Bazedoxifene/Conjugated Estrogens (BZA/CE) Improvement of Metabolism

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1. Study aim, background, and design

With the dramatic increase in life expectancy, many women will spend half of their lives in a post-menopausal state of estrogen deficiency that increases risk for metabolic syndrome, type 2 diabetes (T2D) and non-alcoholic fatty liver diseases (NAFLD).[1] Overall, the role of estrogen deficiency to the pathophysiology of chronic metabolic diseases in women is emerging as a novel therapeutic challenge. In 1995, approximately 38% of postmenopausal women in the U.S. used **hormone therapy (HT)**, consisting of estrogen with or without progestin, to treat symptoms of menopause and to prevent chronic conditions such as cardiovascular disease (CVD) osteoporosis, and Alzheimer's [2]. It was not until the Women's Health Initiative (WHI) was abruptly halted in 2002 as a result of a link between HT and increased risk of coronary heart disease events, stroke and breast cancer that the health benefits of HT were seriously questioned.[3] Results of the WHI led many women and their physicians to overestimate the individual-level risk associated with HT use. Fortunately, the period after 2010 saw a detailed analysis of the WHI and led to a stratification of the absolute risk of HT by age. Recently, the benefits of estrogen therapy have been reevaluated by international menopausal societies leading to a consensus on the benefits of HT pending that it is administered in early menopausal women and is not associated with a progestin.[4] The most promising HT involves the use of tissue selective estrogen complexes (TSECs) combining a conjugated estrogen (CE) and the selective estrogen receptor modulator (SERM) bazedoxifene (BZA) in a single tablet. The major innovation of TSEC BZA/CE is that it provides all the advantages of CE treatment without the use and side effects of a progestin.[5,6] Another advantage is that it contains BZA, a SERM that has estrogen antagonistic activity in breast and uterus and estrogen agonistic activity in bone thus preventing osteoporosis while protecting the breast and uterus from estrogenic stimulation.[6] BZA/CE (DUAVEE) is this novel menopausal therapy approved by the FDA for the treatment of postmenopausal symptoms and the prevention of menopausal osteoporosis. However, an important beneficial effect of estrogen is also to prevent postmenopausal metabolic disorders. [1,7,8] There is currently no information on the efficacy of BZA/CEin preventing postmenopausal metabolic disorders. Our recent preliminary data, derived from a mouse model of post-menopausal metabolic syndrome, demonstrate that the combination BZA/CE prevent estrogen deficiency-induced metabolic dysfunction-including obesity, T2D and NAFLD- and without uterus stimulation.[9] Because SERMs like tamoxifen or Raloxifene have never shown such a beneficial metabolic effect in preclinical models and in humans,[1] these data underscore the clinical significance of this line of investigation and they also provide a firm rationale for proposing a pilot study to assess the efficacy of BZA/CE in preventing metabolic dysfunction in postmenopausal women.

The goal of this pilot clinical study is to perform a **randomized placebo-controlled study** to assess the beneficial effect of a **3 month-treatment** with BZA/CE *vs. placebo* on glucose homeostasis and body composition in 30 post-menopausal women. The recruitment will be performed at Tulane Health Sciences Center. The following endpoint will be measured ***at the beginning of the study and after 3 months of treatment***. Patients will have a monthly visit with the clinical coordinator.

a) ***Effect of DUAVEE™ on visceral adiposity***. ***Rationale:*** Our preliminary data show that TSEC treatment prevents visceral fat accumulation in ovariectomized female mice with diet-induced metabolic dysfunction. [9] It also decreases hepatic and skeletal muscle triglyceride accumulation [9] in these ovariectomized mice. ***Hypothesis***: BZA/CE will improve visceral obesity in postmenopausal women. ***Design:*** We will determine the effect of a 3 month- treatment with DUAVEE on body mass index (BMI), waist to hip circumference (WHR)-a clinical index of visceral fat accumulation- and

directly quantify abdominal fat by DEXA Scan**. *We expect*** *that BZA/CE treatment will improve WHR and decrease abdominal fat in postmenopausal women.*

