RELATIONSHIP BETWEEN AUTONOMIC NERVOUS SYSTEM FUNCTION AND BONE MINERAL DENSITY IN TYPE 1 DIABETIC INDIVIDUALS

by

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A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Master of Science in Exercise Science.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Objective: To determine the influence of autonomic nervous system function (ANSF) on bone mineral density (BMD) in type 1 diabetic individuals. **Research Design and Methods:** Sixteen subjects were grouped based on normal (n = 11, mean age = 38.7 ± 10.5 years, mean duration of diabetes = 20.1 ± 14.3 years, mean weight = 77.6 ± 16.2 kg) or abnormal (n = 5, mean age 50.4 ± 9.3 years, mean duration of diabetes = 32.2 ± 14.1 years, mean weight = 73.9 ± 7.3 kg) ANSF as determined by measurement of the mean circular resultant (MCR) of heart rate variability. Additional measures of ANSF included the following: expiration to inspiration ratio during deep breathing, valsalva maneuver, heart rate and blood pressure response to standing, power spectral density analysis of heart rate response to deep breathing, normal breathing, and paced breathing; and spontaneous baroreflex sensitivity during normal breathing and paced breathing. Past physical activity was measured via questionnaire. Current physical activity was measured for three days by an accelerometer. Participants also completed food records on the days that they donned accelerometers. Dual energy x-ray absorptiometry assessed BMD and bone mineral content (BMC) at the left hip, lumbar spine, and total body. **Results:** There was no significant difference in BMD or BMC at the left hip ($p = 0.635$, $p = 0.119$), lumbar spine ($p = 0.722$, $p = 0.572$), or total body ($p = 0.654$, $p = 0.606$) between type 1 diabetic individuals with normal (mean MCR = 56.82 ± 21.70) and abnormal (mean

MCR = 11.62 ± 5.88) ANSF. Measures of physical activity and diet were also not different between groups. Serum creatinine was different between groups ($p = 0.022$). **Conclusions:** These data suggest that there is no difference in BMD between type 1 diabetic individuals with normal and abnormal ANSF. The present findings were likely hampered by an inadequate sample size.

Chapter 1

INTRODUCTION

Type 1 diabetes appears to have detrimental effects on the attainment of peak bone mineral density (BMD) (1, 2), with insufficient BMD potentially leading to osteopenia and osteoporosis (3). Type 1 diabetic postmenopausal women have been found to be over twelve times more likely to experience a hip fracture than those without diabetes (4). In Sweden, Miao et al. found that both men and women with type 1 diabetes are at increased risk for hip fracture. This risk is exacerbated by the presence of microvascular, neurological, or cardiovascular disease (2). Studies (1, 3) have linked various metabolic abnormalities associated with type 1 diabetes to decreased measures of BMD. For instance, insulin deficiency alone may cause a decrease in bone mineral accrual (3, 5). Diabetes may also cause reductions in the metabolism of phosphorus and calcium as well as reductions in collagen proliferation (6). In addition, protein and other nutrients obtained through the diet have been shown to influence BMD (7, 8). Finally, physical activity is recognized to have a fundamental influence on BMD (9, 10, 11) with protective effects evident in premenopausal women (12, 13) and middle-aged men (14).

Type 1 diabetes also has been associated with decreased measures of heart rate variability (HRV), an index of autonomic nervous system function (ANSF) (15).

Decreased measures of HRV are thought to be indicative of decreased parasympathetic nervous system (PNS) activity and increased sympathetic nervous system (SNS) activity (16, 17). In addition, measurement of spontaneous baroreflex (SBR) sensitivity has been considered an index of vagal cardiac control. SNS activity has been thought to depress this reflex (18). Increased SNS activity has been shown to inhibit osteoblasts (19), the cells responsible for bone formation. Meanwhile, decreased SNS activity, via inhibition of beta-adrenergic receptors, has been shown to preserve bone mineral (20) due to decreases in the number and activity of osteoclasts (19, 21), the cells responsible for bone resorption.

The negative effect of type 1 diabetes on BMD is of growing concern with various mechanisms having been implicated. In particular, it appears that the role of the autonomic nervous system warrants further investigation. HRV has been substantiated as a measure of ANSF, especially in those with diabetes mellitus (15, 17, 22, 23). The purpose of this investigation therefore was to use several indices of ANSF, including HRV, to assess the influence of the autonomic nervous system on BMD in type 1 diabetic individuals. We hypothesized that type 1 diabetic individuals with depressed measures of ANSF would demonstrate decreased levels of BMD when compared with type 1 diabetic individuals with normal ANSF measures.

Chapter 2

METHODS

Participants

Twenty-one volunteers were recruited over a twelve-month period from the Diabetes and Metabolic Research Center, Newark, DE, the Diabetes and Metabolic Diseases Center, Wilmington, DE, the University of Delaware campus community, and via advertisements in local periodicals. Type 1 diabetic volunteers were required to be men or premenopausal females of at least 18 years of age. Volunteers were excluded from the study for the following reasons: 1) if they had type 2 diabetes, 2) if they had known coronary artery disease, atrial fibrillation, frequent atrial arrhythmias, frequent ventricular arrhythmias, a pacemaker, or acute myocardial ischemia, 3) if they had clinically significant renal dysfunction, 4) if they were pregnant or lactating, 5) if they were postmenopausal, 6) if their treatment dosage of antihypertensive medications or other medications affecting the autonomic nervous system changed within two months prior to the study, or 7) if they were smokers. Participants were divided into two groups based on the assessment of abnormal or normal ANSF via reference to age-adjusted normative data for the mean circular resultant (MCR) (24).

This investigation was approved by the University of Delaware Institutional Review Board. Participants provided written consent to the investigation

(Appendix A). Participants were compensated for their time and travel expense upon successful completion of all study requirements.

Procedures

Once volunteers had been cleared for participation, they reported to the Human Performance Lab at the University of Delaware for the first of two visits. Participants were instructed to abstain from the following prior to the first visit: medications and nonprescription drugs 8 hours prior to testing, food and caffeinated beverages 10 hours prior to testing, alcoholic beverages 24 hours prior to testing, and vigorous exercise 48 hours prior to testing (25). Trained personnel administered all tests and instructions.

A pilot study was conducted before beginning the full investigation. Healthy volunteers underwent all tests in order to acquaint investigators with protocol procedures.

VISIT ONE – AUTONOMIC FUNCTION TESTING

Intake

A medical history questionnaire (Appendix B) was administered and reviewed. Height and weight were measured on a Healthometer mechanical beam scale (Sunbeam Products, Inc., Boca Raton, Florida). Waist-to-hip ratio was determined by measuring the waist with a soft measuring tape at the narrowest part between the lowest rib and the iliac crest and at the hip at the widest part of the gluteal region. A 12-lead electrocardiogram (ECG) was applied to participants before allowing ten minutes of rest in a supine posture.

Data Acquisition & Analysis

Data for HRV were acquired and analyzed using an ANS2000 ECG Monitor and Respiration Pacer (D.E. Hokanson, Inc., Bellevue, Washington). Concurrently, respiration, electrocardiography, and beat-to-beat blood pressure data were captured at 500 Hz using Windaq software (DATAQ Instruments, Akron, Ohio).

E/I Ratio & Mean Circular Resultant

An ECG provided a measure of heart rate as participants breathed as deeply as possible during five-second cycles for six minutes. If necessary, the deep breathing was repeated and the better of two trials was used for statistical analysis. R-R interval is the interval between two successive R waves on an ECG tracing. The shortest R-R interval during inspiration (I) and the longest R-R interval during expiration (E) were used to calculate the E/I ratio, a measure of RR-variation (26, 27). An E/I ratio above 1.10 was considered normal while an E/I ratio less than or equal to 1.10 was considered indicative of abnormal HRV (26). As a measure of HRV, E/I ratio decreases the influence of intrinsic heart rate, but it is affected by ectopic beats (27). The mean circular resultant, however, serves as a more sensitive measure of HRV while also being robust to ectopic beats. Heartbeats are plotted as events on a timeline that are then plotted around a unit circle that is equivalent to one breath. Normal HRV is observed as a random distribution of heartbeats around the circle. Abnormal HRV is observed as a uniform distribution of heartbeats around the circle. With uniform distribution, vector analysis is implemented to obtain a vector mean, a value that is proportional to the degree of HRV (27). Both E/I ratio and MCR provide

an index of PNS activity (28, 29). The E/I ratio of one subject from the abnormal ANSF group was excluded from the results due to the presence of ectopic beats.

