Received: 10 April 2019 | Revised: 29 July 2019 | Accepted: 2 August 2019

DOI: 10.1111/chd.12836

ORIGINAL ARTICLE

Congenital Heart Disease WILEY

Prevalence and risk factors for low bone density in adults with a Fontan circulation

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Funding information
NHMRC grant APP1065794: 'Functional outcomes after Fontan Surgery'.
NHMRC partnership grant 1076849.

Abstract

Objective and Patients: This study aimed to characterize bone mineral density abnormalities and pathophysiological associations in young adults living with a Fontan circulation.

Design: Participants underwent bone mineral density measurement using dual-energy X-ray absorptiometry and serum biochemical analysis, cardiopulmonary exercise and strength testing and transthoracic echocardiography.

Results: In our cohort (n = 28), 29% had osteopenic-range bone mineral density and one patient was osteoporotic (average hip t score: −0.6 ± 1.1; spine t score: −0.6 ± 0.9). Four patients (14%) had z scores < −2.0. Parathyroid hormone levels were increased compared with laboratory median (6.1 ± 3.5 vs 4 pmol/L, P = .01) and 27% had 25-hydroxy-vitamin D < 50 nmol/L. 25-hydroxy-vitamin D negatively correlated with parathyroid hormone (ρ = −0.53, P = .01) suggesting secondary hyperparathyroidism. Atrioventricular valve systolic to diastolic duration ratio, an echocardiographic measure of diastolic dysfunction, inversely correlated with hip t and z scores (P < .01). Hip

All authors takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

t scores were positively associated with oxygen saturations (ρ = 0.45, P = .05) and tended to be inversely associated with parathyroid hormone levels (ρ = −0.44, P = .07) and N-Terminal pro b-type natriuretic peptide (ρ = −0.42, P = .08).

Conclusions: Many young adults with a Fontan circulation have abnormal bone mineral density. The underlying pathophysiology is likely multifactorial. Possible contributors include secondary hyperparathyroidism, hypoxemia, diastolic cardiac dysfunction and neurohormonal activation. As low bone mineral density is clinically relevant and potentially treatable, assessment of bone mineral density should be part of routine care in this cohort.

KEYWORDS

biochemistry, bone density, congenital heart disease, Fontan, hormones, single ventricle

1 | INTRODUCTION

For children born with complex congenital heart disease (CHD) resulting in single ventricle physiology with low pulmonary vascular resistance, Fontan palliation is the surgical pathway of choice. In the current era, the majority of these children are surviving into adulthood but with reduced quality of life, exercise capacity and life expectancy.[1,2]

In light of improved late survival, recent work has focused on the long-term morbidities of Fontan physiology where low cardiac-output and chronically elevated systemic venous pressures are key substrates for chronic end-organ dysfunction.[3,4] Fontan physiology has recently been associated with abnormal bone mineral density (BMD).[5] The pathophysiology that contributes to reduced bone density is poorly understood; proposed risk factors include decreased weight bearing activities and lower levels of moderate to vigorous exercise (especially during childhood), hepatic dysfunction, vitamin D deficiency and treatment with medications including loop diuretics and warfarin.[6-8] Recently, a predisposition to subclinical hypothyroidism has been associated with Fontan physiology that likely reflects chronic venous hypertension and end-organ fibrosis.[9] Endocrinological dysfunction is poorly characterized in this setting and the implications for bone health are unknown as is the impact of raised venous pressure, which is known to affect bone blood and interstitial fluid flow.[10,11]

In view of current uncertainties, we undertook an exploratory analysis to characterize BMD abnormalities in a group of young adults living with a Fontan circulation and describe possible novel pathophysiological associations.

2 | METHODS

2.1 | Study design and population

Eligible participants (Fontan circulation ≥16 years residing within New South Wales, Australia) were prospectively enrolled during a pre-specified 24-month period from the Adult Congenital Disease Database at Royal Prince Alfred Hospital (RPAH) and the Australia and New Zealand Fontan Registry. Exclusion criteria were pregnancy, major intellectual disability, non-English speaking background and severe physical disability.

Informed consent was obtained from all participants ≥18 years of age and assent as well as parental consent was obtained in those <18 years. In accordance with the ethical guidelines of the 1975 Declaration of Helsinki, the study protocol was pre-approved by the Sydney Local Health District Ethics Review Committee. Participant demographics and clinical information, including fracture history, were obtained from the medical record and confirmed in interviews.

