = 5)	CE/BZA (n = 7)	
Race, n, Caucasian/black	10/2	5/0	5/2	0.470
Age, y	54.5 (53, 56.5)	55 (54, 58)	54 (52, 56)	0.188
Years since LMP	2.7 (1.7, 4.4)	4.5 (2.1, 4.8)	2.0 (1.4, 3.8)	0.123
Waist-to-hip ratio	0.85 (0.82, 0.94)	0.84 (0.82, 0.85)	0.9 (0.82, 0.95)	0.290
BMI, kg/m <sup>2</sup>	32.4 (30.7, 37.8)	31.2 (30.7, 38.6)	33 (30.6, 37)	0.935
WC, cm	108.0 (91.7, 115.6)	106.7 (92, 116.8)	109.2 (91.4, 114.3)	0.808
Fasting glucose, mg/dL	93.5 (83.5, 108)	88 (84, 98)	94 (83, 114)	0.465
TC, mg/dL	211.5 (190.5, 231.5)	216 (202, 245)	208 (167, 218)	0.291
HDL, mg/dL	63 (49.5, 71)	66 (58, 73)	60 (45, 69)	0.370
LDL, mg/dL	124 (111.5, 147.5)	128 (115, 147)	120 (99, 148)	0.465
Triglycerides, mg/dL	113.5 (82.5, 128.5)	103 (102, 125)	124 (81, 131)	0.935

Data are expressed as median (IQR).

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LMP, last menstrual period; TC, total cholesterol; WC, waist circumference.

<sup>a</sup>From Wilcoxon rank sum test.

IVGTT followed by mathematical modeling using the MINMOD Millennium software. Unlike results obtained by HOMA-B, and HOMA2-B, the AIRg was not different between the CE/BZA arm and the placebo [CE/BZA vs placebo: 189 (−31 to 444) vs −25 (−47 to −9), *P* = 0.273] (Table 3). However, unlike results obtained with HOMA-IR and HOMA2-IR, *S*<sub>I</sub> increased in the placebo arm. No changes were seen between groups in *D*<sub>I</sub>, *S*<sub>G</sub>, the basal insulin concentration, the removal rate of insulin from the interstitial space, the movement of circulating insulin to the interstitial space, glucose at time zero, and *S*<sub>G</sub> at zero insulin. FIC [CE/BZA vs placebo: −3.50 (−11.56 to 0.78) vs −0.25 (−0.70 to −0.0), *P* = 0.715] and GSIC [CE/BZA vs placebo: −32.8 (−123.6 to 6.9) vs −1.85 (−20.5 to 25.8), *P* = 0.201] were not different in the CE/BZA arm compared with placebo (Table 3). The effect of the treatment-related differences on serum markers of IR and inflammation are shown in Table 4. There were no differences between the two groups in systemic markers of IR (leptin, adiponectin, osteocalcin, RBP4, FGF-21), inflammation (LCN2, PAI-1, CRP), or oxidative stress (TBARS).

### 3. Discussion

The main finding of this study is treatment of obese menopausal women with the combination of CE with BZA improves  $\beta$  cell function [calculated by HOMA-B (*P* = 0.045) and to a lesser extent by HOMA2-B (*P* = 0.068)] and lowers *G*<sub>p</sub>. Large randomized controlled trials suggest estrogen therapy with or without a progestin reduces the incidence of T2D in postmenopausal women [1–3, 6, 34–36]. The exact mechanism of this antidiabetic effect is not understood clearly but could involve the beneficial effect of estrogen therapy on pancreatic  $\beta$  cell function and insulin secretion in postmenopausal women [6]. Indeed, our findings are consistent with preclinical studies showing that estradiol provides pancreatic islet protection from metabolic injuries in culture and *in vivo* [17, 37–43]. Estrogens also promote human islet protection in an *in vivo* diabetic environment [37, 44]. Additionally, BZA, a SERM, displays estrogen agonistic activity in female  $\beta$  cells, thus providing the beneficial effects of estrogens on  $\beta$  cell survival and insulin secretion [17]. Accordingly, treatment with CE/BZA prevents T2D induced by estradiol deficiency and Western diet in female mice [16], and mitigates  $\beta$  cell apoptosis, thus maintaining insulin secretion and delaying diabetes onset in female diabetic mouse models of  $\beta$  cell failure [17, 45]. CE and BZA provide similar protection in cultured islets from female human donors [17]. Therefore, the observed improvement in HOMA-B in