Valsalva Maneuver

Next, while remaining supine, the heart rate response to a Valsalva maneuver was measured as the better of two trials. Participants exhaled into the mouthpiece of an open manometer, maintaining a pressure of 40 mmHg for 15 seconds, thereby inducing a rise in intrathoracic pressure. The ratio of the longest R-R interval after the maneuver to the shortest R-R interval during the maneuver was used to calculate the Valsalva ratio (30). Normal heart rate response to the maneuver manifests as a tachycardia and accompanying peripheral vasoconstriction during the strain and a bradycardia and an accompanying brief rise in blood pressure upon release (25). Thus, the Valsalva invokes a complicated reflex arc mediated by both the PNS and SNS with affects on both the heart and the vasculature (29). A ratio greater than 1.21 was considered normal (24). An increase in intraocular and intracranial pressure occurs as a result of a hemodynamic response precipitated by the increase in intrathoracic pressure. Therein lies a risk for intraocular hemorrhage and lens dislocation; although the maneuver is comparable to pressures experienced as a result of normal daily activities (31). Therefore, a letter requesting permission to perform the valsalva maneuver was sent to participants' eye doctors (Appendix C). The maneuver was only performed upon receipt of written approval from participants' eye doctors. The data for two subjects from the abnormal ANSF group were excluded from the results due to aberrant heart rhythms.

Blood Draw

After the valsalva maneuver, a venous blood sample was collected from the antecubital vein in order to measure the following: glycated hemoglobin, thyroid stimulating hormone, serum creatinine, parathyroid stimulating hormone, and vitamin D. Estimated glomerular filtration rate was calculated using an equation derived from the Modification of Diet in Renal Disease Study (32). Five volunteers were excluded from data analysis due to some manifestation of the following abnormal blood test results: depressed vitamin D levels, elevated serum creatinine, or abnormal parathyroid hormone levels.

Normal & Paced Breathing

At this time, beat-to-beat finger arterial blood pressure was measured using a Finometer (Finapres Medical System, Arnhem, The Netherlands). A finger cuff was placed around the middle phalanx of either the second or third digit of the left hand. The device was calibrated using its built-in return-to-flow calibration. Although finger arterial blood pressure monitoring may lack pure diagnostic value, it is considered a reliable tool for tracking blood pressure changes (33). ECG was acquired through Windaq via a Dinamap Dash 2000 (GE Medical Systems Information Technologies, Milwaukee, Wisconsin). Respiration was monitored via two stretch-sensitive bands placed around the chest and abdomen and interfaced with an Inductotrace (Ambulatory Monitory Inc., Ardsley, New York). Five minutes of data were captured as subjects rested quietly while supine. Next, subjects were instructed to breath at a rate of 0.25 Hz for five minutes. The Finometer's physiocal

function was disabled during periods of data acquisition and enabled between periods of data acquisition.

SBR sensitivity was calculated via a time domain sequence technique using custom software packaged with the Finometer hardware. Linear regression was applied to individual sequences of corresponding changes in systolic blood pressure and pulse interval over three or more successive heartbeats (18, 34). SBR sensitivity was considered the average regression slope from all sequences during total Finometer recording time, during five minutes of normal breathing, and during five minutes of paced breathing. This technique quantifies baroreceptor reflex control of heart rate and is primarily considered an index of vagal tone (18, 34). The SBR sensitivity during normal and paced breathing of one subject from the abnormal ANSF group was excluded due to decreased data acquisition.

Power spectral density (PSD) analysis of an electrocardiogram using fast Fourier transform is widely used in the assessment of ANSF (16). PSD analysis provides an estimation of how the variance of a signal distributes as a function of its frequency (16). Very-low frequency (VLF, 0.0033-0.04 Hz), low frequency (LF, 0-0.08 Hz), and high frequency (HF, 0.15-0.60 Hz) components of the computed spectrum may be differentiated from the short-term recordings made in the present investigation: six minutes of deep breathing, five minutes of normal breathing, and five minutes of paced breathing at 0.25 Hz. In addition, the mid-frequency band (0.08-0.15 Hz) of HRV was calculated and considered an index of PNS activity (35). Although the VLF component of HRV has been considered an index of SNS activity, this may breach the boundaries of PSD analysis (36). The LF component of HRV is influenced by both parasympathetic and sympathetic activity (16). The HF component

is an indicator of efferent vagal activity alone (16). This has been corroborated by pharmacological blockade of the PNS by atropine and the subsequent abolishment of the HF component of PSD analysis (37). Finally, the ratio of LF to HF components is thought to reflect sympatho-vagal balance (16). A ratio greater than two indicates increased sympathetic activity (38). Due to time constraints during the preparation of this manuscript, PSD analysis was not performed on a single subject from the normal ANSF group. Also, PSD analysis of deep breathing for one subject from the abnormal ANSF group was excluded due to errors in data acquisition. The Ansar Group, Inc. (Philadelphia, Pennsylvania) provided additional PSD analysis of the five-minute period of normal breathing via continuous wavelet transformation (39). This method is well suited to HRV analysis because of its adaptability to changing signal characteristics. Three frequency bands were provided: very-low frequency (0.0033-0.04 Hz), low frequency (0.04-0.15 Hz), and high frequency (0.15-0.40 Hz). An error in data analysis for The Ansar Group, Inc. precluded the use of two subjects from the normal ANSF group.

Orthostasis and 30:15 Ratio

With the subjects still in the supine posture, blood pressure was measured four times at one-minute intervals using a Dinamap Dash 2000 (GE Medical Systems Information Technologies, Milwaukee, Wisconsin). The participant then stood in order to obtain four additional blood pressure measurements at one-minute intervals. All values were recorded by hand. Orthostatic hypotension was identified as a greater than 20 mmHg drop in average upright systolic blood pressure or a greater than 10

mmHg drop in average upright diastolic blood pressure (30) and was indicative of sympathetic impairment.

Another bout of standing was used to measure the longest and shortest R-R intervals at approximately the thirtieth and fifteenth beats respectively to determine the 30:15 ratio. Normal HRV was defined by a ratio greater than or equal to 1.04, and abnormal HRV was defined by a ratio less than or equal to 1.00 (26). The actual occurrence of the shortest and longest R-R intervals may range between the 5^{th} and 25^{th} beats and the 20^{th} and 40^{th} beats, respectively (35). This measure is considered an index of PNS activity due to depressed HRV as a result of pharmacological blockade with atropine (40).

BETWEEN VISITS – NUTRITION & PHYSICAL ACTIVITY

Actical & Food Diary

The first visit concluded with instruction on the use of the Actical accelerometer (Mini Mitter Co., Inc., Bend, Oregon). The Actical provided a three-day measure of physical activity. The Actical has been well correlated with energy expenditure ($r = 0.83$) and activity energy expenditure ($r = 0.85$) as measured by room and portable calorimetry (41). Participants wore the accelerometer on the right hip above the superior iliac crest (41). They received written instructions on the use of the Actical as well as a log sheet to record activity that the Actical may have been less sensitive to detecting (Appendix D). Concurrently, participants recorded everything that they ate, drank, and chewed for three days (Appendix E). They received a sample food diary and a guide for accurately determining portion sizes (Appendix E). Food diaries were analyzed using the Food Processor Nutrition

Analysis and Fitness software package (Version 8.0, ESHA Research, Salem, Oregon) to account for possible dietary influences on BMD. Participants were encouraged to don the Actical and record food intake for two working days and one non-working day in an attempt to best account for any diurnal changes in diet and activity behaviors.

Baecke Physical Activity Questionnaire

The Baecke physical activity questionnaire (Appendix D) was administered to measure present and past history of physical activity (42). The Baecke physical activity questionnaire has been used successfully in various populations including those with type 1 diabetes (43). It has been validated against physical activity as measured by doubly labeled water ($r = 0.69$, $p < 0.001$) (44). The questionnaire provides four indices of physical activity: the work activity index, the sport activity index, the leisure-time activity index, and the past activity index.

VISIT TWO – BONE MINERAL DENSITY

The second visit commenced with the collection of food diaries and accelerometers. Accelerometer data was transferred to a PC via the ActiReader (Mini Mitter Co., Inc., Bend, Oregon). The ActiReader was connected to a PC via an RS-232 serial cable and read data from the Actical via a short-range telemetric link.

Dual Energy X-Ray Absorptiometry (DXA)

Next, the BMD and bone mineral content (BMC) of participants was measured at the left hip, lumbar spine, and total body using a Hologic Delphi QDR Series 4500 DXA (Hologic, Inc., Bedford, Massachusetts). A radiological technician

with limited scope certification performed all DXA measurements for all participants. Detailed procedures for the assessment of the left hip, lumbar spine, and total body accompany the DXA equipment. Soft tissue lean body mass and percent body fat were also assessed at this time. Participants were required to abstain from calcium supplementation for 24 hours prior to BMD assessment via DXA. All female participants underwent pregnancy testing (Mainline Confirms hCG, Mainline Technology, Inc., Ann Arbor, Michigan) before the DXA scan. The total body BMD and BMC results for one volunteer from the abnormal ANSF group were excluded due to metal in the antebrachium of one upper extremity.

Statistical Analysis

The Kolmogorov-Smirnov test for normality was performed for all data before proceeding with analysis using SPSS statistical software for Windows (Version 13.0, SPSS, Chicago, Illinois). An alpha of 0.05 delineated significance. Differences in BMD between type 1 diabetic individuals with normal ANSF and abnormal ANSF were determined via independent t-tests. Pearson's correlation coefficients were used to investigate relationships among BMD and BMC relative to measures of ANSF, physical activity, and nutritional quality. A Chi-square test was implemented to determine differences in the gender composition of each group.