b) ***Effect of*** *BZA/CE* ***on glucose homeostasis. Rationale.*** Our preliminary data shows that TSEC treatment decreases fasting and fed blood glucose levels (as a result of improved insulin sensitivity) in ovariectomized female mice with diet-induced metabolic dysfunction. [9] ***Hypothesis:*** DUAVEE™ treatment will decrease glucose and insulin levels in postmenopausal women. ***Design:*** We will determine the effect of a 3 month- treatment with DUAVEE on fasting and fed glucose and insulin levels. ***We expect*** *that BZA/CE treatment will decrease glucose and insulin levels in postmenopausal women.*

c) ***Clinical modelization of the impact of*** *BZA/CE* ***on glucose homeostasis. Rationale.*** Our preliminary data shows that TSEC treatment improves insulin resistance and glucose tolerance in ovariectomized female mice with diet-induced metabolic dysfunction. [9] ***Hypothesis:*** DUAVEE treatment will ameliorate insulin sensitivity and glucose tolerance in postmenopausal women. ***Design:*** We will determine the effect of a 3 month- treatment with DUAVEE™ on glucose homeostasis following an IV-Glucose Tolerance Test (IVGTT) which will be studied using the minimal model. In the minimal model, computer software is used to calculate insulin sensitivity and secretion from the glucose and insulin dynamics observed during the IVGTT. [10-12] Three major independent parameters can be derived: *The insulin sensitivity index (S_I)* is the measure of the capability for insulin-stimulated glucose uptake*; the acute insulin response to glucose (AIRg)* which is the total insulin release during the first phase of insulin secretion and *the disposition index (DI)* which is the ability of an individual to control blood glucose taking into consideration insulin secretion in the face of the prevailing degree of insulin resistance.[13] Therefore, the DI a major therapeutic index when assessing a new drug. The IVGTT coupled to the minimal model is the gold standard and provides the most clinically relevant comprehensive metabolic portrait of an individual.[10] ***We expect*** *that BZA/CE treatment will enhance insulin sensitivity (S_I) and the disposition index (DI) in postmenopausal women.*

d) ***Effect of DUAVEE™ on systemic inflammation. Rationale.*** Our preliminary data show that TSEC treatment improves systemic markers of inflammation and metabolic dysfunction in ovariectomized female mice with diet-induced metabolic dysfunction. Metabolic dysfunction is characterized by recruitment and release of pro-inflammatory cytokines from WAT that cause insulin resistance and a general, low-grade systemic inflammatory state. Our preliminary data show that serum leptin levels and the leptin/adiponectin ratio — a marker of insulin resistance and atherogenesis — were markedly decreased by TSEC treatment in ovariectomized female mice fed a HFD. [9] In addition, TSEC treatment decreased serum levels of RBP4, an adipokine that is elevated in insulin resistance and type 2 diabetes.[9] Further, TSEC treatment reduced serum levels of Lcn2,[9] an adipokine that is also elevated in adipose tissue and in serum of obese and insulin resistant rodents and humans. Finally, serum levels of TBARS, a marker of oxidative stress and lipid peroxidation, were significantly decreased in OVX mice by TSEC treatment.[9] Thus, in ovariectomized female mice fed a HFD, TSEC improves adipose dysfunction and systemic inflammation and protect against lipid peroxidation and oxidative stress. ***Hypothesis:*** BZA/CE treatment will improve systemic markers of inflammation in postmenopausal women. ***Design:*** We will determine the effect of a 3 month- treatment with DUAVEE on serum cytokines measurement (Leptin - Insulin – adiponectin – RBP4 - LCN2 – TNFα - IL6 – PAI-1 – FGF21). ***We expect*** *that BZA/CE treatment will improve systemic markers of inflammation, lipid peroxidation and oxidative stress in postmenopausal women.*