**Table 2. Effect of CE/BZA on Body Composition**

	Baseline			Week 12			Change from Baseline to Week 12			<i>P</i> <sup>a</sup>
	Placebo (n = 5)	CE/BZA (n = 7)	Placebo (n = 5)	CE/BZA (n = 7)	Placebo (n = 5)	CE/BZA (n = 7)	Placebo (n = 5)	CE/BZA (n = 7)		
<b>Anthropometric measurements</b>										
WC, cm	106 (93.5, 114.3)	109.2 (91.4, 116.8)	107.4 (91, 111.8)	106 (86.4, 112)	-2.5 (-2.5, 0.8)	-4.2 (-7.6, 0)	-2.5 (-2.5, 0.8)	-4.2 (-7.6, 0)	0.290	
Weight, kg	83.8 (75.8, 105.9)	90.5 (79.5, 104.9)	77.6 (75.6, 104.5)	92.3 (78.2, 103.8)	-0.9 (-1.4, -0.2)	-0.4 (-1.4, -0.5)	-0.9 (-1.4, -0.2)	-0.4 (-1.4, -0.5)	0.372	
WHR	0.87 (0.80, 0.90)	0.92 (0.8, 0.96)	0.82 (0.8, 0.92)	0.86 (0.8, 0.97)	-0.00 (-0.02, 0.00)	-0.05 (-0.06, 0.03)	-0.00 (-0.02, 0.00)	-0.05 (-0.06, 0.03)	0.465	
BMI, kg/m <sup>2</sup>	31.6 (30.4, 38.9)	32.6 (31.1, 37.0)	31.0 (30.1, 38.4)	32.9 (30.5, 36.8)	-0.5 (-0.6, -0.3)	-0.2 (-0.5, 0.3)	-0.5 (-0.6, -0.3)	-0.2 (-0.5, 0.3)	0.223	
<b>DXA</b>										
<b>Body composition</b>										
Total fat mass, g	33,163 (31,749, 49,295)	36,918 (33,221, 38,895)	33,630 (31,199, 47,887)	36,864 (34,883, 39,869)	-1408 (-1932, 467)	273 (-571, 974)	-1408 (-1932, 467)	273 (-571, 974)	0.361	
Total mass in g	84,587 (75,177, 105,711)	9,3007 (78,332, 104,466)	78,073 (75,141, 105,095)	93979 (78,280, 104,520)	-616 (-670, -36)	75 (-52, 282)	-616 (-670, -36)	75 (-52, 282)	0.273	
Total % fat	44.1 (41.4, 46.6)	41.2 (37.8, 45.3)	44.8 (42.3, 45.6)	41.1 (38.8, 44.6)	-1 (-1.3, 0.7)	0 (-0.7, 0.9)	-1 (-1.3, 0.7)	0 (-0.7, 0.9)	0.361	
A fat mass, g	3227 (2596, 4608)	3276 (2473, 4199)	3096 (2804, 4522)	3631 (3084, 3975)	-131 (-452, -86)	1.5 (-226, 611)	-131 (-452, -86)	1.5 (-226, 611)	0.465	