Chapter 3

RESULTS

Descriptive statistics for the investigative samples are provided in Table 1. Although not significant, both age and duration of diabetes were greater in diabetic individuals with abnormal ANSF when compared to diabetic individuals with normal ANSF. Although Table 1 displays a difference in percent body fat between groups, this difference is absolved when considered relative to gender. Males from the normal ANSF group ($n = 5$) averaged 23.62 ± 5.41 percent body fat while males from the abnormal ANSF group ($n = 5$) averaged 19.74 ± 6.84 percent body fat ($p = 0.349$). Females from the normal ANSF group ($n = 6$) averaged 32.05 ± 6.95 percent body fat. There were no females in the abnormal ANSF group.

Table 1: Descriptive statistics.

	Normal ANSF (n = 11)	Abnormal ANSF (n = 5)	p
Age (years)	38.7 ± 10.5	50.4 ± 9.3	.051
M:F Gender Ratio	5:6	5:0	.093
Age of Diabetes Onset (years)	18.63 ± 13.42	18.16 ± 7.44	.942
Diabetes Duration (years)	20.08 ± 14.25	32.24 ± 14.06	.135
Weight (kg)	77.5 ± 16.2	73.9 ± 7.3	.641
Height (cm)	168.04 ± 10.25	172.50 ± 8.86	.417
BMI (kg/m^2)	27.61 ± 5.96	24.91 ± 2.82	.358
Waist:Hip Ratio	0.83 ± 0.08	0.85 ± 0.06	.606
% Body Fat	28.21 ± 7.43	19.74 ± 6.84	.048
Lean Body Mass (kg)	51.90 ± 12.78	55.39 ± 8.53	.590

All values presented as means ± standard deviations. ANSF, autonomic nervous system function; M, male; F, female; BMI, body mass index.

Bone Mineral Density & Bone Mineral Content

There was no significant difference ($p > 0.05$) in BMD or BMC at the hip, lumbar spine, or total body between type 1 diabetic individuals with normal and abnormal ANSF (Figures 1a-1c). According to clinical criteria (T-score < -1.0), 18.8% of the subjects displayed osteopenia at the hip and lumbar spine. No osteoporosis (T-score < -2.5) was evident in this sample.

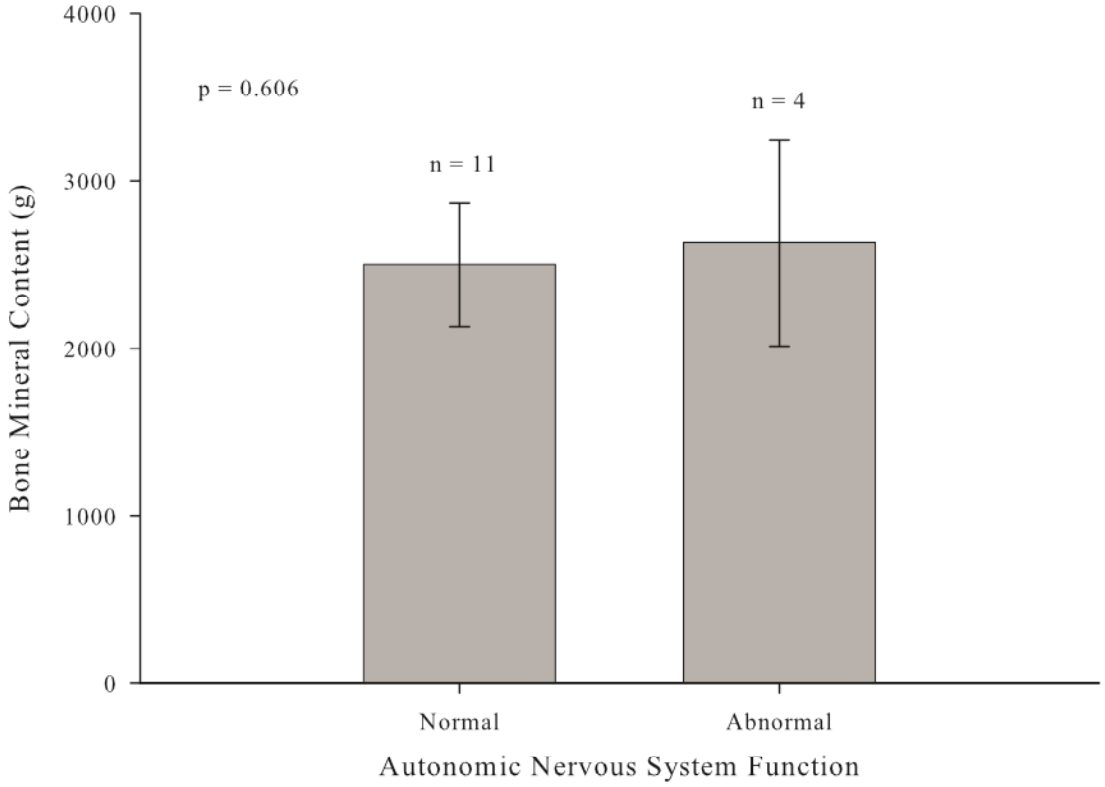


Figure 1a: Total body bone mineral content between type 1 diabetic subjects with normal and abnormal autonomic nervous system function. Error bars represent standard deviation from the mean.

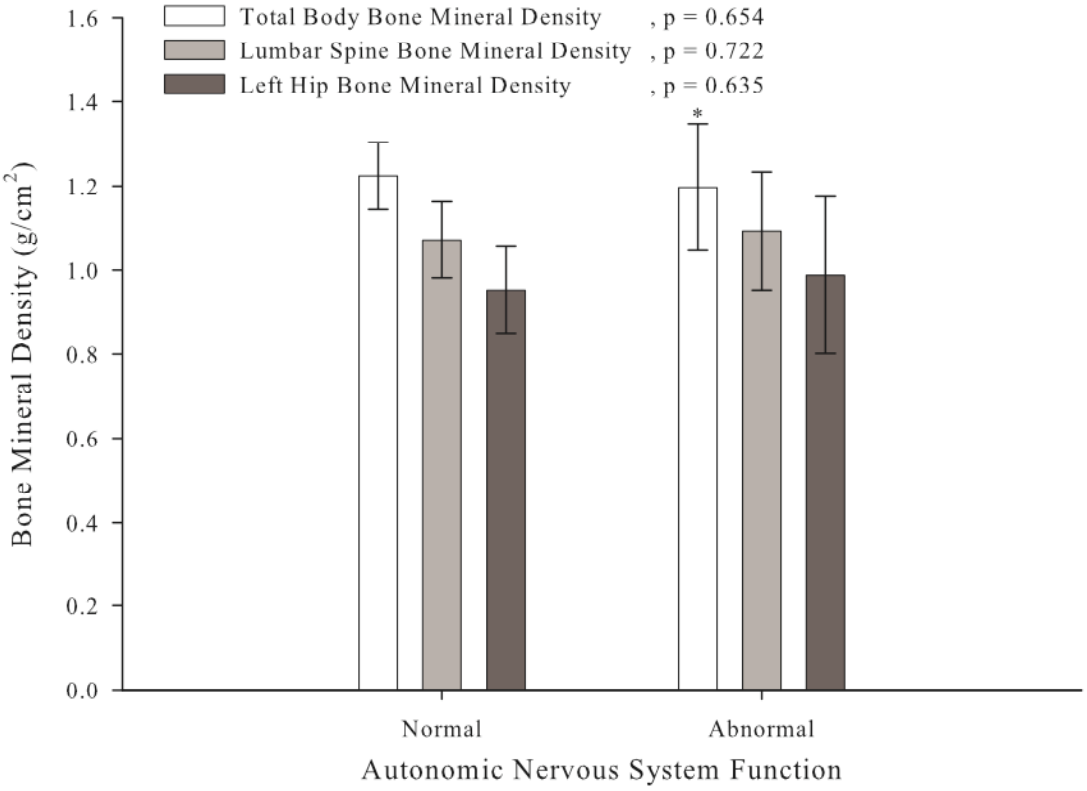


Figure 1b: Bone mineral density between type 1 diabetic subjects with normal and abnormal autonomic nervous system function. Error bars represent standard deviations from the mean (*n = 4).

