RESEARCH PROTOCOL

Title	Effect of GLP-1 receptor agonists on trabecular bone score and visceral adiposity in postmenopausal women with type 2 diabetes mellitus.
Principle Investigator/Co-investigators	PI: Vishnu Garla Co-Investigators: Licy Yanes Cardozo, Candace Howard-Claudio, Lillian Lien, Vikas Majithia, Damian Romero, Erika Wachs, David Gordy Shreena Dhawan, Abhishek Chhabra
Abstract	Postmenopausal women with diabetes mellitus have a higher risk of osteoporotic fractures, with significant associated mortality and morbidity. Osteoporosis is underdiagnosed in diabetes, as the bone mineral density (BMD) as currently measured is often normal despite underlying abnormalities. The trabecular bone score (TBS) is a novel modality to assess bone microarchitecture and accurately assess fracture risk in patients with diabetes. Due to increased co-prevalence of osteoporosis and diabetes mellitus, the potential effects of antidiabetic medications on fracture risk assume importance. Based on findings in animal studies, we hypothesize that GLP-1 receptor agonists increase TBS in postmenopausal women with type 2 diabetes mellitus (T2DM). We propose a prospective non-randomized case-control study by enrolling 48 patients (24 in the GLP group and 24 in the non GLP group). DXA scans, markers of bone formation, and resorption, and selected inflammatory markers will be assessed at baseline, six months and one year. We expect to see a significant improvement in TBS in the GLP group as compared to the non GLP group. We also expect to see a decrease in bone resorption markers, inflammatory mediators, and visceral adiposity and an increase in bone formation markers in the GLP group as compared to the non GLP group.

Background	Osteoporosis is characterized by compromised bone strength and predisposition to fractures. It affects 200 million women worldwide; one in three women over age 50 will suffer an osteoporotic fracture. [1, 2]Osteoporotic hip fractures are expected to increase from 1.6 million to 4.5 million worldwide by 2050. [3] T2DM affects 422 million people worldwide, including about 25% of adults in the United States. Several studies have reported a 20-30% increased risk of osteoporotic fracture with T2DM. Importantly, with increased life expectancy the co-prevalence of T2DM and osteoporosis with resultant fractures, is expected to increase. [4, 5] Typically, osteoporosis is diagnosed by assessing BMD on a DXA scan. T2DM patients often have normal or increased BMD by DXA despite abnormalities of trabecular bone that can lead to increased fracture risk. A TBS can be derived by analyzing pixel gray level variations in DXA image to assess the bone microarchitecture. Higher scores are seen in patients with dense trabecula and lower scores in those with sparse trabeculae. T2DM is known to be associated with low TBS even when BMD is normal. [6] Several T2DM medications can affect bone metabolism. Thiazolidinediones (TZD) are PPAR-γ agonists which are used in the treatment of T2DM. They inhibit osteocyte formation and are associated with decreased BMD and increased risk of fractures. [7] Sodium glucose co-transporter (SGLT2) canagliflozin has also been associated with increased fractures. [8] GLP-1 receptor agonists are FDA approved for the treatment of T2DM. In mice, GLP-1 receptor agonists have been shown to decrease visceral adiposity, reduce levels of inflammatory markers and improve trabecular bone microarchitecture but their effect on humans has not been assessed. [9] This is the objective of the current proposal.
Purpose	Hypothesis: “Administration of GLP-1 receptor agonists will increase TBS in postmenopausal women (age > 55) with T2DM by reducing visceral fat mass and associated production of inflammatory mediators(IL-1, IL-6 and TNF-alpha), leading to a decrease in bone resorption (assessed by measuring C-telopeptide). We further hypothesize that GLP-1 receptor agonists will decrease sclerostin levels, leading to increased bone formation (assessed by measuring osteocalcin and P1NP).”