A total mass, g	6821 (6307, 9303)	6965 (6065, 10375)	6725 (6515, 9079)	8855 (6622, 9276)	-224 (-445, -96)	280 (-74, 1183)	-224 (-445, -96)	280 (-74, 1183)	0.201	
A % fat	47.3 (42.6, 47.5)	42.6 (39.4, 45.5)	45.3 (43, 46)	42 (40.9, 47.3)	-1.3 (-2.2, 0.3)	1.1 (-3.5, 3.5)	-1.3 (-2.2, 0.3)	1.1 (-3.5, 3.5)	0.234	
G fat mass, g	6098 (5620, 7879)	5959 (5567, 6916)	5648 (5464, 7701)	5505 (5461, 6370)	-178 (-189, -116)	-26 (-550, 279)	-178 (-189, -116)	-26 (-550, 279)	0.361	
G total mass, g	14926 (12,485, 17,238)	14896 (12,570, 16,043)	13780 (12,121, 17,181)	13901 (12,762, 16,668)	-57 (-364, -45)	89 (-1134, 403)	-57 (-364, -45)	89 (-1134, 403)	0.584	
G % fat	45.7 (45, 48.1)	42.8 (38, 50)	46.6 (44.8, 47.3)	42.3 (38.2, 49.7)	-0.90 (-1.30, -0.80)	0.35 (-0.30, 2)	-0.90 (-1.30, -0.80)	0.35 (-0.30, 2)	0.144	
<b>Adipose indices</b>										
Total body % fat	44.1 (41.4, 46.6)	43.2 (39.1, 45.3)	44.8 (42.3, 45.6)	40.8 (38.8, 44.6)	-1 (-1.3, 0.70)	-0.30 (-0.70, 0.30)	-1 (-1.3, 0.70)	-0.30 (-0.70, 0.30)	0.602	
A/G ratio	0.95 (0.95, 0.98)	1.04 (0.97, 1.16)	0.93 (0.92, 0.97)	1.06 (0.98, 1.07)	-0.02 (-0.03, -0.01)	0.01 (-0.05, 0.02)	-0.02 (-0.03, -0.01)	0.01 (-0.05, 0.02)	0.602	
VAT mass, g	731 (332, 947)	584 (539, 629)	708 (285, 813)	630 (543, 680)	-47 (-134, -23)	46 (-63, 118)	-47 (-134, -23)	46 (-63, 118)	0.289	
VAT volume, cm <sup>3</sup>	790 (359, 1023)	631 (583, 680)	766 (308, 879)	681 (587, 735)	-51 (-144, -24)	50 (-68, 127)	-51 (-144, -24)	50 (-68, 127)	0.289	
VAT area, cm <sup>2</sup>	152 (69, 196)	121 (112, 131)	147 (59, 169)	131 (113, 141)	-10 (-27, -5)	10 (-13, 25)	-10 (-27, -5)	10 (-13, 25)	0.289	

