Diabetic Complications Consortium

Application Title: The Role of Hormones in the Premature Vascular and Bone Disease in Women with Type 1 Diabetes

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1. Project Accomplishments:

Over the past year of this research award, we have purchased and used the Trabecular Bone Score (TBS) software from Insight on our DXA scanner, and have been able to measure TBS on all DXA scans performed at our Center on our studies of women with and without type 1 diabetes (T1D). In addition, we have examined differences in sex hormones and menstrual dysfunction in women with T1D compared to age-matched controls without diabetes and how these differences relate to the accelerated vascular complications in women with T1D. Lastly, we have measured bone turnover markers to examine how these markers are related to the increased risk for bone disease and CVD in women with T1D.

2. Specific Aims:

Aim-1: To evaluate differences in sex hormones and menstrual dysfunction in women with T1D compared to age- matched controls without diabetes

Results for Aim 1:

We examined differences in sex hormones and menstrual dysfunction between women with T1D and similarly aged women without diabetes. As shown in Table 1, women with T1D were slightly younger than the women without diabetes who were recruited, but had significantly more coronary artery calcium, a marker of subclinical atherosclerosis. Women with T1D were slightly more overweight and had a larger waist circumference, but a similar volume of intra-abdominal fat. Total and LDL cholesterol and triglycerides were al significantly lower in women with T1D than in non-diabetic women, but HDL cholesterol was not different. Women with T1D were more likely to report amenorrhea and menstrual dysfunction, and reported longer menstrual cycles on average than women without diabetes.

Table 1 ***Characteristics of Study Participants***	Type 1 Diabetes (n=160)	Control (n=151)
Age (years)	37 ± 7*	40 ± 8
Duration of diabetes (years)	25 ± 8	N/A
Insulin dose per kg body weight per day	0.60 ± 0.31	N/A
Glycosolated hemoglobin A1c ($HbA1_c$) (%)	7.7 ± 1.3**	5.2± 0.4
Coronary calcium score (Agatston Units)	32.4 ± 113.0**	2.12 ± 9.7
Progression of coronary calcium from baseline to 1st follow-up visit ∓ (%)	16.3%**	3.6%
Body Mass Index (kg/m^2)	26.4 ± 4.6*	25.1 ± 5.5
Average Waist Circumference (cm)	81.1 ± 11.4*	77.3 ± 12.3
Intra-abdominal fat volume, lumbar 4-5 (cm^2)	28 (20-42)	32 (21-52)
Subcutaneous fat volume, lumbar 4-5 (cm^2)	149 (101-207)	129 (85-192)
Total Cholesterol (mg/dl)	177 ± 32**	191 ± 33

LDL-Cholesterol (mg/dl)	99 ± 28**	113 ± 30
HDL-Cholesterol (mg/dl)	65 ± 18	62 ± 17
Triglycerides (mg/dl)	54 (44-71)**	67 (48-94)
Hypertension (% yes)	39%**	9%
Systolic / Diastolic Blood Pressure (mm/Hg)	108*/72	105/72
Current smoker (% yes)	10 %	9%
Age at menarche (years)	13.3 ± 2.0	13.0 ± 1.5
Average length of menstrual cycle (days)	31.6 ± 20.0*	28.2 ± 4.7
Irregular menstrual periods (% by self report)	17%	11%
Ever had amenorrhea (<3 cycles in one year) (% by self report)	16%*	5%
Menstrual dysfunction (irregular periods and/or amenorrhea)	24%*	15%
Ever used hormonal birth control (% yes)	79%*	89%
Ever been pregnant (% by self report)	65%	67%
Ever had a miscarriage or stillbirth (% by self report)	34%	34%
Total number of pregnancies	2.4 ± 1.3	2.4 ± 1.1
Number of live births	1.6 ± 0.90	1.7 ± 1.1

* p<0.05, ** p< 0.001; Data are means ± SD or median (25^{th} – 75^{th} percentile)
Ŧ Progression was defined as an increase of 2.5 or greater in square root transformed coronary calcium volume from baseline to visit 2.

