"Radiographic and proteomic characterization of bone health in patients with type 2 diabetes and type 1 diabetes of extreme duration"

Specific Aims:

Specific Aim 1: Characterize bone health by DEXA and HR-pQCT in those with extreme duration type 1 diabetes (Medalists);

Specific Aim 2 Characterize bone health by DEXA and HR-pQCT in a matched group of individuals with type 2 diabetes and nondiabetic controls, and compare to those with extreme duration type 1 diabetes.

Specific Aim 3 Characterize and compare the plasma protein profile associations with structural features of type 1 diabetes, type 2 diabetes and non-diabetic controls.

Summary of Goals:

The goal of this study was to provide pilot data to better characterize skeletal health in an aging population with type 1 diabetes relative to those with type 2 diabetes and those without the disease in the same age group. This was to be done by collecting radiographic data and plasma protein profiles from individuals belonging to each of these groups. All of these goals were accomplished.

Accomplishments:

As detailed in Table 1 below, specimens were collected for analysis from 15 individuals with 50 or more years of type 1 diabetes, 15 individuals with type 2 diabetes and 10 individuals without diabetes. It was a surprise to the investigators that the group without diabetes was the most difficult to recruit. Due to the expected difficulty of the other groups (type 1 and type 2), those with diabetes were recruited first and the controls were recruited secondarily. It was also expected that as the funding started one month later than expected, one more month would be available for recruiting, which in the end was not available.

The results from the radiographic imaging, both dual energy x-ray absorptiometry and high resolution peripheral quantitative computed tomography, provide quantitative evidence of that which we hypothesized based on evidence from the literature. There is a slight preservation of bone mineral density amongst those with type 1 diabetes relative to those without diabetes, but these individuals without diabetes do not experience the same increase in BMD as those with type 2 diabetes. This difference is additionally supported by the higher cortical BMD and lower

	Controls			**Medalists**			**Type 2**			
	N	Mean	Std Dev	N	Mean	Std Dev	N	Mean	Std Dev	P-value
Age	11	61.56	7.49	14	64.26	4.63	14	62.37	9.50	0.37
HbA1c (%)	10	5.59	0.24	13	7.12	0.79	15	7.07	0.96	<0.001
HDL-C (mg/dL)	10	73.09	18.49	13	74.54	14.64	15	54.73	16.63	<0.01
LDL (mg/dL)	10	94.45	31.50	13	77.54	18.07	15	96.27	32.13	0.11
Total Cholesterol (mg/dL)	10	187.36	36.04	13	166.62	27.10	15	181.73	40.19	0.18
Serum Creatinine	10	0.82	0.16	12	0.82	0.14	15	0.77	0.28	0.81
DEXA measures	N	Mean	Std Dev	N	Mean	Std Dev	N	Mean	Std Dev	P-value
Femoral Neck										
Bone Mineral Density	11	0.73	0.09	15	0.74	0.14	15	0.84	0.16	0.02
Z-Score	11	0.17	0.64	15	0.48	1.27	15	0.98	1.20	0.20
T-Score	11	-1.16	0.75	15	-1.04	1.26	14	-0.51	1.25	0.15
Total Lumbar Spine										
Bone Mineral Density	11	0.98	0.20	15	1.01	0.19	15	1.26	0.64	0.08
Z-Score	11	0.85	1.55	15	1.49	1.69	15	1.70	1.17	0.37
T-Score	11	-0.68	1.70	15	-0.15	1.85	14	0.27	1.53	0.39
1/3 Radius										
Bone Mineral Density	11	0.67	0.07	9	0.65	0.10	15	0.66	0.10	0.64
Z-Score	11	-0.38	1.05	9	-0.68	1.64	15	-0.49	1.62	0.67
T-Score	11	1.89	3.14	9	0.94	1.61	15	0.97	1.70	0.93
Basic HR-pQCT measures	N	Mean	Std Dev	N	Mean	Std Dev	N	Mean	Std Dev	P-value
Radius										
Trabecular Area (mm^2)	10	243.36	62.04	14	234.15	31.00	14	259.10	52.11	0.61
Trabecular Bone Density (mgHA/cm^3)	10	146.62	40.12	14	155.60	46.50	14	159.39	49.62	0.5
Cortical Bone Mineral Density (mgHAcm^3)	10	951.52	71.56	14	947.45	48.56	14	939.04	55.37	0.7
Cortical Thickness (mm)	10	0.70	0.21	14	0.77	0.13	14	0.76	0.16	0.4
Number of Trabeculae (1/mm)	10	1.75	0.42	14	1.80	0.46	14	1.80	0.48	0.7
Interhomogeneity of Network (mm)	10	0.30	0.15	14	0.28	0.24	14	0.29	0.26	0.5
Tibia										
Trabecular Area (mm^2)	10	658.67	167.00	15	636.52	112.28	15	669.33	140.78	0.8
Trabecular Bone Density (mgHA/cm^3)	10	160.42	31.60	15	181.61	42.12	15	175.89	42.12	0.4
Cortical Bone Mineral Density (mgHAcm^3)	10	863.60	83.41	15	790.46	81.94	15	865.53	71.47	0.03
Cortical Thickness (mm)	10	0.97	0.28	15	0.87	0.23	15	1.15	0.33	0.04
Number of Trabeculae (1/mm)	10	1.76	0.24	15	1.87	0.41	15	1.86	0.38	0.5
Interhomogeneity of Network (mm)	10	0.24	0.10	15	0.26	0.21	15	0.23	0.11	0.5
Porosity measures										
Radius	N	Mean	Std Dev	N	Mean	Std Dev	N	Mean	Std Dev	
Cortical Pore Volume (mm^3)	10	10.58	6.19	14	15.62	12.01	14	13.08	8.11	0.3
Cortical Porosity (%)	10	2.73	1.89	14	3.37	2.57	14	2.79	1.91	0.5
Cortical Pore Diameter (mm)	10	0.18	0.02	14	0.20	0.05	14	0.18	0.03	0.8
Cortical Pore Diameter Distribtion (mm)	10	0.08	0.02	14	0.09	0.03	14	0.08	0.02	0.6
Endocortical Perimeter (mm)	10	94.10	13.20	14	61.86	5.53	14	65.90	8.83	0.47
Tibia										
Cortical Pore Volume (mm^3)	10	64.94	32.82	15	94.60	35.59	15	76.67	26.66	0.09
Cortical Porosity (%)	10	7.56	3.56	15	11.63	4.54	15	7.64	2.69	0.03
Cortical Pore Diameter (mm)	10	0.21	0.03	15	0.22	0.03	15	0.20	0.02	0.03
Cortical Pore Diameter Distribtion (mm)	10	0.09	0.02	15	0.10	0.01	15	0.09	0.01	0.05
Endocortical Perimeter (mm)	10	94.09	13.20	15	94.06	9.93	15	93.67	12.84	0.97