Data are expressed as median (IQR).

Abbreviations: A, android; BMI, body mass index; G, gynoid; VAT, visceral adipose tissue; WC, waist circumference; WHR, waist-to-hip ratio.  
<sup>a</sup>From Wilcoxon rank sum test.

Table 3. Effect of CE/BZA on Glucose Homeostasis

	Baseline			Week 12			Change from Baseline to Week 12			P <sup>a</sup>
	Placebo (n = 5)	CE/BZA (n = 6)	Placebo (n = 5)	CE/BZA (n = 6)	Placebo (n = 5)	CE/BZA (n = 6)	Placebo (n = 5)	CE/BZA (n = 6)		
IVGTT <sup>b</sup>										
AIRg, $\mu\text{U/L}^{-1} \cdot \text{min}^{-1}$	331 (190, 374)	179 (135, 272)	181 (68, 382)	634 (104, 716)	-25 (-47, -9)	189 (-31, 444)			0.273	
G <sub>b</sub> , mg/dL	87.3 (81.7, 88.8)	90.9 (84.5, 100.8)	92 (78.5, 93.8)	85.0 (81.3, 90.3)	2.7 (0.9, 4.9)	-5.2 (-9.2, -1.7)			0.029	
DI	658 (497, 946)	651 (286, 1051)	1519 (395, 1733)	892 (595, 1892)	267 (28, 572)	500 (-19, 841)			0.855	
S <sub>I</sub> , $\mu\text{U/L}^{-1} \cdot \text{min}^{-1}$	3.42 (1.76, 3.94)	3.59 (1.25, 6.53)	4.54 (2.85, 5.77)	3.26 (1.60, 5.79)	1.35 (1.12, 1.82)	-0.24 (-1.50, 0.19)			0.029	
S <sub>G</sub> , $\text{min}^{-1}$	0.01 (0.007, 0.016)	0.016 (0.006, 0.018)	0.016 (0.014, 0.024)	0.013 (0.010, 0.018)	0.008 (0.008, 0.009)	0.002 (-0.009, 0.005)			0.068	
I <sub>b</sub> , $\mu\text{U/mL}$	8.1 (7.3, 16.3)	6.7 (2.3, 18.8)	8.9 (6.5, 14.9)	14.0 (2.6, 37.0)	0 (-0.8, 0.8)	1.2 (-0.7, 2.9)			0.465	
P2, $\text{min}^{-1}$	0.033 (0.025, 0.053)	0.026 (0.020, 0.031)	0.032 (0.015, 0.04)	0.018 (0.005, 0.045)	-0.010 (-0.010, -0.000)	-0.001 (-0.019, 0.025)			0.855	
P3, $\mu\text{U/L} \cdot \text{min}^{-2}$	9.97e-6 (5.72e-6, 1.83e-5)	8.53e-6 (2.58e-6, 1.99e-5)	9.14e-6 (8.84e-6, 1.81e-5)	4.59e-6 (1.96e-6, 7.81e-6)	-1.38e-7 (-8.73e-7, 3.43e-6)	-1.99e-7 (-8.75e-6, 8.53e-7)			0.584	
G0, mg/dL	200 (198, 219)	249 (228, 254)	217 (215, 254)	251 (234, 268)	14.6 (1.5, 46.2)	4.2 (-12.7, 30.0)			0.273	
GEZI, $\text{min}^{-1}$	5.40e-3 (3.88e-3, 1.32e-2)	9.93e-3 (5.31e-3, 1.57e-2)	1.15e-2 (9.74e-3, 1.96e-2)	8.85e-3 (6.94e-3, 9.26e-3)	6.84e-3 (6.45e-3, 7.61e-3)	-5.80e-4 (-7.29e-3, 3.23e-3)			0.100	
HOMA										
$\beta$ cell, $\mu\text{U/mM}$	110.7 (101.3, 432.4)	61.8 (32.9, 315.6)	110.8 (75.8, 392.6)	299.1 (52.0, 636.2)	-25.5 (-39.9, 0.1)	18.5 (-0.9, 320.6)			0.045	
IR, $\text{mM} \cdot \mu\text{U/L}^{-2}$	1.78 (1.59, 2.98)	1.67 (0.51, 3.93)	2.03 (1.50, 2.75)	2.81 (0.53, 8.01)	-0.01 (-0.10, 0.24)	0.15 (-0.18, 0.34)			0.855	
HOMA2										
% $\beta$ cell	126 (125, 178)	104 (83, 125)	128 (101, 171)	134 (77, 154)	-9.9 (-22.8, 1.7)	12.1 (-0.4, 42.9)			0.068	
IR	1.91 (1.75, 2.02)	1.50 (1.4, 2.14)	1.75 (1.66, 1.95)	1.76 (1.61, 1.88)	-0.25 (-0.27, 0.20)	0.09 (-0.11, 0.31)			0.584	
Insulin clearance										
FIC <sup>c</sup>	13.9 (8.2, 16.6)	13.6 (4.9, 26.8)	13.9 (8.0, 15.9)	10.5 (3.9, 20.7)	-0.25 (-0.70, -0.00)	-3.50 (-11.56, 0.78)			0.715	
GSIC <sup>d</sup>	127 (93, 219)	154 (79, 355)	153 (134, 217)	122 (57, 236)	-1.85 (-20.5, 25.8)	-32.8 (-123.6, 6.9)			0.201	

Data are expressed as median (IQR). Note that for technical reasons, the IVGTT serum was processed in only 11 of 12 subjects (CE/BZA n = 6).