Study Duration: *Duration of the recruitment + treatment of patients: 1 year*

Target Start and End Dates: *October, 2014 – September, 2015.*

Predicted outcome: We can foresee two possible outcomes***.*** The most likely is that a 3 month-treatment with BZA/CE in postmenopausal women decreases visceral adiposity and markers of systemic inflammation and improves insulin resistance and glucose homeostasis. There are several reasons to anticipate these positive results. *First,* CE in already known to improve glucose and energy homeostasis in postmenopausal women[1] and should continue to have the same activity in a TSEC. *Second,* an importantly SERMs like tamoxifen or raloxifene have never shown any beneficial metabolic effect in rodents and humans.[1] Conversely, BZA exhibits a powerful metabolic effect in female mice and accordingly, we expect that it will translate to women. Finally, TSEC shows a beneficial effect on HFD-induced obesity, independently from estrogen deficiency in mice, suggesting that it may also improve obesity in women. For all these reasons, we are confident that BZA/CE anti-diabetic and anti-obesity effects will translate to postmenopausal women. Acquisition of this information is critical to open novel avenues of therapies for postmenopausal women. The second possible outcome is that although our data generated in pre-clinical models are quite convincing and strongly suggests that BZA/CE will improve metabolic health in women, our hypothesis may not prove correct. This pilot study needs however to be performed because if successful, such preliminary data can lead to new therapeutic avenue for post-menopausal metabolic disturbances.

Statistical Methods: According to our preliminary data obtained in mice, [9] TSEC treatment increased whole body glucose disposal by 30% and enhanced hepatic insulin sensitivity by 75% compared to vehicle. Although a formal power analysis was not feasible in the pilot study, in light of the high precision that is achieved using the minimal model, we can expect similar improvements to be detectable with 30 patients for whole body insulin sensitivity (Si and DI) in this randomized trial. Our statistician will conduct our data analysis. Briefly, a mixed effects statistical model will be used to investigate the within subject differential response to BZA/CE compared to placebo. Statistical significance will be set at $p<0.05$. Analysis will be carried out using the software package SAS V 9.3, SAS Institute, Inc. Cary, NC.

2. Subject Population

We will recruit and screened participants with the aim of randomizing 30 women (10 per group). After referral and clearance (see below), the final assessment of eligibility will be performed during a **screening visit** (blood, medical history and physical examination, barriers to participation, questionnaires). Breast exam and/or mammography will be part of the screening to assess eligibility.

Inclusion:

- Post-menopausal women (<5y since final menstrual period) with age between 50-60y
- Symptomatic (hot flashes, vaginal dryness) or asymptomatic
- BMI 26-45 kg/m^2 (Overweight, Obesity I and Obesity II)
- fasting glucose <125mg/dl
- Triglycerides < 200 mg/dl
- Normal mammogram within past 12 months
- Physician clearance

Exclusion:

- Amenorrhea from other causes (Hyperandrogenemia and anovulation)
- Type 2 and Type 1 diabetes
- Medications: diabetes or diabetic drugs, estrogen/progestin therapy, antidepressants and antipsychotics, antiretroviral (HIV), oral steroids, weight loss drugs.
- ≤ 3 month washout of birth control pill (often prescribed for postmenopausal symptoms)
- Hysterectomy (partial or complete)
- Contraindications to estrogen treatment (unusual vaginal bleeding, blot clots, hepatic disease, bleeding disorder, past/present history of breast or uterine cancer, pregnant, breastfeeding)

Screening paradigm: Subjects will be recruited through endocrine and other medicine clinics at Tulane University Hospital & Clinic, Tulane-Lakeside Hospital and through referral from the Department of Medicine at Tulane Hospital & Clinic. Importantly, our recruitment efforts will be done with the Section of Reproductive Endocrinology and Infertility in the Department of Obstetrics and Gynecology at Tulane University Health Science Center. We will also use community advertisements and promotional materials (attached).