As shown in **Table 2**, we examined levels of sex hormones by diabetes status and phase of the menstrual cycle at the time of the visit among premenopausal women. Women with T1D had higher total testosterone and SHBG compared to women without diabetes, but there was no difference in total estradiol (E2). However, when free estradiol index (FEI) was calculated from estradiol and SHBG, women with T1D had significantly lower FEI than women without diabetes.

Table 2	**T1D (n=160)**				**Non-DM (n=151)**				**Least Square Means Difference (95% CI)**	**P-value**
Menstrual cycle Phase	EF (n=27)	LF (n=61)	LU (n=58)	AN (n=14)	EF (n=26)	LF (n=54)	LU (n=68)	AN (n=3)	T1D vs. Non-DM	
T (ng/dL)	41.5	52.7*	45.6	46.9	31.7	42.5	38.0	36.6	9.7 (3.5, 15.8)	0.002
SHBG (nmol/L)	175*	153*	164*	143	128	118	101	106	49 (34, 64)	< 0.001
FAI	1.05	1.48	1.11	2.23	1.11	1.51	1.54	1.18	0.17 (-0.22, 0.56)	0.40
E2 (pg/ml)	46.8	147.7	130.5	54.0	46.0	164.3	111.4	67.3	-0.19 (-14.9, 14.5)	0.98
FEI	0.10	0.34*	0.30*	0.16	0.14	0.47	0.42	0.23	-0.32 (-0.55, -0.10)	0.003
LH (mIU/ml)	5.9	9.7	4.4	3.7	4.6	10.0	3.9	9.5	-0.2 (-0.5, 0.08)	0.15
FSH *mIU/ml)	9.7	8.2	4.5	5.4*	8.1	8.3	4.4	14.7	-0.001 (-0.12, 0.11)	0.90
Progesterone (ng/ml)	0.69	0.98	11.31	0.86	0.84	0.88	10.53	1.23	0.09 (-1.29, 1.47)	0.89

EF = Early Follicular, LF = Late Follicular, LU = Luteal, AN = Anovulatory; * p < 0.0125, ** p < 0.001

We then examined whether the sex hormone levels were associated with coronary artery calcium (CAC) progression, as shown in Table 3. Higher levels of SHBG and FSH predicted progression of CAC, as did lower levels of FEI and FAI.

Table 3 ***Association of Hormones with CAC Progression***§	**Odds Ratio (95% CI)***	**P-value**
SHBG (square root transformed)	**2.0 (1.2-3.3)**	**0.007**
E2 (log transformed)	0.6 (0.3-1.1)	0.10
T (cubic transformed)	1.1 (0.7-1.9)	0.67
LH (log transformed)	1.2 (0.6-2.3)	0.59
FSH (log transformed)	**2.2 (1.2-4.4)**	**0.02**
FEI (log transformed)	**0.3 (0.2-0.7)**	**0.001**
FAI (log transformed)	**0.6 (0.4-1.0)**	**0.048**

* Standardized odds ratios per SD in transformed units (SHBG=2.8, E2=0.62, T=0.49, LH=0.86, FSH=0.64, FEI= , FAI= 0.63)

§Adjusted for age, diabetes, baseline calcium volume, follow-up time, BMI, HDL-cholesterol, LDL-cholesterol, systolic and diastolic blood pressure, and menstrual cycle phase

Aim-2: To study the differences in bone health in women with T1D compared to age- matched controls without diabetes

Results for Aim 2: We have examined bone density at the hip, lumbar spine and forearm and lumbar spine TBS in women with T1D compared to age-matched controls without diabetes. As shown in Table 4, women with T1D were younger by an average of 2 years than women without T1D, but in univariate analysis had significantly lower left and right hip and forearm bone mineral density (BMD) at all sites (upper, mid and lower third of the forearm). There was no difference in BMD at the lumbar spine or trabecular bone score (TBS) by diabetes status in univariate analysis. By self-report, women with T1D were twice as likely (49% vs. 24%, p=0.01) to have fractured a bone, confirming that fracture is more common in women with T1D than women without diabetes.