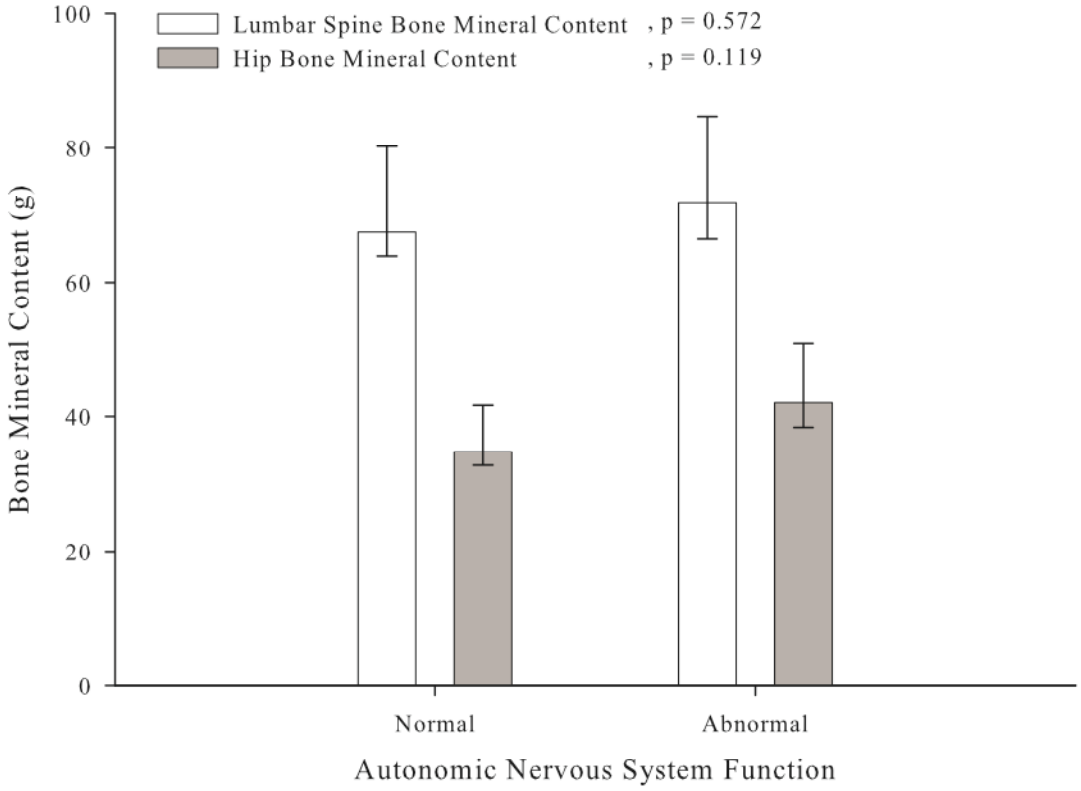


Figure 1c: Bone mineral content at the lumbar spine and hip between type 1 diabetic subjects with normal and abnormal autonomic nervous system function. Error bars represent standard deviations from the mean.

Autonomic Nervous System Function

Table 2 provides a thorough review of the indices of ANSF used in this investigation. Parasympathetic function was clearly different between type 1 diabetic individuals with normal ANSF and those with abnormal ANSF ($p < 0.05$). Differences in sympathetic function were less distinct with only the mean change in systolic blood pressure and the low frequency power spectral component of deep

breathing as significant findings ($p < 0.05$). Finally, our single index of sympatho-vagal balance was significantly different between groups ($p < 0.05$).

Table 2: Indices of autonomic nervous system function (ANSF).

	Normal ANSF (n = 11)	**Abnormal ANSF (n = 5)**	**p**
Parasympathetic Function			
E/I Ratio – 6 breaths	1.333 ± 0.118	1.070 ± 0.044**	.001
MCR	56.8 ± 21.7	11.6 ± 5.8	.000
Valsalva Ratio	2.047 ± 0.396	1.627 ± 0.371*	.125
30:15 Ratio	1.574 ± 0.602	1.158 ± 0.244	.164
SBR – Total (ms/mmHg)	10.650 ± 3.592	4.585 ± 3.198	.006
SBR – Normal (ms/mmHg)	11.402 ± 3.726‡	4.032 ± 3.234**	.005
SBR – Paced (ms/mmHg)	9.698 ± 3.634‡	3.689 ± 2.448**	.011
Deep Breathing HF (ms^2)	839.765 ± 805.954‡	51.045 ± 82.619**	.081
Normal Breathing HF (ms^2)	646.649 ± 730.290‡	24.568 ± 20.206	.084
Paced Breathing HF (ms^2)	830.998 ± 622.726‡	108.520 ± 86.400	.005
Ansar HF (ms^2)	456.691 ± 419.016†	32.000 ± 36.684	.016
Deep Breathing MF (ms^2)	439.679 ± 217.670‡	33.102 ± 52.981**	.004
Normal Breathing MF (ms^2)	497.147 ± 725.137‡	94.882 ± 145.010	.294
Paced Breathing MF (ms^2)	305.132 ± 568.640‡	13.040 ± 14.960	.280
Sympathetic Function			
Mean Change SBP (mmHg)	3.568 ± 4.377	–26.400 ± 23.483	.046
Mean Change DBP (mmHg)	1.636 ± 3.180	–3.700 ± 8.245	.226
Deep Breathing LF (ms^2)	790.982 ± 285.260‡	93.567 ± 151.760**	.001
Normal Breathing LF (ms^2)	345.136 ± 330.769‡	81.596 ± 79.399	.108
Paced Breathing LF (ms^2)	216.435 ± 246.781‡	53.228 ± 46.122	.174
Ansar LF (ms^2)	390.453 ± 337.991†	178.404 ± 246.901	.244
Ansar VLF (ms^2)	437.205 ± 421.109†	109.706 ± 62.410	.115
Sympatho-vagal Balance			
Ansar LF/HF	1.468 ± 1.071†	5.710 ± 2.090	.000

All values presented as means ± standard deviations (*n = 3, **n = 4, †n = 9, ‡n = 10). E/I, expiration/inspiration; MCR, mean circular resultant; SBR, spontaneous baroreflex sensitivity; HF, high frequency band (0.15-0.60 Hz, Ansar: 0.15-0.40 Hz); MF, mid-frequency band (0.08-0.15 Hz); LF, low frequency band (0-0.08 Hz, Ansar: 0.04-0.15 Hz); VLF, very-low frequency (0.0033-0.04 Hz); SBP, systolic blood pressure; DBP, diastolic blood pressure.

Physical Activity & Nutrition

Table 3 highlights the lack of significant nutritional differences between groups. Analysis of dietary records indicate that calcium intake in the abnormal ANSF group failed to reach the United States Department of Agriculture's (USDA) recommended daily allowance (RDA) of 1200 mg for those aged 50 years. Both groups failed to meet the USDA's RDA of 310-420 mg of magnesium. Vitamin D intake was adequate within the normal ANSF group. However, subjects comprising the abnormal ANSF group fell short of the USDA's RDA of 200-400 IU for Vitamin D.

Table 3: Nutrition characteristics.

	Normal ANSF (n = 11)	**Abnormal ANSF (n = 5)**	**p**
Energy Intake (kcal)	2000.90 ± 645.23	2156.16 ± 758.92	.678
Calcium (mg)	1085.41 ± 341.97	930.59 ± 484.00	.472
Magnesium (mg)	217.29 ± 91.22	230.44 ± 134.71	.821
Phosphorus (mg)	1057.93 ± 424.09	1600.66 ± 1039.34	.150
Vitamin D (IU)	235.37 ± 206.68	189.15 ± 228.88	.694
Protein (g)	81.25 ± 28.63	175.16 ± 192.20	.377

All values presented as means ± standard deviations. ANSF, autonomic nervous system function.

Total energy expenditure (TEE) and activity energy expenditure (AEE) measured by the Actical were not significantly different between groups (Figure 2a, 2b). However, there was a positive correlation between hip BMD and both Actical TEE ($r = 0.626$, $p = 0.009$) and the Sport Index of the Baecke Physical Activity Questionnaire ($r = 0.516$, $p = 0.041$) (Table 4). Also, hip BMC was related to Actical

TEE (r = 0.564, p = 0.023), Actical AEE (r = 0.507, p = 0.045) and the Sport Index of the Baecke Physical Activity Questionnaire (r = 0.514, p = 0.042) (Table 4).