3. Procedure

Treatment plan: Subject will participate in a randomized scheme as follows:

a) PLACEBO for 3 months (N=10 patients)
b) BZA/CE for 3 months (N=10 patients)

BZA/CE group: Participants assigned to *BZA/CE* will receive a daily tablet containing conjugated estrogens 0.45 mg and bazedoxifene 20 mg. BZA/CE (bazedoxifene/conjugated estrogens) tablets, 0.45 mg/20 mg are oval, biconvex, pink tablets, branded with "0.45/20" in black ink on one side. The recommended and only FDA approved dosage is one BZA/CE tablet daily, taken without regard to meals [14]. Tablets should be swallowed whole. If a dose of BZA/CE is missed, participants will be instructed to take it as soon as remembered unless it is almost time for the next scheduled dose. They should not take two doses at the same time. The dose is one tablet per day independent of weight and fat mass. Participants will be provided with information about BZA/CE and its potential side effects and contraindications. Pfizer will provide the medication.

PLACEBO group: In addition to receiving a placebo tablet (provided by Pfizer), participants assigned to the placebo group will be counseled on how to adapt to changes associated with menopause.

DEXA scans: will be performed using a Hologic QDR 4500A whole-body scanner. The scan takes about 5 – 15 minutes and the radiation dose is less than 1 mrem, equal to about 12-h of background radiation. The scans are analyzed with the QDR for Windows V11.1 software. In our hands, the SD is 320 g (CVs of 0.6%) and 300 g (CVs of 1.1%) for FFM and FM, respectively. We will also use the DXA data to estimate muscle mass.

Glucose and insulin: Fasting glucose and insulin will be measured after the 12-h overnight fast. Insulin levels will be collected at each time point during the IVGTT which is described below.

IV-Glucose Tolerance Test (IVGTT): The intravenous glucose tolerance test (IVGTT) interpreted with the minimal model provides individual indexes of insulin sensitivity (S_I) and glucose effectiveness (S_G).

Patients will have intravenous catheters inserted in a vein in each arm. One will be used for blood samples and the other to administer the IVGTT required medications - glucose and insulin. Baseline blood samples will be taken at -15, -10, and -1 min. before glucose is injected intravenously in the dose of 11.4 g/m^2 body surface area over 1 min. beginning at time 0. Blood samples will then be taken at timed intervals to check glucose levels via the YSI glucose Analyzer Insulin will be administered intravenously over 30 seconds in the dose of0.03 U/kg body weight twenty minutes after the glucose administration. Again, blood samples will be taken at timed intervals until the 3 hour IVGTT is completed. A total of 33 blood samples will be collected for both glucose and insulin according to the chart below.

Time (min)	**Glucose and Insulin**
-15	X
-5	X
-1	X
0	Glucose IV over 60 seconds – 11.4 g/m^2 body surface area
+2	X
+3	X
+4	X
+5	X
+6	X
+8	X
+10	X
+12	X
+14	X
+16	X
+19	X
+20	Insulin iv over 30 seconds - -0.03 U/kg body weight
+22	X
+23	X

+24	X
+25	X
+27	X
+30	X
+35	X
+40	X
+50	X
+60	X
+70	X
+80	X
+90	X
+100	X
+120	X
+140	X
+160	X
+180	X

Biomarkers of Inflammation: We will measure serum cytokines (Leptin – Insulin – adiponectin – RBP4 - LCN2 – TNFa - IL6 – PAI-1 – FGF21) through high sensitivity ELISAs. These biomarkers will be collected prior to any testing at Visits 2 and 5 after a 12 hour fast.

Endothelial Function Test: This test will be done at Visits 2 and 5 after a 12 hour fast. It is a non-invasive test that uses Peripheral arterial tonometry (EndoPAT) to assess endothelial function.

Specifics of this Endothelial Function Test can be found in the "Addendum to BIM Study" document that is attached to this protocol.

4. Risks

Risks to Subjects: This Human Subjects Research meets the definition of a Clinical Trial.

Sources of Materials

All data and specimens will be obtained solely for research purposes. These will include 1) detailed medical history, 2) physical exam including BMI and WHR measurement, 3) multiple blood samples to assess complete blood count, complete metabolic panel, lipid profile; glucose and insulin blood samples from IV-GTT testing, and cytokines(biomarkers of inflammation), 4) body composition measures by DXA (for details see section 3c. Approach), Endothelial Testing using the EndoPAT machine and 5) stored blood specimens used to study metabolic health and inflammation. All data from individual subjects will be maintained for confidentiality and names and identities will not be disclosed in any published document.