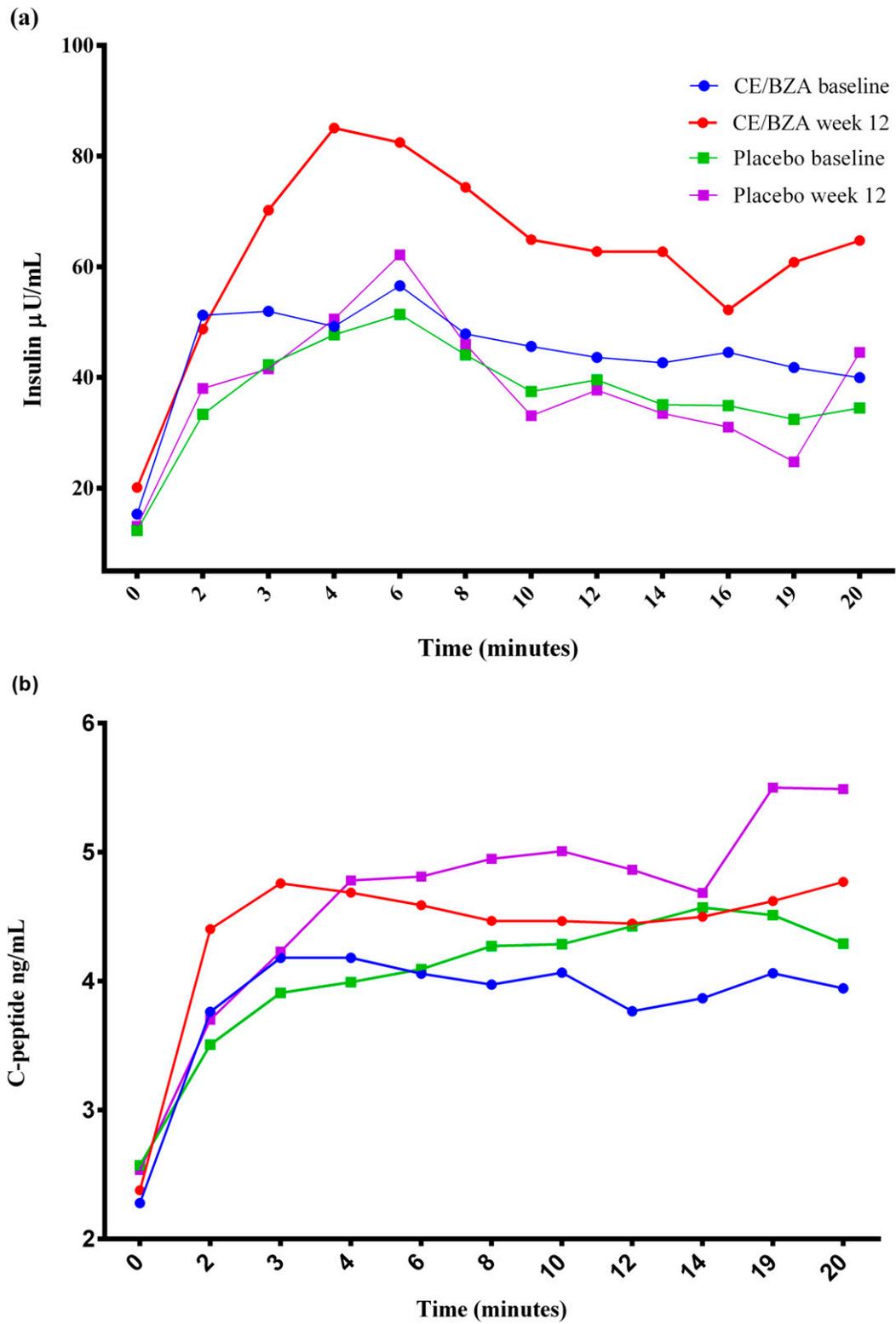
Abbreviations: G0, glucose at time zero; GEZI, glucose effectiveness at zero insulin; I<sub>b</sub>, basal insulin concentration; P2, removal rate of insulin from the interstitial space; P3, movement of circulating insulin to the interstitial space.