Table 1. Summary of clinical and radiographic measurements of controls, Medalists and individuals with type 2 diabetes

cortical thickness relative to the two other groups (Table 1). This is consistent with hypotheses in the literature which suggests that insulin resistance, a hallmark of T2DM, reduces the turnover of bone by osteoclasts. Yet, amongst individuals with type 1 there is a higher cortical

porosity which may contribute to the greater fragility experienced by this group relative to both those with type 2 diabetes and age matched controls, despite other markers.

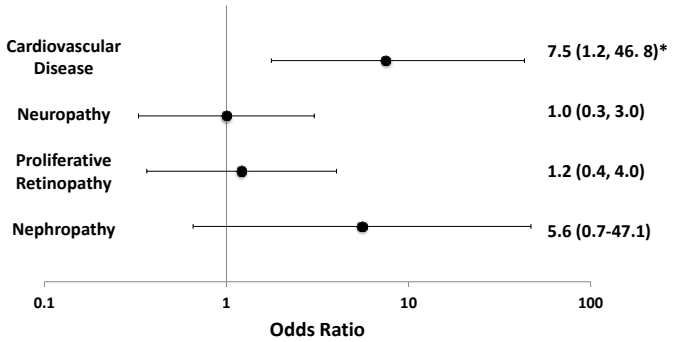

Figure 1. Odds ratio of diabetic complications and low BMD (T < -1.0 SD) at the femoral neck in Medalists *Adjusted for statin use

Analysis for the third specific aim which was characterization of plasma protein targets across the three groups associated with bone phenotypes yielded the expected results - association of known bone turnover/ formation factors having a different degree of association in each group. A caveat which must be noted is the low power in the analysis. The relationships with proteins will be discussed individually in each group with emphasis on the cortical porosity of the tibia, as it has been the primary factor associated with bone fragility and fracture risk.

There are several factors which are both strongly negatively and positively correlated with cortical porosity in to the tibia (Table 2); however, none are consistently related in the three groups to the same degree. This is consistent with the hypothesis of the proposal. Further analyses are planned to compare the relative contribution to porosity across the three groups.