This study does not involve major risk to screeners and trial participants. To minimize the potential risks of the assessment methods and outcome variables, investigators will frequently monitor the study to assure that no volunteer suffers any adverse effects from participating in the research. Risks of complications will be reduced by carefully selecting participants for enrollment as per our inclusion/exclusion criteria. Other than participants being Overweight, with Obesity I and Obesity II at the time of enrollment, the inclusion and exclusion criteria were created to ensure women with low risk for bone fractures, have no diabetes and are not on medications such as diabetic drugs, dyslipidemia, estrogen/progestin therapy, antidepressants and antipsychotics. The Principal Investigator or its representative(s) will obtain medical clearance prior to each participant's enrollment.
Potential risks associated with the study procedures include:

a) Analysis of archived of blood specimens: There is no risk for participants to have their blood collected and stored for future use pertaining to metabolic health and inflammation. All specimens will be de-identified and kept in a secure location.
b) Blood pressure testing: Participants may experience temporary discomfort during blood pressure recordings due to the pressure of the cuff inflating on their arm.
c) Blood draw: There is the possibility of pain and bruising at the vein on your arm where the needle is inserted. Multiple sticks may be necessary if there is difficulty in drawing participants blood. Aseptic (sterile) technique and trained personnel minimize these risks.
d) Body composition assessment: The DXA scan involves extremely low levels radiation exposure. This technique has been demonstrated to be safe and approved for children. There are no known risks of the body scan
e) IV Glucose Tolerance Test: The IV glucose tolerance test is the gold standard for assessing insulin sensitivity and secretion from the glucose and insulin dynamics observed during the IVGTT which are computerized as a minimal model using software. Some people feel nauseated or sweaty after receiving the glucose. Some people develop hypoglycemia. The IV(s) may be associated with pain or bruising where the needle(s) are inserted and the development of a bruise at these sites with a minimal possibility of infection. Trained personnel and aseptic (sterile) techniques are used to minimize these risks. The intended sites of catheterization will be cleaned thoroughly with an antiseptic and, after insertion; the area will be dressed so that it is not in contact with the outer environment. Also, the subject will be supervised, and all sites will be monitored for any signs of infection (increase in body temperature, swelling, redness, etc., around the insertion sites), upon which the catheter(s) will be removed immediately. Trained, credentialed personnel under the supervision of PIs will perform all procedures.
f) Endothelial Function Test: The EndoPAT is performed with the participant in a lying or sitting position for approximately 15 minutes. The subject may become uncomfortable for that length of time. Finger probes are placed on each index finger and minimally inflated which may pose some discomfort to their fingertips. The finger probes are deflated after the testing is

completed. A cuff is placed on their non-dominant arm and inflated for about 5 minutes during the testing period. This may cause some discomfort and/or tingling in there arm, however it is considered very safe and should pass with the release of the cuff. Only Trained personnel will be performing this test.

Protection against Risks

Confidentiality

The study investigators will treat participant identity with professional standards of confidentiality. Each participant will be assigned an identification number and all participant protected health information/records/questionnaires/data will be coded and identified using these numbers rather than participant names. The coded participant list identifying participants by name will be stored separately from the number coded records/questionnaires/data. All participant records/data will be stored in locked file cabinets. Study results will be reported as group data and/or by participant identification number only. Study results may be presented in publications or at meetings/conferences, however participant identity will not be disclosed. A participant may request their personal health information or their personal study data/results at any time during the study and will be given copies.

All volunteers are assured of their confidentiality both verbally and in the informed consent form. The clinical facilities are strictly limited to the staff of the research institution and to research volunteers. This is accomplished by a variety of stringent security measures. All medical records are stored in locked areas. Access to these areas is limited to the clinical support staff, director of the clinical facilities, and the PIs. Electronic data storage is similarly restricted with only the PIs and authorized persons having access to databases containing confidential clinical records, i.e. those containing name OR other identifying information.