Table 4	Women with T1D (n=68)	Non-diabetic Women (n=78)	p-value
Age	57 ± 10	59 ± 9	0.02
Left hip Total BMD (g/cm^2)	0.82 ± 0.12	0.86 ± 0.13	0.01
Left hip Neck BMD (g/cm^2)	0.69 ± 0.12	0.76 ± 0.11	0.0003
Right hip Total BMD (g/cm^2)	0.80 ± 0.15	0.89 ± 0.11	0.0001
Right hip Neck BMD (g/cm^2)	0.69 ± 0.11	0.77 ± 0.11	<0.0001
Forearm Upper BMD (g/cm^2)	0.38 ± 0.07	0.42 ± 0.15	0.004
Forearm Mid BMD (g/cm^2)	0.54 ± 0.08	0.59 ± 0.06	<0.0001
Forearm lower third BMD (g/cm^2)	0.63 ± 0.10	0.69 ± 0.08	0.0002
Lumbar spine BMD (g/cm^2)	0.98 ± 0.13	0.99 ± 0.14	0.54
Lumbar spine TBS (g/cm^2)	1.36 ± 0.09	1.38 ± 0.09	0.27

In multivariate linear regression, adjusted for age, body fat and lumbar spine BMD, women with T1D had significantly lower TBS than women without diabetes (1.33 ± 0.01 vs. 1.36 ± 0.01, p=0.03). Higher levels of SHBG were negatively correlated to BMD of the right (r=-0.45, p=0.02) and left hip (r=-0.54, p=0.005) and FAI was positively correlated to BMD of the right (r=0.56, p=0.003) and left hip (r=0.65, p=0.0004) and the BMD of the lower third of the forearm (r=0.48, p=0.01).

Aim-3: To study how sex hormones and bone turnover markers are related to the development of bone disease and CVD in women with T1D

Results for Aim 3: We have completed measuresment of bone markers included in the Meso Scale Diagnostics (MSD) Human Bone Panels 1 and 2, which include alkaline phosphatase (ALP), sclerostin (SOST), osteoprotegerin (OPG), osteocalcin (OCL), osteonectin (ONN), and osteopontin (OPN). In addition, we have measured additional bone related markers including Vitamin D, insulin-like growth factor (IGF)-1, amino-terminal propeptide (P1NP), and c-terminal telopeptide (CTx1).

In our preliminary analysis, we have found significant differences in bone markers between women with and without type 1 diabetes, as shown in Table 5, including higher ALP, OPG, and OPN but lower CTx-1 and IGF-1.

Table 5	**Women with T1D (n=68)**	**Non-diabetic Women (n=78)**	**p-value**
ALP (ng/mL)	**13.3 ± 5.8**	**11.2 ± 4.5**	**0.02**
Sclerostin (ng/mL)	0.045 ± 0.02	0.050 ± 0.02	0.10
OPG (ng/mL)	**0.38 ± 0.11**	**0.32 ± 0.08**	**<0.0001**
ONN (ng/mL)	1192 ± 328	1193 ± 379	0.98
OPN (ng/mL)	**21.6 ± 10.0**	**18.1 ± 7.3**	**0.01**
OCL (ng/mL)	34.6 ± 16.8	33.3 ± 12.9	0.61
P1NP (ng/mL)	24 ± 2.6	20 ± 1.7	0.18
CTx-1 (ng/mL)	**0.35 ± 0.02**	**0.40 ± 0.02**	**0.01**
IGF-1 (pg/mL)	**0.44 ± 0.02**	**0.64 ± 0.03**	**<0.0001**
Vitamin D (ng/mL)	14.8 ± 5.3	16.2 ± 6.9	0.17

Higher levels of OPG are associated with cardiovascular disease, and have previously been reported in adults with type 1 diabetes. Lower levels of IGF-1 have also been reported in type 1 diabetes, perhaps due to the lack of portal vein insulin and related effects on the liver. As shown in **Table 6**, there were significant associations between higher bone density measures and lower levels of ALP, OPG, OPN and OCL in this cohort, but no significant associations with ON, Vitamin D P1NP, CTx-1 or IGF-1.