One of the most interesting and supportive pieces of data previously seen in our study is of Alpha-2-HS-glycoprotein, also known as Fetuin-A, among the 50 –Year Medalists. In our initial research on bone mineral density (BMD) amongst the Medalists and vascular complications, we saw an increased risk for cardiovascular disease with lower BMD (Figure 1). This is consistent with what has been found in other studies, including the Framingham Heart Study. Due to this finding, we not only furthered our search for factors in bone, we also directly examined potential factors contributing to coronary artery calcification including osteocalcin (OCN) in the Medalists, a key factor in hydroxyapatite deposition (Figure 2). During this examination, we identified a significant difference in the levels of OCN positive monocytes amongst those Medalists positive for CVD

Type 2	rho	Unadjusted p	
SPARC-related modular calcium-binding protein 1	0.593	0.0223	Calcium binding in bones and eye/ recessive genetic disease
Fibrinogen	0.607	0.0187	Improved bone healing
Pleiotrophin	0.614	0.0171	growth factor
Lipopolysaccharide-binding protein	0.643	0.0117	MMP10/ macrophage activation - heterotopic ossification;
Megakaryocyte-associated tyrosine-protein kinase	0.686	0.00617	Co-receptor for tyrosine phosphorylation of VEGF, regulates bone metabolism.
Neuropilin-1	0.686	0.00617	The cell surface receptor through which GDF9 generates a signal is the bone morphogenetic protein type II receptor (BMPR2)/ BMP15
Medalists			
Platelet glycoprotein Ib alpha chain	0.579	0.0264	Osteoclasts both resorb bone and provides inhibitory and stimulatory signals,
Tyrosine-protein phosphatase non-receptor type 1	0.596	0.0213	Implicated in the differentiation of neuronal and bone marrow
Contactin-2	0.707	0.00432	binds to TGF-beta/BMP cytokines and blocks TGF-beta1 binding to cell surface receptors
Alpha-2-HS-glycoprotein	0.818	0.000297	Fetuin- A associated with vascular calcification
Controls			
DNA topoisomerase 1	0.794	0.00984	Receptor on the TGF-beta super family Interacts with BMP receptor IA/ normal blood vessel development
Chitinase-3-like protein 1	0.806	0.00824	Associated with decreased joint mobility, particularly cartilage degradation
Chymase	0.806	0.00824	Interaction with mast cells and Notch pathway for differentiation into osteoblasts
Interleukin-8	0.806	0.00824	Suppression of this factor by zingerone has be associated with suppression of inflammation with evidence in the cartilage by p38 and JNK pathways
Vascular endothelial growth factor D	0.83	0.00556	Induces osteoclast differnetiation in the presence of Nfkappa-B ligand, Down regulation abolishes differentiation

Table 2. Correlations and significance levels by disease status with cortical porosity at the tibia

compared to those without the disease. In further studies examining the mechanism of OCN, we identified the role of HDL-c, one of the receptors, and OCN's ability to suppress it (data not shown). The positive correlation demonstrated herein of a marker of decreased bone strength (porosity) and increased levels of Fetuin-A continues to support this model.

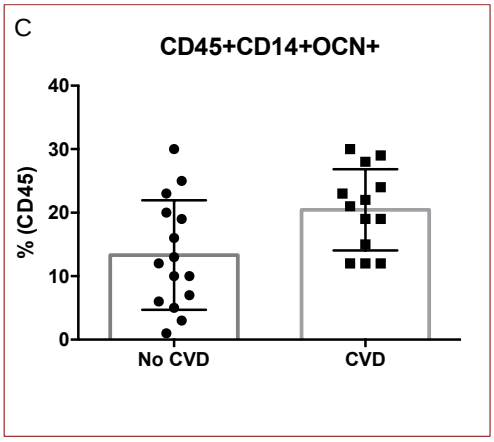

Figure 2. Percent OCN+ cells by CVD status among Medalists

In the other groups examined, several other previously reported proteins were found to be associated with porosity. We are currently examining the relationship between groups and will be doing pathway analyses, we plan to publish these results and make data available to others.

Publications:

Keenan, HA, Maddaloni E. Bone Microarchitecture in Type 1 Diabetes: It's complicated. Curr Osteoporos Rep. 2016 Oct 4 PMID:27704394

Resource Sharing

All data and useful radiologic images resulting from this research will be made available according to the rules of this funding mechanism. All detailed resources will be released within five years of completion of the study. Material Transfer and Intellectual Property: My institution and I will adhere to the NIH Grants Policy on Sharing of Unique Research Resources including the "Sharing of Biomedical Research Resources: Principles and Guidelines for Recipients of NIH Grants and Contracts" issued in May, 2004: http://grants.nih.gov/grants/policy/model_organism/. Specifically, material transfers would be made with no more restrictive terms than those found in the Simple Letter Agreement or the UBMTA and without reach-through requirements. Should any intellectual property arise which requires a patent, we would ensure that the technology remains widely available to the research community in accordance with the NIH Principles and Guidelines document. Regarding any DNA constructs generated with funds from this grant, we will adhere to the NIH Grants Policy on Sharing of Unique Research Resources including the "Sharing of Biomedical Research Resources Principles and Guidelines for Recipients of NIH Grants and Contracts".