HIPPA Compliance

Tulane University Health Sciences Center complies with the federal 1996 Health Insurance Portability and Accountability Act (HIPPA). Specifically, Tulane University Health Sciences Center protects the privacy and confidentiality of medical records and information contained in medical records of persons who are subjects of research projects, including all Protected Health Information (PHI) as defined by the HIPAA Privacy Regulations. PHI of research subjects and the use or disclosure of such information is governed by PBRC research policies, as well as Common Rule, FDA regulations and other applicable laws.

Tulane University Health Sciences Center and the Principal Investigators (the persons chiefly responsible for the record) protect the privacy of research subjects and their PHI collected during a research project. Tulane University Health Sciences Center will not use or disclose existing PHI or PHI created during a research project, unless:

- The subject signs both (a) a HIPAA Authorization for use and disclosure of PHI using an approved Authorization Form or other form containing all the elements of legally effective HIPAA authorization and (b) the informed consent to participate in research form approved by the Institutional Review Board (IRB), or
- The IRB grants a waiver to the requirement of obtaining a signed HIPAA Authorization Form, or
- The IRB-approved protocol uses properly de-identified PHI

- The IRB-approved protocol uses the limited data set and the Principal Investigator signs a limited data use agreement with the entity that maintains the designated record set.

5. Benefits

We cannot promise benefits from participating in this study but we expect clear improvements of metabolic health for all the women randomized to pharmacological interventions. The results of the study will provide important information about pharmacological intervention with BZA/CE that may help in controlling weight/fat gain in women during early post menopause. This information can contribute to the development of evidence-based recommendations to help manage weight during menopause.

6. Remuneration

Subjects will receive compensation for their time and travel in the form of a Clincard. They will receive $25.00 for study visits 1, 3, and 4 and $50.00 for study visits 2 and 5. The Clincard is a reloadable debit/credit card that will be given to each subject upon completion of their first visit. After each completed study visit thereafter, the card will be uploaded with the amount allowed for that visit. The subject will received compensation for each completed study visit. If they complete all study visits, they will receive up to a total of $175.00. If a subject is withdrawn from the study by either the investigator or sponsor, or withdraws voluntarily, the subject will be compensated only for the study visits they complete.

Tulane University is required to report payments of $600 or more to the Internal Revenue Service (IRS). This means that if you receive $600 or more from Tulane during the calendar year, your compensation will be reported to the IRS and you will receive an IRS 1099 Form.

7. Costs

There will be no costs to the subject for participating in this research study.

8. Alternatives

The alternative is that the subjects do not have to participate in the research.

9. Consent process and documentation

The objectives of the project, all experimental procedures, all requirements for participation, and any possible discomforts, risks, and benefits of participation will be clearly explained in writing and orally, in lay terms, to the subject by the study coordinator, the Principal Investigator or its representatives. Subjects will be informed orally and in writing that they are free to withdraw from the study at any time for any reason without bias or prejudice. After all questions have been answered and the subject agrees to participate in the study as described, he/she will sign a written informed consent document.

10. Qualifications of the investigators

We have assembled a synergic team.

The Principal Investigator, Dr. Mauvais-Jarvis, MD, PhD is a physician scientist who received his medical degree from the University of Paris School of Medicine in France and completed clinical

training in endocrinology in Paris teaching hospitals. He is a world-renowned expert in the action of estrogen on metabolism with 15 years experience in the conduct of metabolic studies in preclinical models. Dr. Mauvais-Jarvis also has extensive experience in clinical studies in patients with diabetes with over 15 publications involving human subjects.

The Co-Investigator, Dr. Vivian A. Fonseca, MD, is the Chief of the Section of Endocrinology. He is a world-renowned expert in the conduct of clinical investigation of novel diabetes drugs in obese and diabetic human subjects and has been conducting such studies at Tulane Health Science Center for the past 20 years

Roberta McDuffie, MSN, ACNS-BC, BSBA, ADN, CDE, Instructor in Medicine, is the principal research coordinator for endocrine research at Tulane University. She will be involved in overseeing patient recruitment.