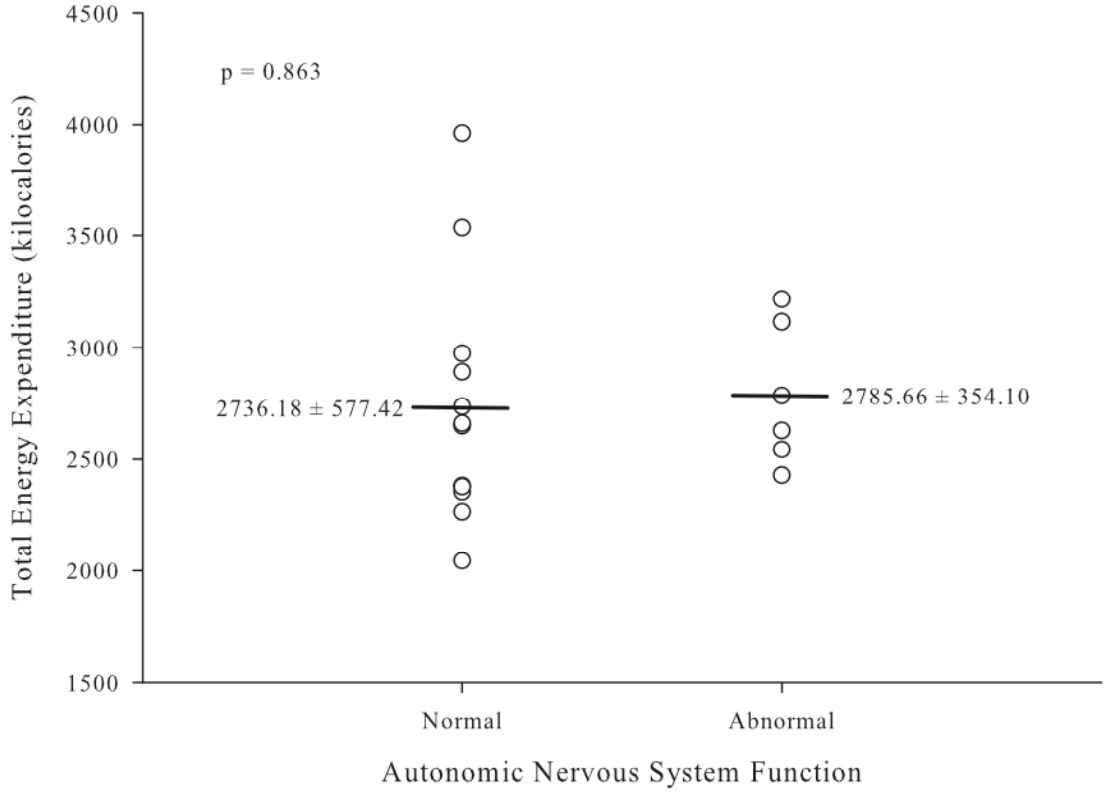


Figure 2a: Actical total energy expenditure between type 1 diabetic subjects with normal and abnormal autonomic nervous system function. All values presented as means ± standard deviations.

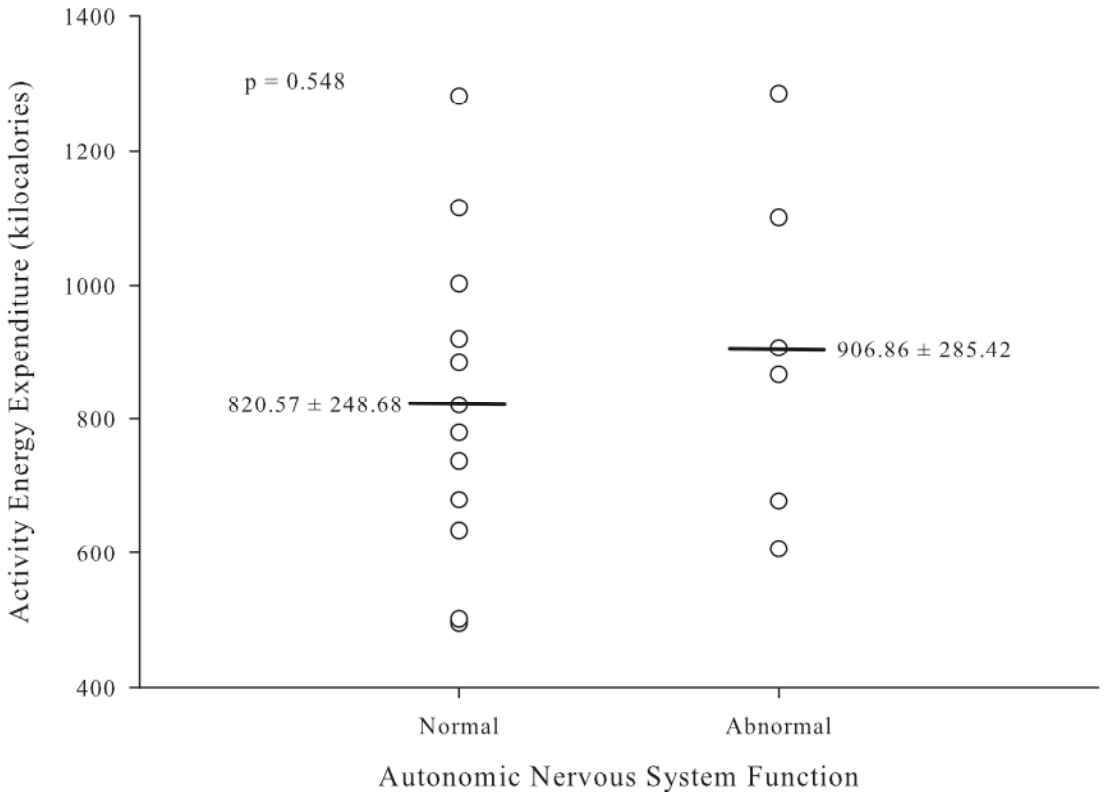


Figure 2b: Actical activity energy expenditure between type 1 diabetic subjects with normal and abnormal autonomic nervous system function. All values presented as means ± standard deviations.

Table 4: Pearson's correlation coefficients for measures of bone health and anthropometrics, physical activity, nutrition, and blood assays.

	Hip BMC	Hip BMD	Total Body BMC*	Lumbar Spine BMD	Lumbar Spine BMC
Age	r = .508	—	—	—	—
	p = .044	—	—	—	—
Height	r = .716	—	r = .723	r = .642	—
	p = .002	—	p = .002	p = .007	—
Weight	—	r = .515	—	—	—
	—	p = .041	—	—	—
% Body Fat	r = -.795	—	r = -.691	r = -.660	—
	p = .000	—	p = .004	p = .005	—
Lean Body Mass	r = .730	r = .686	r = .686	—	—
	p = .001	p = .003	p = .005	—	—
Actical TEE	r = .564	r = .626	—	—	—
	p = .023	p = .009	—	—	—
Actical AEE	r = .507	—	—	—	—
	p = .045	—	—	—	—
BSI	r = .514	r = .516	—	—	—
	p = .042	p = .041	—	—	—
Magnesium	r = .568	—	r = .536	—	—
	p = .022	—	p = .040	—	—
Phosphorous	r = .621	r = .622	r = .597	—	—
	p = .010	p = .010	p = .019	—	—
TSH	—	—	—	r = .516	—
	—	—	—	p = .041	—
Serum creatinine	r = .845	r = .646	r = .760	—	r = .602
	p = .000	p = .007	p = .001	—	p = .014
eGFR	r = -.505	—	r = -.579	—	—
	p = .046	—	p = .024	—	—

BMC, bone mineral content; BMD, bone mineral density; TEE, total energy expenditure; AEE, activity energy expenditure; BSI, Baecke activity questionnaire sport index; TSH, thyroid stimulating hormone. *n = 15.

Blood Tests

Table 5 reveals no significant difference in glycated hemoglobin between groups ($p = 0.253$). Also, most participants displayed adequate diabetic control. Serum creatinine did appear significantly different between groups ($p = 0.022$). Remaining blood assays were not different between groups (Table 5). Estimated glomerular filtration rate was negatively correlated with total body BMD ($r = -0.559$, $p = 0.030$, $n = 15$).

Table 5: Blood assays.

	Normal ANSF (n = 11)	**Abnormal ANSF (n = 5)**	**p**
HbA_{1c} (%)	7.26 ± 1.29	8.04 ± 0.95	.253
TSH (mIU/l)	2.08 ± 1.04	2.52 ± 1.57	.514
Serum creatinine (mg/dl)	0.87 ± 0.12	1.06 ± 0.15	.022
eGFR (ml/min/1.73 m^2)	90.3 ± 12.4	80.6 ± 14.5	.188
PTH (pg/ml)	41.63 ± 13.84	43.6 ± 13.68	.796
Vitamin D (ng/ml)	36.18 ± 9.91	39.8 ± 11.07	.524

All values presented as means ± standard deviations. HbA_{1c}, glycated hemoglobin; TSH, thyroid stimulating hormone; eGFR, estimated glomerular filtration rate (expressed as ml/min/1.73 m^2 of body surface area); PTH, parathyroid stimulating hormone.

Chapter 4

DISCUSSION

The present investigation attempted to elucidate the potential detrimental influence of the SNS on the skeletal retention of bone mineral in type 1 diabetic individuals. BMC and BMD were not significantly different between type 1 diabetic individuals with normal and abnormal ANSF at all anatomical locations measured. The affect of the SNS on bone was shown previously in mice (19). In addition, Togari et al. (45) offer a well-substantiated argument on the influence of the SNS on bone. A review of several *in vivo* and *in vitro* studies provided evidence that SNS stimulation of beta-adrenergic receptors increased bone resorption through stimulation of osteoclast activity and formation. This stimulation decreased bone accrual by inhibiting osteoblasts. Following suit, investigations into the effectiveness of pharmacologic beta-blockade proved cumulatively inconclusive with benefits detected among some populations and no change among others (45). The rationale for intervention with beta-blockers remains sound and more stringent protocols should be considered for the future (45).

