**Diabetic Complications Consortium**

**Application Title:** Investigation of Mechanisms Underlying Diabetic Skeletal Fragility
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## 1. Project Accomplishments:

We have enrolled 54 adults (n=21 T2D, n=33 non-diabetic) who were undergoing total hip replacement surgery due to osteoarthritis (Table 1), acquiring a specimen from the proximal femur and a blood sample. We extracted a section from the femoral neck and a trabecular core from the femoral head and imaged these specimens using micro-computed tomography (μCT) to assess cortical and trabecular microarchitecture, mechanically tested them using cyclic reference point indentation (cRPI) and uniaxial compression to assess cortical and trabecular mechanical properties, and performed fluorometric assay to measure advanced glycation endproduct (AGE) content. Serum samples were used to measure pre-operative glycated hemoglobin (HbA1c) and content of advanced glycation endproducts (AGE).

We have been granted a renewal of our IRB protocol as of 9/29/2017 to continue subject recruitment in order to collect data on bone pentosidine (a specific AGE) and skin AGEs. Results from this work has been presented both orally and in poster format at annual meetings of the American Society for Bone and Mineral Research (ASBMR) and the Orthopaedic Research Society (ORS). We are currently preparing a manuscript for submission.

## 2. Specific Aims:

**Aim 1**: **To determine contribution of AGE accumulation to bone microstructure and bone biomechanical properties at the proximal femur in adults with T2D.**

**Progress / Results:** In the specimens collected to date**,** we found no differences in cortical porosity at the femoral neck between T2D and non-diabetic subjects. Although previous studies have reported increased cortical porosity in subjects with T2D, these measurements taken from the distal radius and tibia. Our measurements are taken at the femoral neck, a site where, to our knowledge, cortical porosity has not yet been quantified.

Cortical bone AGEs tended to be higher in T2D (+21.3%, p=0.10), while trabecular bone AGEs were similar in the two groups. These findings are similar to those reported by other studies.
Compressive mechanical testing of trabecular bone specimens from the femoral head did not reveal any differences between groups, with the exception of compressive toughness-to-yield, which was 60.0% lower in T2D subjects than in non-diabetic subjects (p=0.06). Cyclic reference point indentation of femoral neck cortical bone revealed higher creep indentation distance (+15.9%, $p \leq 0.05$) and indentation distance increase (+19.9%, $p = 0.058$) in T2D subjects compared to the non-diabetic subjects. No other cRPI variables differed significantly between groups, but indentation distances trended in the same direction (i.e., worse outcomes in T2D vs. controls). These findings illustrate that probes indented deeper into T2D cortical bone compared to non-diabetic bone, which suggests that T2D cortical bone has reduced ability to resist the initiation and propagation of microscale cracks. Together, our results from mechanical testing of cortical and trabecular bone are consistent with worse cortical bone indentation properties in T2D subjects.

We did not observe any relationships between bone AGEs and cRPI variables. It is possible that the lack of relationships may be due to the small and heterogeneous sample size and/or the fact that hyperglycemia in our subjects was fairly well-controlled.

**Specific Aim 2: To determine the associations between glycemic control and AGE levels in bone, skin and serum. Specifically, we hypothesized that bone levels of pentosidine and total AGE content would be positively correlated with skin AGE content, serum pentosidine, and poor glycemic control as reflected by higher serum HbA1c levels.**

**Progress / Results:** We are currently working on developing a protocol to measure pentosidine content in bone. In addition, we received our AGE reader device in later stages of subject recruitment. Thus, we have not yet collected sufficient bone pentosidine or skin AGE data to be able to report correlations with the variables stated in Aim 2. Notably, we found no association between HbA1c and either total cortical bone AGE content or total trabecular bone AGE content. However, total cortical bone AGE content was positively correlated with serum pentosidine ($r=0.39$, $p \leq 0.05$), whereas total trabecular bone AGE content was unrelated to serum pentosidine. These preliminary findings suggest that serum measures of glycation do not serve as good predictors of glycation content in bone. However, further work with a larger sample size and wider range of AGEs are necessary to confirm the lack of correlations detected here.

Table 1. Demographics and clinical characteristics of non-diabetic and T2D subjects enrolled in the study, expressed as mean ± SD for continuous variables and number of subjects for categorical variables.

| | **Non-Diabetic (n = 33)** | **Type 2 Diabetic (n = 21)** |
|---|---|---|
| **Sex** | | |
| Male (n, %) | 19 (58%) | 12 (57%) |
| Female (n, %) | 14 (42%) | 9 (43%) |
| **Race** | | |
| White / Caucasian | 27 (82%) | 17 (81%) |
| Black / African-American | 5 (15%) | 4 (19%) |
| Asian | 1 (3%) | 0 |
| **Basic Clinical Characteristics** | | |
| Age (yrs) | 64.3 ± 10.9 | 66.1 ± 9.8 |
| Height (m) | 1.70 ± 0.11 | 1.68 ± 0.07 |
| Weight (kg) | 86.2 ± 23.2 | 92.9 ± 15.7 |
| BMI ($kg/m^2$) | 30.0 ± 8.0 | 33.0 ± 5.7 |
| **Diabetic Status** | | |
| HbA1c ( %) | 5.70 ± 0.24 | 7.00 ± 1.31 |
| **Diabetes Medication Used** | | |
| Metformin | - | 11 (52%) |
| Insulin | - | 7 (33%) |
| Other | - | 3 (14%) |

## 3. Troubleshooting and Future Work:

In the initial phases of the study, we lost several specimens due to mishandling and/or miscommunication with the pathology department. Accordingly, the some of the results decribed above earlier were based on subsets of the total enrolled number of T2D and non-diabetic subjects. In addition, we were not able to obtain the skin AGE reader or a protocol to evaluate pentosidine in the bone. Therefore, we applied for extended IRB approval to recruit more subjects to complete these analyses and approval was granted recently. We will continue to enroll subjects for the next year, as requested in the no-cost extension.

## 4. Publications:

Abstracts

1. Karim L, Van Vliet M, Velie K, Abdeen A, Ayres D, Bouxsein ML. Role of Advanced Glycation End-Products and Cortical Porosity in Type 2 Diabetes. Abstracts of the 38th Annual Meeting for the American Society for Bone and Mineral Research, Atlanta, GA, 2016. Selected for poster presentation.
2. Karim L, Velie K, Van Vliet M, Abdeen A, Ayres D, Bouxsein ML. Cortical Bone Tissue Properties are Deteriorated in Adults with Type 2 Diabetes Mellitus. Transactions of the 62nd Annual Meeting of the Orthopaedic Research Society, Orlando, FL, 2016. Selected for oral presentation.
3. Karim L, Moulton JN, Abdeen A, Ayres, D, Bouxsein ML. Pre-Yield Trabecular Bone Mechanical Properties are Altered in Adults with Type 2 Diabetes. Submitted to the Orthopaedic Research Society Annual Meeting. (under review)