Tina Thethi, MD, MPH is an Associate Professor and attending physician is the Department of Medicine, Section of Endocrinology at Tulane University Health Sciences Center. She is currently the principal investigator of the LEADER study, a multicenter study examining the effects of liraglutide versus placebo on cardiovascular events and will be involved in patient recruitment.

Dragana Lovre, MD is an Assistant Professor of Medicine in the Department of Medicine, Section of Endocrinology at Tulane University Health Sciences Center, and will be involved in patient recruitment.

11. References

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Addendum to the Bazedoxifene/Conjugated Estrogens (BZA/CE) Improvement of Metabolism Study

We will add two markers to the labs that we are drawing at visit 2 and 5:

A. Osteocalcin (Total Active and Inactive)

This will be in collaboration with Dr. Gerard Karsenty at Columbia University, New York as that is the only location where the assay is available. Samples will be processed in the Department of Genetics and Development at Columbia University.

We are interested in Osteoacalcin as a recent study uncovered that a novel pathway taking place in osteoblasts which results in secreting a hormone Osteocalcin which improves glucose homeostasis, this adds more confidence to support the concept of bone and energy metabolism regulate one another. [1] The regulation of bone remodeling by an adipocyte-derived hormone implies that bone may exert a feedback control of energy homeostasis.[1] One of the features of Osteocalcin especially relevant to our study is that it possibly mediates the metabolic function (observed in study with mice) and it may be responsible for changes in visceral fat, beta cell proliferation, insulin secretion, and insulin sensitivity. [1] Given that out study will be looking at DUAVEE effect on glucose homeostasis, visceral adiposity and estrogens' key role in regulation of bone mass and strength (by controlling activity of bone-forming osteoblasts and bone-resorbing osteoclasts) it would be very important to see what happens to the osteocalcin during Estrogen therapy in postmenopausal women.

Therefore, this marker for bone (Osteocalcin) is particularly of interest to us and our study as we are conducting a study that is giving conjugated estrogen and bazedoxifene in postmenopausal women when they are losing bone.

B. Telomere Length

This will be in collaboration with Dr. Stacey Drury at Tulane University Behavioral and Neurodevelopmental Genetics Laboratory. Samples will be processed in the Genetics Laboratory at Tulane.

This Telomere length represents a novel biomarker that may represent one mechanism linking environmental stress exposure and biological outcomes at the cellular level. One retrospective study showed telomere lengths were longer in postmenopausal women who had a history of long-term HT than in postmenopausal women without HT. [2] With the dramatic increase in life expectancy, many women will spend a large part of their lives in a post-menopausal state[3] and apart from causing degeneration of the cardiovascular, skeletal and central nervous systems[3-5] estrogen deficiency also increases risk for metabolic syndrome and type 2 diabetes.[3] Overall, the role of estrogen deficiency to the pathophysiology of chronic diseases in women is emerging as a novel therapeutic challenge therefore; we would like to examine the effect of short-term hormone therapy on telomere length in postmenopausal women. We are interested the effect of conjugated estrogen and bazedoxifene on telomere length in our study with postmenopausal women.

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Addendum to BIM study

1. Addition of a noninvasive endothelial function assessment using Peripheral arterial tonometry (EndoPAT) as a part of Visit 2 and visit 5 assessments.

EndoPAT is FDA cleared non-invasive test which evaluates the level of Endothelial Function.
The test will take approximately 15 minutes. During the test patient should be lying or sitting in a relaxed position and recording will be taken from both index fingers via thimble-like sensors that are non invasive and positioned on patient's fingertips. After 5 minutes, a blood pressure cuff will be inflated on non-dominant arm for 5 minutes; this might be associated with some discomfort and/or tingling in the arm, however it is considered very safe and should pass with the release of the cuff. Recording of the arterial tone will continue for 5 more minutes after the cuff is released.