Specific Aim(s)	Aim 1: To test the hypothesis that administration of GLP-1 receptor agonists will increase trabecular bone score in postmenopausal women (age >55 years) with T2DM. Aim 2: To test the hypothesis that administration of GLP-1 receptor agonists will decrease visceral fat mass, and decrease production of inflammatory markers (IL-1, IL-6, TNF-alpha) in postmenopausal women (age>55 years) with T2DM, and that this will be associated with decreased bone resorption (assessed by measuring C-telopeptide). Aim 3: To test the hypothesis that administration of GLP-1 receptor agonists will decrease sclerostin, with an associated increase in bone formation (assessed by measuring osteocalcin and P1NP), in postmenopausal women (age >55 years) with T2DM.
Study Period (inclusive years)	Prospective (June 1, 2019- December 31, 2022)

Study Design	This is a prospective non-randomized study of one year duration. A total of 48 (24 in the GLP group and 24 in the non GLP group) postmenopausal females will be enrolled in the study. This study design will allow us to study the effects of GLP-1 receptor agonists on bone metabolism (formation and resorption); and bone microarchitecture (TBS) and also provide evidence regarding the potential role of visceral adipose tissue in bone metabolism in postmenopausal women with T2DM. All UMMC providers in the division of endocrinology will be briefed about the study and all the participants will be drawn from the UMMC Endocrinee, Family Medicine, and Internal Medicine clinics. The treating providers will have sole discretion in the choice of medications to be used for the management of each patient's type 2 diabetes mellitus. Patients who are enrolled in the study will be placed in the GLP group if they are on GLP-1 receptor agonists (for the treatment of their diabetes mellitus as decided by their endocrine provider) or in the non GLP group if they are not on GLP-1 receptor agonists. Throughout the study, the management of the each patient's T2DM will be according to standard of care as decided by the treating provider, for both the groups. Methodology: Eligible participants will be identified by designated Research Personnel, who are listed on the delegation log, by going through the UMMC Endocrine, Family Medicine, and Internal Medicine clinic schedules using the inclusion and exclusion criteria. Once a potential participant is identified, the provider (Faculty, resident, nurse practitioner or fellow) scheduled to see the patient would be notified. Of note, Dr. Garla's and Dr. Cardozo's clinic patients will not be considered for the study. The treating provider will independently make the decision regarding the treatment of the patient's diabetes mellitus (whether GLP-1 receptor agonist or other medication needs to be started or not), then the treating provider will enquire if the patient is interested in participating in the study.

	If the patient is interested, the treating provider will notify the research personnel who would see the patient promptly in the privacy of the examination room and then explain to the patient about the study in detail (objectives, design, tests involved and follow up, etc.) and seek their consent. Once consent is obtained then the research personnel will enroll the participant to the appropriate group (GLP vs non GLP) based on the medications prescribed by the treating provider. The research personnel will coordinate with radiology to schedule the baseline, 6 months and 1 year DXA scan. We will obtain research blood draws at baseline, 6 months and 1 year utilizing the same venipuncture done for routine clinical labs at these visits. A $10 gift card will be given to participants after completion of each of the three DXA scan amounting to a total of 30$ for the entire study. Please see the Schedule of events document for a detailed description of study procedures.
Inclusion Criteria	Criteria that will identify the study population -A diagnosis of Type 2 Diabetes Mellitus -Postmenopausal female -Age >55 years - Hemoglobin A1c between 7 and 10 within 6 months of the first visit.
Exclusion Criteria	List all exclusion criteria* -A diagnosis of Type 1 Diabetes mellitus -History of GLP-1 receptor agonist/DPP4 inhibitor use -eGFR <30 ml/min in the last 3 months -History of pancreatitis -Documented personal or family history of medullary thyroid cancer -Documented history of treatment with anti-osteoporosis agents - Documented secondary osteoporosis -Documented presence of prosthesis or devices in the spine -Unwilling or unable to consent

Number of Participants/ Records to be reviewed (anticipated)	48
Outcome Measures	The primary outcome will be change in TBS from baseline to six months and one year after the initiation of a GLP-1 receptor agonist. TBS will be assessed by DXA scans done at baseline, six months and one year. The secondary outcomes are - Change in visceral fat mass (measured by DXA) at 6 and 1 year after starting GLP-1 receptor agonists as compared to baseline. - Change in inflammatory markers and bone resorption markers (as measured by commercial assays) at 6 and 12 months after starting GLP-1 receptor agonists as compared to baseline. - Changes in levels of sclerostin, osteocalcin, and P1NP (as measured by commercial assays) from baseline to six months and one year after starting GLP-1 receptor agonist. Statistical methods to assess changes in individual measures and possible correlations among different measures (e.g. TBS, visceral fat mass, and biomarkers) are described below.
Study Endpoints	Outcomes will be assessed at six months and one year and will be compared to baseline values.