<sup>a</sup>From Wilcoxon rank sum test.

<sup>b</sup>IVGTT data derived by MINMOD Millennium software.

<sup>c</sup>FIC derived from fasting C-peptide to insulin ratio.

<sup>d</sup>GSIC derived from molar ratio of C-peptide to insulin AUC over first 20 min of IVGTT.



**Figure 2.** Insulin and C-peptide concentrations. (a) Mean serum insulin concentrations first 20 min of the IVGTT. (b) Mean serum C-peptide concentrations first 20 min of the IVGTT.

Table 4. Effect of CE/BZA on Insulin Resistance and Inflammation Markers

	Baseline		Week 12		Change from Baseline to Week 12		<i>P</i> <sup>a</sup>
	Placebo (n = 5)	CE/BZA (n = 7)	Placebo (n = 5)	CE/BZA (n = 7)	Placebo (n = 5)	CE/BZA (n = 7)	
Serum biomarkers							
Leptin ng/mL	54.4 (53.1, 62.1)	54.3 (38.7, 60.1)	64.5 (46.8, 72.6)	56.6 (41.4, 68.5)	0.7 (-0.1, 10.5)	-1.6 (-7.2, 9.5)	0.465
Adiponectin µg/mL	12.9 (7.7, 13.1)	7.5 (4.4, 14.6)	10.3 (5.9, 11.9)	7.4 (3, 10.2)	-2.5 (-2.6, -0.4)	-1.4 (-2.8, -0.5)	0.808
LAR	4.12 (3.87, 8.06)	7.99 (5.06, 8.80)	7.93 (5.42, 8.15)	8.92 (5.59, 21.90)	1.55 (0.02, 4.03)	2.71 (-0.04, 4.96)	0.808
PAI-1 ng/mL	10.1 (10.1, 10.6)	14 (9.4, 16.7)	11.4 (9.4, 13.4)	8.9 (8.8, 14.9)	1.3 (-0.4, 3.3)	-1.8 (-5.2, -1.4)	0.088
FGF-21 pg/mL	361 (316, 556)	261 (189, 415)	241 (227, 330)	171 (149, 315)	-89 (-226, -48)	-134 (-208, 24)	0.685
LCN2 ng/mL	17.9 (16.7, 22.7)	14.8 (13.5, 15.7)	17.8 (12.9, 20.9)	13.8 (11.5, 24.1)	-3.8 (-4.9, -2.2)	-1.7 (-3, 3.5)	0.223
CRP mg/mL	4.58 (1.83, 6.78)	3.49 (2.34, 4.59)	4.67 (1.44, 5.96)	2.82 (1.93, 3.49)	-0.39 (-0.82, -0.06)	-0.41 (-2.66, 0.45)	0.935
RBP4 µg/mL	40.2 (32.7, 46.9)	32.9 (15.9, 35.3)	28.7 (28.6, 45.8)	28.1 (19.7, 44.6)	-4.0 (-24.1, 4.6)	-0.4 (-5.6, 14.5)	0.465
TBARS nmol/mL	6.1 (5.5, 8.4)	6.5 (4.0, 8.2)	5.5 (4.9, 5.9)	3.8 (2.2, 7.1)	-1.2 (-1.2, 0.0)	-1.7 (-3.3, -0.4)	0.372
Intact OCN ng/mL	10.1 (7.4, 11.3)	5.2 (1.5, 9.5)	10.4 (6.3, 10.6)	4.6 (4.3, 10.3)	-0.9 (-1.1, 2.7)	0.8 (-0.7, 3.1)	0.465
Glu-OC ng/mL	4.6 (4.6, 8.6)	4.4 (2.5, 5.9)	3.8 (3.4, 24.4)	4.8 (1.8, 11.4)	-1.2 (-1.9, 2.1)	0.0 (-0.7, 4.7)	0.372
Glu-OC ng/mL	4.0 (3.4, 4.4)	4.2 (2.9, 4.3)	5.9 (3.6, 7.0)	4.2 (3.6, 4.9)	1.5 (0.5, 3.0)	0.6 (-0.6, 0.8)	0.168
Glu/Gla ratio	1.05 (0.66, 1.36)	0.98 (0.80, 1.30)	0.9 3 (0.44, 1.28)	0.98 (0.58, 2.27)	-0.10 (-0.42, 0.62)	0.04 (-0.14, 1.29)	0.685