There exists a consensus of some impact of the SNS on bone (19, 45, 46). However, its involvement in type 1 diabetes remains unclear. A review by Carnevale et al. (46) suggests that differences in patient therapy as well as both disease

pathogenesis and duration may confound investigation into bone health in type 1 diabetes. The authors acknowledge an increased fracture risk among individuals with type 1 diabetes. They propose that a component of this may simply be a result of common adjuncts of the disease such as retinopathy, neuropathy, nocturia, and decreased muscle strength and the subsequent predisposition to functional imbalance and falls. Carnevale et al. (46) suggest precise metabolic control in order to decrease the negative impact of type 1 diabetes on bone. They go further to highlight the need for rigorous research that includes adequate sample size and control for the many confounding characteristics of the disease (46). For instance, Rix et al. (47) investigated the influence of peripheral neuropathy on BMD in type 1 diabetes. Biothesiometry was used to measure peripheral neuropathy and DXA was used to measure BMD. The investigators used an un-validated questionnaire to measure physical activity in contrast to the Baecke physical activity questionnaire and Actical accelerometer used to measure physical activity in our study. The authors acknowledge this and speculate that the presence of peripheral neuropathy may have influenced physical activity levels in cases of increased severity. In turn, this could influence measures of BMD. Nonetheless, they conclude that peripheral neuropathy is another confounding adjunct of type 1 diabetes that negatively affects BMD.

Our investigation did not have criteria to exclude individuals based on metabolic control. However, most participants exhibited adequate diabetic control (see HbA_{1c}, Table 5). Yet there was a significant difference in serum creatinine between groups ($p = 0.022$, Table 5). Although still within normal reference ranges, this may be suggestive of decreased kidney function among those individuals with abnormal ANSF compared to individuals with normal ANSF in our sample. There

were also significant positive correlations between serum creatinine and hip BMC, hip BMD, and lumbar spine BMC (Table 4). In addition, estimated glomerular filtration rate was negatively correlated with total body BMD ($r = -0.559$, $p = 0.030$, $n = 15$). In contrast, Smets et al. (48) discovered decreased BMD at the femoral neck and lumbar spine in type 1 diabetic individuals with end-stage renal failure six months after simultaneous pancreas kidney transplantation.

Bridges et al. investigated bone health via DXA of the distal radius in type 1 diabetic men to determine differences when compared to healthy men and type 2 diabetic men and to investigate potential causal mechanisms of decreased BMD in type 1 diabetic men (49). Body mass index was the only positive correlate of BMD in type 1 diabetic men. However, there was no significant difference in BMD among groups (49). Rakic et al. conducted a similar investigation of both men and women with type 1 diabetes, type 2 diabetes, and healthy individuals from the Fremantle Diabetes study. BMD was measured by DXA at the hip, lumbar spine, and forearm. With respect to type 1 diabetes, the investigators discovered decreased BMD among men at the hip, lumbar spine, and femoral neck but not at the forearm when compared to men with and without type 2 diabetes. The authors suggest that their sample size of 23 type 1 diabetic men was inadequate to detect differences in BMD (50). Strotmeyer et al. used DXA to compared BMD at the hip, femoral neck, spine, and total body between premenopausal, middle-aged, type 1 diabetic women and non-diabetic female counterparts. They also measured calcaneal quantitative ultrasound. After controlling for several potential confounders and mediators, all measurements of bone health were significantly decreased among women with type 1 diabetes. The investigators were unable to display increased fracture risk due to bone loss. In addition, their sample

was largely free of serious disease complications thus hindering their consideration of the potential mechanisms of decrease BMD (51). These studies further illustrate the discrepancies in bone health research in type 1 diabetes. None of these studies used a direct measure of physical activity such as the Actical accelerometer used in the present investigation. Furthermore, insufficient sample sizes and incongruent anatomical DXA measurement sites complicate adequate comparisons among the published data on bone health in type 1 diabetes.

In support of the present findings, Ingberg et al. (52) compared BMD in type 1 diabetic individuals and healthy age- and gender-matched counterparts. This was a population-based investigation that found no difference in BMD between groups as measured at the femoral neck, total femur, and lumbar spine (52). Unfortunately, this study did not have a measure of physical activity. The authors also reported a lack of statistical power from their sample size (52).

Considering the predominant importance of physical activity and nutrition in bone health, the present study design may simply have lacked the control and/or adequate sample size to identify a relationship between ANSF and bone health. This may remain true even with consideration for physical activity and dietary makeup via a three-day record. Indeed, our data display what appears to be an outlier in the abnormal ANSF group (Figures 2a, 2b). This subject also carried the most BMC of all subjects measured. However, removal of the subject during data exploration did not alter our results. This individual exhibited an extraordinary amount of physical activity that demonstrates the beneficial affect of exercise on BMD. Here, perhaps contributing to its overriding influence on bone health when attempting to measure the possible affect of the SNS.

It is important to recognize the inadequate sample size of the present investigation. A power calculation based on a study by Liu et al. (1) required 17 individuals in each group in order to detect a difference in BMD. Time and resource limitations prevented the acquisition of the proposed sample size for the present manuscript. Furthermore, there was an imbalance in the number of diabetic individuals comprising each group, thus complicating statistical analysis. In addition, inadequate sample size did not allow for subject matching relative to age, duration of diabetes, and gender between groups. In lieu of these factors, there were still no detectable trends in support of the hypothesis.

In conclusion, based on the present data and the limitations discussed, there is no difference in BMD between type 1 diabetic individuals with normal and abnormal ANSF. Future efforts must be focused on tight control of the population sample by accounting for as many confounding variables as is feasible. Subjects must be matched on as many criteria as possible and an adequate sample size must be achieved. In addition, an uncompromised analysis of physical activity and dietary quality must be conducted.

Appendix A: Informed Consent

INFORMED CONSENT

Study Title: **Autonomic Nerve Fiber Dysfunction (Decreased Parasympathetic/Increased Sympathetic Activity) and Reduced Bone Mineral Density: Is There a Link?**

A. INTRODUCTION/PURPOSE:
You are being invited to take part in a research study that will examine the relationship between heart rate variability (ability of the heart to speed up and slow down) and bone loss. It is possible that type 1 diabetic individuals with reduced heart rate variability have lower bone mineral density which may put them at greater risk for the development of osteoporosis. Your participation in this study is expected to last about 1 month. It may be necessary to test 68 individuals with type 1 diabetes in order to get enough information. This study is being conducted by Raelene E. Maser Ph.D., William B. Farquhar Ph.D., Michelle Provost-Craig Ph.D., and M. James Lenhard M.D..

B. STUDY DESCRIPTION:
Visit 1: You must be 18 years of age or older, have type 1 diabetes, and be male or a premenopausal female to take part in this study. A letter or direct verbal permission will be obtained, prior to coming to this study visit, from your eye doctor giving you clearance to take part in one of the tests listed below. You will come to the University of Delaware's Human Performance Laboratory for this visit. You will be asked not to eat or drink anything except water and refrain from taking your routine medications 8 hours prior to this appointment. You should take your bedtime insulin dose as usual. In addition, you should refrain from taking any nonprescription drugs in the last 18 hours, avoid alcoholic beverages in the last 12 hours, and you should not engage in any vigorous exercise that you usually do not do for 24 hours before testing.

During this visit, an electrocardiogram (EKG, usual heart rhythm test performed in a doctor's office) will be performed, your weight, height, and waist and hip circumference will be measured and you will be asked to fill-out a medical history questionnaire. A fingerstick blood sugar level will be performed.

During the tests described below you will be wearing 5 to 8 electrodes to monitor heart rate, a blood pressure cuff around the upper arm and finger to measure blood pressure and elastic-like bands around the chest and stomach to measure breathing rate. Your blood pressure and heart rate response to normal and paced breathing will be measured for approximately 5 minutes. For paced breathing, you will be asked to control your breathing rate by listening to a tape (example: in 2,3, out 2,3, in 2,3 out 2,3, etc.). Your heart rate will also be measured for six minutes while breathing as deeply as possible by following a set of lights. As the lights go up you will inhale and as the light go down you will exhale. This test may be performed twice. You will be asked to blow into a tube for 15 seconds (this is the test for which we will obtain your eye doctor's permission). Your heart rate will be measured during and for 60 seconds after you have finished blowing. This test will be performed twice while you are lying down. Your blood pressure will be measured four times, one minute apart, while you are lying down. You will then be asked to stand up and your blood pressure will again be measured four more times, each time one minute apart. These eight blood pressures will be measured by the cuff around the upper arm while your finger blood pressure will be monitored continuously during this time. Change in your heart rate will also be determined as you go from lying down to standing.