Protected Health Information (PHI)	PHI to be accessed: Patient's medical record PHI to be collected: Name, Address, phone number and email address Medical record number Date of birth Race (African American or Caucasian) Duration of menopause (Years and months) Duration of T2DM (Years and months) Smoker (Yes/No) and duration Alcohol use (Yes/No) (Years and months) Presence of diabetic retinopathy (Yes/No) Presence of diabetic neuropathy (Yes/No) History of falls and/or fractures (Yes/No) List of anti-hyperglycemic medications List of current medications Weight Height BMI Hip circumference Waist circumference Blood pressure Date of DXA scan Visceral adiposity index Subcutaneous adiposity Lumbar trabecular score Hemoglobin A1c (%) Low density lipoprotein (LDL) High density lipoprotein (HDL) Total cholesterol Triglycerides Microalbuminuria/Creatinine ratio Interleukin-1 (IL-1) Interleukin-6 (IL-6) Tumor necrosis factor-alpha C-telopeptide Sclerostin P1NP Osteocalcin. RedCap will be utilized to record all study date. Access to study data will be restricted to members of the study team.

Statistical Methodology	Statistical analyses were designed in collaboration with Dr. Wondwosen Yimer, PhD (Statistics). Means and standard deviations of baseline, six month and one year TBS values will be reported. Changes in TBS will be analyzed using a linear mixed model contrasting the GLP and non GLP groups. Means and standard deviations of baseline, and 6 and 12-month values of visceral fat mass, IL-1, IL-6 , TNF-α and C-telopeptide levels will be reported. Changes in TBS will be analyzed for any correlation the changes in visceral fat mass, levels of inflammatory mediators and C-telopeptide in the GLP and non GLP groups using linear or logistic regression as appropriate. Means and standard deviations of baseline, six month and one-year values of sclerostin, P1NP and osteocalcin will be reported and analyzed for any correlation to the changes in TBS in the GLP and non GLP groups linear or logistic regression as appropriate. Power analysis: The primary endpoint is the change in TBS at 6 and 12 months compared to baseline. From prior studies we expect a 10% increase in TBS (corresponding to 0.117 for human TBS) for the GLP group only. With a difference of 0.117 between 24 cases and 24 controls we have power of >0.8 and < 5% probability of type 1 error. The primary motivation for this pilot study is to assess effect sizes to support more accurate power calculations, to be used in an application for extramural funding to conduct a larger, definitive study.

References	*1) Kanis JA (2007) WHO Technical Report, University of Sheffield, UK: 66.* *2) https://www.iofbonehealth.org/facts-statistics#category-14 as accessed on 4/1/2019.* *3) Gullberg B, Johnell O, Kanis JA (1997) World-wide projections for hip fracture. Osteoporos Int 7:407.* *4) Paschou SA, Dede AD, Anagnostis PG, Vryonidou A, Morganstein D, Goulis DG. Type 2 Diabetes and Osteoporosis: A Guide to Optimal Management. J Clin Endocrinol Metab. 2017 1; 02(10):3621-3634.* *5) Schacter GI, Leslie WD. Diabetes and Bone Disease. Endocrinol Metab Clin North Am. 2017; 46(1):63-85.* *6) Jiang N, Xia W. Assessment of bone quality in patients with diabetes mellitus. Osteoporos Int. 018; 29(8):1721-1736.* *7) Zhu ZN, Jiang YF, Ding T. Risk of fracture with thiazolidinediones: an updated meta-analysis of randomized clinical trials. Bone. 2014; 68: 115-23.* *8) Watts NB, Bilezikian JP, Usiskin K, Edwards R, Desai M, Law G, Meininger G.Effects of Canagliflozin on Fracture Risk in Patients With Type 2 Diabetes Mellitus. J Clin Endocrinol Metab. 2016; 101(1):157-66* *9) Mabilleau G, Pereira M, Chenu C. Novel skeletal effects of glucagon-like peptide-1 (GLP-1) receptor agonists. J Endocrinol. 2018; 236(1):R29-R42.*
Data Collection Sheet	See Case report form attached to the proposal
Funding Source	We have been successful in obtaining an MCCTR pilot grant for this study.