To prepare for the test patients will be asked to avoid the following actions at least 3 hours prior to the test: Eating (anything), Drinking coffee, tea, sodas, juice, alcohol (water is the only drink allowed), Smoking (including any cigarette/nicotine replacements such as electronic cigarettes).

Recommendation on the day of visit: to wear loose clothing around the arms. To remove all jewelry such as a watch, bracelets and rings as these might interfere with blood circulation during the test. Trim nails of index fingers on both hands - we will place thimble-like sensors on index fingers during the test. Long nails might damage the sensor and affect measurements.

EndoPAT assesses digital flow mediated dilation during reactive hyperemia using measurements from both arms – occluded side and control side.

Normal Endothelial Function means that arteries function well, and this has been shown to protect blood vessels from atherosclerosis (hardening of the arteries) and plaque buildup. Results of the Endothelial Function test are automatically calculated and EndoScore is generated, which indicates the present state of arterial health, or how well arteries dilate in response to increased need for blood.

Significance of adding EndoPAT to our study

The disparity between the incidence of cardiovascular disease among pre- and postmenopausal women has been ascribed to the actions of endogenous estrogen on the cardiovascular system and particularly on the vascular endothelium.[1]

As cardiovascular disease remains the leading cause of death in the 21st century,[2] endothelial vascular dysfunction has become suspected as being associated with cardiovascular disease at its very beginning stages.[3] Recent studies have shown that peripheral vascular endothelial function testing in the ambulatory setting correlates with the extent of CAD risk and the presence or absence of CAD, [4, 5] and that recent data suggest that peripheral vascular endothelial function testing is feasible in ambulatory patients, and plays important next step in bringing this technology to clinical applicability.[5] The main actions exerted by the endothelium are selective permeability, maintenance of balance between thrombosis and fibrinolysis, inhibition of cell proliferation of vascular smooth muscles, active participation of immune response through the release of factors leading to active contractions, and modulation of vascular tonus through the production of vasoactive substances.[1] In women, the processes involved in vascular damage are thought to be slowed by the presence of estrogen.[6] There is currently no information on the efficacy of CE/BZA in preventing postmenopausal metabolic disorders. Available data on the effects of estrogens on inflammatory process are fairly contradictory, with both anti-inflammatory,[7, 8] and proinflammatory[9] effects reported. The pre-clinical study from Mauvais-Jarvis' lab in a mouse model of post-menopausal metabolic syndrome, demonstrates that the

combination CE/BZA prevents estrogen deficiency-induced metabolic dysfunction-including obesity, T2DM and NAFLD- and without uterus stimulation.[10]

Given the fundamental role of endothelial dysfunction in the genesis of atherosclerosis and other chronic disorders, such as diabetes mellitus and hypertension,[1] the significance of preventing initial disturbance of metabolic syndrome through decreasing inflammation and coagulation pathways as well as vascular tone regulation may have an impact on morbidity and mortality in postmenopausal women . Recently, the benefits of estrogen therapy have been reevaluated by international menopausal societies leading to a consensus on the benefits of HRT pending that it is administered in early menopausal women and is not associated with a progestin.[11] The most promising HRT involves the use of tissue selective estrogen complexes (TSECs) combining a conjugated estrogen (CE) and the selective estrogen receptor modulator (SERM) bazedoxifene (BZA) in a single tablet. The major innovation of TSEC BZA/CE is that it provides all the advantages of CE treatment without the use and side effects of a progestin.[12, 13] Another advantage is that it contains BZA, a SERM that has estrogen antagonistic activity in breast and uterus and estrogen agonistic activity in bone thus preventing osteoporosis while protecting the breast and uterus from estrogenic stimulation.[13] DUAVEE (BZA/CE) is this novel menopausal therapy approved by the FDA for the treatment of postmenopausal symptoms and the prevention of menopausal osteoporosis. An important beneficial effect of estrogen is also to prevent postmenopausal metabolic disorders which may be associated with vascular tone regulation and may have an impact on morbidity and mortality in postmenopausal women.

References:

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