You will have about 5 tablespoons of blood drawn to test for substances (e.g., vitamin D, thyroid hormones, testosterone level [men only]) in the blood that may be related to heart rate variability and bone mineral density. A urine sample will also be examined for microalbuminuria (protein in the urine), marker of bone breakdown, creatinine (marker of kidney function), and calcium. Blood and urine will be stored (frozen) for consideration of future study of factors that may be related to heart rate variability and bone mineral density. Samples will be stored as long as the sample remains stable enough to provide accurate results. If your vitamin D level is low, your thyroid

hormone levels are abnormal, your kidney function is abnormal, or your testosterone level is low (men only), not all the lab tests will be performed nor will your participation in visit 2 be needed. Your results for the other tests describe under visit 1 and your stored blood and urine samples may be used in future analyses.

At the end of this visit, you will be instructed how to collect a 3-day food record. You will be asked to record everything you eat/drink/chew, including the amount, for 3-days. You will be given a questionnaire that will ask you to provide information about your past physical activity level. In addition, you will be given a small device, which you will wear around your hip for 72-hours. This device will monitor your current physical activity level. You should return this device, your completed physical activity questionnaire and your 3-day food record when you come back for visit 2. Visit one will last approximately 3 hours.

Visit 2: Your second visit to the Human Performance Laboratory will consist of three assessments of bone mineral density (total bone mineral density and bone mineral density of the hip and lumbar spine) using an X-ray procedure referred to as a DXA scan. Prior to the scan, females will be asked to give a urine specimen so that an over-the-counter pregnancy test can be performed. You and a member of the research staff will read the results of the pregnancy test. The DXA scans will not be performed if the pregnancy test is positive. The DXA scans will be performed by an x-ray technician with limited scope certification for the DXA scanner. During each scan, you will lie on your back on the scanning bed. The DXA scanner sends an x-ray beam from under the scanning bed to the scanning arm, which is located above you. The scanning arm measures the strength of the beam, which is used to differentiate bone mineral and soft tissue in your body. The DXA software then determines how much of your body is fat, muscle, and bone. Visit 2 will last approximately 45 minutes.

C. CONFIDENTIALITY:

All information collected by the research staff during this study will be confidential. However, the findings of the research may be reported as group data. Your data will be kept in a locked file cabinet and will be entered on secured computers where a case number will identify all subjects. Only laboratory personnel will have access to the data. Following completion of this project, the data will be transferred to a long-term storage medium, such as a CD, and stored indefinitely in a secured file cabinet in Dr. Maser's office.

Your personal health information is health information about you that could be used to identify you. This information may include demographics (such as your age, sex, height, weight), information about your health now and in the past, and other facts about you collected for the purposes of this research study. The information that will be collected will be the minimum needed to meet the goals of this research study and will be used only for the study described in this consent. If you decide not to allow this use of your information, this will prevent you from taking part or continuing to take part in the research study, since the researcher needs this information to meet the study goals.

D. BENEFITS:

There may be no direct benefit to you for participating in this research study. Your taking part in this study may, however, help improve medical knowledge about the effect of heart rate variability on bone mineral density and may therefore benefit yourself or others in the future. Some of the data collected during the first visit includes routine blood work. This information will be provided to you and/or sent to your physician if you request so on your medical history questionnaire.

E. RISKS:

Risks and discomfort, such as soreness, bruising, or infection, may come from putting a needle in your arm or lancing the finger for the blood tests. There is little risk associated with procedures of heart rate variability but some individuals may experience dizziness with the test that requires blowing into a plastic tube for 15 seconds or when standing to test a change in blood pressure.

There is a theoretical risk of bleeding in the back of the eye with the test that requires blowing into a tube, because of pressure changes, but this risk is comparable to pressure changes that occur in the performance of daily activities. Your eye doctor will be contacted to indicate whether you should be excluded or not from doing this particular test.

The DXA is a machine that uses X-rays and emits radiation. However, the radiation given off during these scans is equal to the radiation received during 2 round-trip, trans-United States commercial flights.

F. RIGHT TO WITHDRAW:

You may refuse to take part in this study at any time. You are free to withdraw consent and stop participation in this study. The decision to withdraw will not affect your participation in other current research. Your taking part in this study may be discontinued by any of the investigators without your consent.

G. COMPENSATION FOR ILLNESS OR INJURY:

In the event of physical injury during these research procedures, you will receive emergency medical treatment. If you require additional medical treatment, you will be responsible for the cost.

H. COSTS:

Procedures related to the study (e.g., blood tests) will be provided at no charge.

I. PAYMENT:

There will be no payment for taking part in visit 1 of this study. You will receive payment of $50.00 for taking part in visit 2 of this study.

J. VOLUNTARY CONSENT:

A member of the research staff has given you the opportunity to ask questions and has answered any that you might have now. The advantages and disadvantages of your participation in this project have been explained. The possible harm that might happen to you by taking part in this study has also been explained. If you have questions at any time, wish to withdraw from the project, or have concerns during the course of the project you may call, Dr. Raelene E. Maser (302-831-8400). If you have questions regarding your rights as a research subject, you should contact the Chairperson of the Human Subjects Review Board, Office of the Vice Provost for Research, at (302) 831-4007. A copy of this consent form will be given to you. Your signature below means that you have freely consented to take part in this study and will allow the use of the described information for the purposes of research.

_____Yes _____No Please check whether your blood and urine can be stored (frozen) for consideration of future study of factors that may be related to heart rate variability and bone mineral density.

_____Yes _____No Please check whether you would like to have the results of your bone mineral density sent to your physician.

_____ _____
Date Participant's Signature

_____ _____
Date Witness's Signature

_____ _____
Date Investigator's Signature

Appendix B: Medical History Questionnaire

MEDICAL HISTORY QUESTIONNAIRE

Directions: We ask that you complete this form carefully. All information will be treated as strictly *CONFIDENTIAL*.

I. Personal Information

Name/Last	First	Middle Initial	Date of Birth	Sex
Address (Street Number)	City and State	Zip	Home Phone	

Please list any medication you have been on or presently take.

Type of Medication Dosage and Frequency How Long Reason

_____
_____
_____
_____
_____
_____
_____

II. MEDICAL HISTORY

A. Illness

Please check if you have had any of the following:

Illness	Present	Past	Dates	Illness	Present	Past	Dates
1. Heart Attack	❑	❑	_____	7. Stroke	❑	❑	_____
2. Anemia	❑	❑	_____	8. Diabetes	❑	❑	_____
3. Asthma	❑	❑	_____	9. Thyroid Disease	❑	❑	_____
4. Epilepsy/Convulsions	❑	❑	_____	10.	❑	❑	_____
5. Back or/Disc Condition	❑	❑	_____	11.	❑	❑	_____
6. Kidney Disease	❑	❑	_____				

B. Symptoms

During the last twelve months have you experienced:

Condition	Yes	No	Condition	Yes	No
1. High Blood Pressure	❑	❑	8. Shortness of Breath	❑	❑
2. Swelling of Hands or Feet	❑	❑	9. Numbness/Tingling in Arms, Hands, Legs or Face	❑	❑
3. Pain or Cramps in Legs	❑	❑	10. Unusual Fatigue or Dizziness, Light Headed	❑	❑
4. EKG Abnormalities	❑	❑	11. Significant Weight Fluctuation (5 lbs or more)	❑	❑
5. Blurred Vision	❑	❑	12. High Triglycerides	❑	❑
6. Skpd. Beats/Palpitations	❑	❑	13. High Cholesterol	❑	❑
7. Chest Pain, Pressure	❑	❑			

III. HEALTH HABITS HISTORY

A. Nutrition/Weight

1. List your height_____ List your weight: Now _____lbs. One year ago _____lbs. At age 21 _____lbs. Graduated High School _____lbs.
2. Length of time at current weight_____
3. Do you regard yourself as overweight/underweight? ❑ Yes ❑ No ❑ Not Sure

B. Smoking/Alcohol

1. Did you ever smoke cigarettes? ❑ Yes ❑ No If yes, how many packs per day: _____
2. Do you currently smoke cigarettes? ❑ Yes ❑ No If yes, how many packs per day: _____
3. If you have stopped smoking, what was the approximate date? _____
4. Do you smoke cigars or pipes? ❑ Yes ❑ No If yes, how many per day: _____
5. How much alcohol do you drink per week? (*Please specify if beer, wine, or hard liquor)*_____

IV. MENSTRUAL HISTORY (PLEASE ANSWER IF THE FOLLOWING QUESTIONS APPLY TO YOU)

❑	POSTMENOPAUSAL	(>6 months without a menstrual period)
❑	SURGICALLY STERILE	(tubal ligation, partial or total hysterectomy)

1. At what age did your period begin? _____
2. During the past year, what is the approximate regularity of your menstrual cycles (cycles occur approximately every 28 days)?
   A. Fairly regular (same number of days + or – 3 days)
   B. Somewhat irregular (variation 4-10 days)
   C. Very irregular (variation greater than 10 days)
   D. Do not menstruate
3. How many menstrual cycles have you missed in the past year? _____
4. During most of your life, were your periods regular: that is, did they occur about once a month? Do not include any time when you were pregnant or taking birth control pills.)
   A. Not regular