Table 6	ALP	OPG	SOST	ONN	OPN	OCL	VitD	P1NP	CTX-1	IGF-1
BMD R Neck	**-0.17**	**-0.28**	**0.22**	0.02	**-0.32**	**-0.37**	0.10	-0.11	0.10	-0.11
BMD R Total Hip	**-0.20**	**-0.23**	0.13	0.03	**-0.37**	**-0.35**	0.09	-0.08	0.11	-0.14
BMD L Neck	-0.11	**-0.22**	0.16	0.05	**-0.34**	**-0.35**	0.11	-0.07	0.11	-0.14
BMD L Total Hip	**-0.16**	**-0.19**	0.08	0.06	**-0.39**	**-0.36**	0.13	-0.04	0.12	-0.21
Forearm Upper	-0.10	**-0.24**	0.05	0.005	**-0.33**	**-0.27**	0.08	0.04	0.15	-0.15
Forearm Mid	**-0.18**	**-0.35**	0.06	0.05	**-0.44**	**-0.40**	0.15	-0.02	0.09	-0.23
Forearm lower third	**-0.22**	**-0.33**	0.13	0.006	**-0.42**	**-0.37**	0.13	-0.08	0.07	-0.22
Lumbar spine BMD	-0.03	-0.10	0.29	0.20	**-0.21**	**-0.23**	-0.10	-0.03	-0.02	-0.34
Lumbar spine TBS	**-0.18**	**-0.29**	**0.22**	-0.10	**-0.23**	**-0.19**	0.02	-0.12	-0.005	**-0.23**

Women with T1D in this cohort had greater arterial stiffness as measured by pulse wave velocity (PWV) and carotid intima-media thickness (cIMT), as shown in Table 7.

Table 7	**Women with T1D (n=45)**	**Non-diabetic Women (n=51)**	**p-value**
PWV (m/s)	8.9 ± 1.7	7.9 ± 1.7	0.009
cIMT (mm)	0.68 ± 0.12	0.63 ± 0.09	0.02

In multivariable analysis adjusting for age and diabetes status, the least square mean difference ± Standard Error for cIMT (0.68 ± 0.01 vs. 0.62 ± 0.01, p=0.002) and PWV (9.0 ± 0.26 vs. 7.9 ± 0.26, p=0.005) remained significant. Both PWV and cIMT were negatively correlated to measures of BMD at the mid and lower third of the forearm (r=-0.33, p=0.003 for PWV, r=-0.35, p=0.0006 for cIMT) and the mid forearm (r=-0.30, p=0.006 for PWV, r=-0.20, p=0.05 for cIMT).

Conclusions: From our preliminary analysis, we have confirmed decreased bone density in multiple sites in women with T1D, as well as increased levels of arterial stiffness and subclinical atherosclerosis when compared to women without diabetes. From our analysis to date, it also appears that women with T1D have differences in sex hormones (higher testosterone and SHBG and lower free estradiol index) and bone turnover markers, which may help to explain their increased risk for both osteoporosis and cardiovascular disease.

Publications:

Shah VN, Sippl R, Joshee P, Pyle L, Kohrt WM, Schauer IE, Snell-Bergeon JK, Trabecular bone quality is lower in adults with type 1 diabetes and is negatively associated with insulin resistance, Osteoporosis International, Submitted for Publication, Oct 2017

Ostendorf DM, Shah VN, Schauer IE, Alman AC, Kohrt WM, Bergman BC, Snell-Bergeon JK, Sex Modifies the Association between Measures of Adiposity and Trabecular Bone Score in the CACTI Study: A Cross-Sectional Study, JBMR, Submitted for Publication 10/2017