Data are expressed as median (IQR).

Abbreviations: Gla-OC, carboxylated osteocalcin; Glu-OC, undercarboxylated osteocalcin; LAR, leptin:adiponectin ratio; OCN, osteocalcin.

<sup>a</sup>From Wilcoxon rank sum test.

menopausal women assigned to CE/BZA may reflect protection of  $\beta$  cell function. Another randomized controlled trial found no effect of CE/BZA on blood glucose in postmenopausal women [11]. However, the study participants were not obese and measures of  $\beta$  cell function were not reported. Surprisingly, we observe no effect of CE/BZA on the AIRg. However, the AIRg explores the IV (exogenous) glucose stimulation of the  $\beta$  cell. The HOMA-B reflects a different aspect of  $\beta$  cell function, *i.e.*, the fasting insulin secretion in response to endogenous glucose production. Therefore, it is possible that CE/BZA improves fasting insulin secretion, which is not observed during IV glucose infusion.

Because insulin and C-peptide are secreted in equimolar quantities but only insulin, not C-peptide, is extracted by the liver, one can quantify prehepatic insulin secretion and hepatic insulin clearance from the ratio of plasma insulin and C-peptide concentrations. In menopausal women, studies are consistent with the combination of estrogens and progestins enhancing hepatic insulin clearance [46–48]. Here, women assigned to CE/BZA do not show any difference in insulin clearance compared with women assigned to placebo, both in fasting and glucose-stimulated states (Fig. 2). This may explain why the HOMA2-B, which uses C-peptide, is not increased substantially (Table 3). Although preclinical studies have shown that treatment with the CE/BZA improves markers of inflammation and ameliorates IR [16], our study did not show improvement in IR and inflammation. Insulin sensitivity assessed by mathematical modeling using the MINMOD increased in the placebo arm, which likely occurred by chance as is often the case in small and short-term studies. There are limitations to our pilot study. First, the small sample size ( $n = 12$ ) associated with high dropout rate (33%) limits the power to detect findings and some findings may have occurred by chance. Second, the finding of significant  $P$  values may have been influenced by the multiple comparisons. Finally, treatment with CE and BZA lasted for only 12 weeks and longer duration of treatment may be needed to observe changes.

In conclusion, a 12-week treatment with CE/BZA in obese menopausal women improves  $\beta$  cell function (HOMA-B) and  $G_{75}$  without change in AIRg, HOMA-IR, DI, insulin clearance, body composition, or markers of systemic inflammation. A larger study is warranted.

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**Correspondence:** Franck Mauvais-Jarvis, MD, PhD, or Dragana Lovre, MD, Section of Endocrinology and Metabolism, Tulane University Health Sciences Center, 1430 Tulane Avenue, #8553 New Orleans, Louisiana 70112. E-mail: [fmauvais@tulane.edu](mailto:fmauvais@tulane.edu) or [dlovre@tulane.edu](mailto:dlovre@tulane.edu).

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**Data Availability:** All data generated or analyzed during this study are included in this published article or in the data repositories listed in References and Notes.

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