B. Regular
C. Sometimes regular; sometimes irregular

5. Between the time you had your first period and your last period, did you ever stop having any periods? (Do not include times when you were pregnant or breast-feeding.)
   A. No
   B. Yes
      - If yes, between your first menstrual period and your last, all together, about how long did you go without having your period? (Do not include times you were pregnant or breast-feeding.)
         1. Less than 12 months
         2. 12 to 23 months
         3. 2 to 4 years
         4. More than 4 years

6. Are you presently taking birth control pills, shots, or female hormones:
   A. Yes (If yes, how long have you been taking them: _____)
      (If yes, please indicate the name of the medicine: _____)
   B. No

7. Did you take birth control pills, shots, or female hormones at any time in the past?
   A. Yes (If yes, how long ago: _____)
      (If yes, how long did you use them: _____)
      (If yes, what age did you start using them: _____)
   B. No

8. Please describe any menstrual or gynecological problems not already covered in the above questions: _____
_____

V. EMERGENCY CONTACT INFORMATION

Local Contact: _____ Relationship: _____ Home Phone: _____

VI. DOCTOR INFORMATION

1. Do you have a primary care doctor: ❑ Yes ❑ No
2. Would you like your doctor sent the results of your routine blood tests that will be performed for you as part of this study?
   ❑ Yes ❑ No
3. Primary care physician's name: _____
4. Address: _____
_____

Appendix C: Letter to Eye Doctor for Permission to Perform Valsalva Maneuver

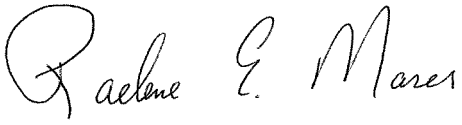
Month Day, Year

Dr. Name
Street
Address, Zip Code

Dear Dr. Name:

Your patient _____ is a participant in a research study entitled: "Autonomic Nerve Fiber Dysfunction (Decreased Parasympathetic/Increased Sympathetic Activity) and Bone Loss: Is There a Link?," being conducted by M. James Lenhard, MD, Raelene E. Maser, PhD, William Farquhar, PhD, and Michelle Provost-Craig, PhD. One test in this investigation is the Valsalva maneuver, in which the participant will expire into the mouthpiece of an open manometer maintaining a pressure of 40 mmHg for 15 seconds. **Participants with active neovascularization or unresolved proliferative retinopathy will be excluded from performing this test.** We would appreciate it if you would please indicate below whether your patient should be excluded or not from the performance of this test and fax this letter back to the Human Performance Laboratory, University of Delaware at 302-831-3693. Your help with this matter is appreciated.

Sincerely,



Raelene E. Maser, PhD

_____The patient noted above **should not** perform the Valsalva maneuver

_____The patient noted above may perform the Valsalva maneuver

_____
Physician Signature

Appendix D: Actical Instructions & Log and Baecke Physical Activity Questionnaire

Actical Instructions

Actical Placement

The Actical must be worn in a manner as closely as possible to that shown during your first visit. It must be worn on the right side of your body just above the boney prominence at your right hip. It must be worn against the skin and secured in place by the belt provided to you. You may remove the Actical for water activities and bathing. Please make note accordingly (see below). You may remove the Actical during sleep. When you are not wearing the Actical, be sure to place the device in a location where it will not be disturbed or misplaced.

Actical Event Marker Log

Your Actical has an "Event Marker" that allows you to mark anything significant that occurs during the recording of your physical activity. An example of a significant event is riding a stationary bicycle. Simply put, the Actical measures the acceleration of your body during your daily activities. Activity on a stationary bicycle does not offer the Actical sufficient information to record your activity accurately. Press the star that the arrow is pointing at to mark an event.

Event #	Date	Time	Description & Length of Event

Physical Activity Questionnaire

(Answers questions 1-16 with regard to your activity level during the last 12 months)

1) What is your main occupation?

---

2) At work I sit……
never/seldom/sometimes/often/always

3) At work I stand……
never/seldom/sometimes/often/always

4) At work I walk……
never/seldom/sometimes/often/always

5) At work I lift heavy loads……
never/seldom/sometimes/often/always

6) After work I am tired..……
very often/often/sometimes/seldom/never

7) At work I sweat……..
very often/often/sometimes/seldom/never

8) In comparison with others of my own age I think my work is physically…….....
much heavier/heavier/as heavy/lighter/much lighter

9) Do you play sports?
yes/no
If yes:
- which sport do you play most frequently?.....
- how many hours a week?
- how many months a year?

If you play a second sport:
- which sport is it?
- how many hours a week?
- how many months a year?

10) In comparison with others of my own age I think my physical activity during leisure time is.....
much more/more/the same/less/much less

11) During leisure time I sweat…….
very often/often/sometimes/seldom/never

12) During leisure time I play sport…….....
never/seldom/sometimes/often/very often

13) During leisure time I watch television…….....
never/seldom/sometimes/often/very often

14) During leisure time I walk……..……
never/seldom/sometimes/often/very often

15) During leisure time I cycle…..……..……..
never/seldom/sometimes/often/very often

16) How many minutes do you walk and/or cycle per day to and from work, school and shopping?
<5/5-15/15-30/30-45/>45

17) **The next two questions (i.e., 17a and 17 b) will ask about participation in physical activities at various ages during your lifetime**.

a. How often did you regularly participate in sports and leisure time activity (EXCLUDING WALKING)? (Please check the appropriate box.)

DURING AGE:	0 TO 1 HOUR/WEEK	2 TO 3 HOURS/WEEK	4 TO 7 HOURS/WEEK	Over 7 HOURS/WEEK
14-17 Years (High School)				
18-21 Years (College)				
20-29 Years				
30-39 Years				
40-49 Years				
50+				

b. How many miles did you normally walk each day outside the house or place of employment? (Please check the appropriate box.)

DURING AGE:	UNDER 1 MILE	1-2 MILES	3-5 MILES	GREATER THAN 5 MILES
14-17 Years (High School)				
18-21 Years (College)				
20-29 Years				
30-39 Years				
40-49 Years				
50+				

NOTE: 12 blocks or 20 minutes of brisk walking is equivalent to approximately 1 mile.

Appendix E: Food Record and Portion Size Guide

Participant's ID _____

FOOD RECORD – Day _____

Please record your food and beverage intake (including water) for the entire day. It is important to record EVERYTHING that you eat and drink. This record ideally needs to be completed from Thursday through Saturday in combination with use of the Actical. However, any combination of two weekdays and one weekend day is acceptable. Be as accurate as possible. For example, specify the type of bread; type of cheese; whether vegetables, fruits, or juices were prepared from fresh, frozen, or canned sources; and whether meats were lean only, or lean with some fat. Remember, this study is only as accurate as your recording.

Name: _____ **Date:** _____

Day of Week: **Mon** **Tues** **Wed** **Thurs** **Fri** **Sat** **Sun**

			Salt	Amount						
Time	**Place: Home/Away**	**Substance Taken In (Preparation Style if applicable)**	**Yes/No**	**Oz.**	**Tbsp.**	**Tsp.**	**Cup**	**Slice/Piece**	**Each**	**Other**

Please list any supplements that you may be taking.

Supplement Name **Dosage** **How often**

_____

_____

A Guide to Portion Sizes

Use the following as guide for measuring the amount of food you consume to record in your food diary.

Food Portion Size	Guide
1 ounce of meat……	Size of a matchbox
3 ounces of meat……..	Size of a deck of cards [recommended portion size for a meal]
8 ounces of meat……..	Size of a thin paperback book
3 ounces of fish……	Size of a checkbook
1 ounce of cheese…….	Size of 4 dice
Medium potato…….	Size of a computer mouse
2 tablespoons of peanut butter…….	Size of a golf ball
1 cup of pasta……...	Size of a tennis ball
Average bagel……..	Size of a hockey puck

The following are serving sizes of some vegetables and fruits.

Vegetable or Fruit	Guide
Medium apple or orange……	Size of a tennis ball
1 cup chopped raw vegetables or fruit……...	Size of a baseball
¼ cup dried fruit (raisins, apricots, mango)……...	A small handful
Lunch-box sized container of unsweetened applesauce…….	1 fruit serving
1 cup of lettuce……...	Four lettuce leaves
Chicken stir-fry with 1 cup of mixed broccoli, carrots, and mushrooms…….....	2 vegetable servings
½ cup cooked or canned legumes (beans and peas)……...	1 vegetable serving
5-6 baby carrots……..	1 vegetable serving

Source: http://www.cancer.org/docroot/PED/content/PED_3_2x_Portion_Control.asp

Appendix F: Certification of Human Subjects Training

Certification of Human Subjects Training

The University of Delaware certifies that John N. Stabley
(Name of researcher)

attended an institutional training session on the use of human subjects in research on

October 27, 2004
(Date)

The session included the following topics:

- The Belmont Report
- Federal regulations for using humans in research (45 CFR 46)
- The University's Federalwide Assurance
- Informed consent
- Institutional procedures
- Sources for additional information

Signatures:

October 27, 2004
Date

Researcher

October 27, 2004
Date

TW Fraser Russell
Vice Provost for Research

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