2.2 | Laboratory studies

Each patient underwent venesection and measurement of a range of biochemical and endocrine markers (see Table 2). These were processed and interpreted by the core laboratory at RPAH. Distribution scores were calculated from the hospital laboratory reference ranges or kit inserts to account for sex differences. Both aldosterone (pmol/L) and renin activity (fmol/L/s) were excluded from analysis if the participant was prescribed angiotensin converting enzyme inhibitors, angiotensin receptor blockers and/or an aldosterone antagonist. Sex hormones were not analyzed in females who were on contraceptives and thyroid stimulating hormone (TSH) was not analyzed in participants on thyroid replacement. Glomerular Filtration rate was estimated in adults from serum creatinine (umol/L) using the CKD-EPI formula.[12] 25-OH vitamin D levels were compared both with the laboratory reference range (>50 nmol/L) and with reported levels in an Australian population.[13]

2.3 | Non-invasive cardiac imaging

Transthoracic echocardiography was performed using Phillips EPIQ 7C and IE33 machines (Phillips; Andover, Massachusetts) according to our standard imaging protocol.[14] Echocardiograms were reported by cardiologists with expertise in adult CHD. Atrioventricular valve systolic to diastolic duration (AVVSD) ratio was manually measured

by trained cardiologists. Diastolic function was assessed using E/A, E/E' and AVVSD ratio as described by Cordina et al.[14]

2.4 | Exercise and strength testing

Each participant underwent supervised and structured skeletal muscle strength testing in a dedicated gymnasium under the supervision of an exercise physiologist and physician. Maximal cardiopulmonary exercise testing (CPET) was performed on an electrically braked bicycle ergometer (Lode Corival; Lobe BV, Groningen, The Netherlands) using an individualized ramp protocol. Breath by breath expiratory gas analysis (Vmax229; SensorMedics; Yorba Linda, California) and ECG monitoring (Cardiosoft version 6.51; GE Healthcare; Waukesha, Wisconsin) were performed. Blood pressure was periodically measured and oxygen saturation measurements (Radical; Massimo Corp; Irvine, USA) were obtained continuously. Heart rate (bpm), blood pressure response (mmHg), work rate (watts), ventilation (VE), oxygen uptake (VO_2), carbon dioxide production (VCO_2), oxygen saturation (SpO2), and ECG morphology were continuously collected during the testing period. Spirometry was performed prior to every exercise test. A blinded respiratory scientist determined peak VO_2 as a 20-second average.[15] Peak VO_2 and peak oxygen pulse values are expressed as percent predicted[16] to account for sex differences. In patients <18 years, equations recommended by Wasserman et al were used.[17]

Dynamic muscle strength was determined by one repetition maximum testing for the leg press and chest fly. Maximal isometric handgrip strength was assessed on both hands using a T.K.K Grip-D handgrip dynamometer (Tokyo, Japan). The highest two values of each hand were averaged and recorded. The peak average was then identified and converted into a percentage of predicted compared to normal reference values.

2.5 | Bone density analysis

Bone density analysis was performed using a GE Lunar Prodigy Advance dual-energy X-ray absorptiometry (DXA) machine (GE Healthcare; Waukesha, Wisconsin; software version 13.60). Measurements were collected from trained staff and performed according to manufacturer guidelines for participant positioning. Hip and spine *t* scores were calculated for all participants aged ≥20 years using Australian anterior-posterior femur and spine reference population (combined Gelong/Lunar; ages 20-40 years, v112).[18,19] Osteoporosis was defined as a *t* score at or below −2.5 and osteopenia as a *t* score between −1.0 and −2.5. Hip and spine *z* scores were calculated for participants of all ages and were matched for age, weight, and ethnicity.[18,19]

2.6 | Statistics

Data are expressed as the mean ± standard deviation (SD) unless specified otherwise. Statistical analysis was performed using commercial software IBM SPSS statistic version 24 (IBM Corporation, New York, USA). A Shapiro-Wilk test was used to assess normal distribution. A one sample t test or one-sample Wilcoxon rank test was used to compare sample values to the estimated or laboratory kit reference medians or means. For females, the value for the follicular phase was used as the reference for sex hormones. Fisher's exact test, independent t test or a Mann-Whitney *U* test were performed as appropriate. For correlation analysis, Spearman's bivariate rank correlation was used due to small sample size and to account for non-normally distributed data. In order to analyze both sexes together in the setting of sex-differentiated values, blood biochemistry was normalized for sex using reference values and converted to a *t* and/or *z* distribution score for correlation analysis. A *P* value < .05 was considered statistically significant and correlations with a *P* value < .10 were reported. Two-sided tests were used throughout.

3 | RESULTS

3.1 | Participant characteristics

Clinical characteristics for the 28 participants in our cohort are shown in Table 1. Thirty-two percent of patients were prescribed warfarin and 18% were prescribed loop diuretics.

3.2 | Laboratory studies

Table 2 illustrates biochemical and hematological results for our cohort. While overall 25-OH vitamin D levels were not significantly lower than the laboratory reference range (>50 nmol/L), 50% of our cohort had vitamin D levels below 69.2 nmol/L (*P* = .54) which is the mean 25-OH vitamin D level in a reference Australian population.[13] Overall average INR is reported for all participants (*n* = 26; 2 did not have INRs collected). The average INR for the 17 patients (65%) not taking warfarin was 1.19 ± 0.15 and for the 9 patients (35%) taking warfarin was 2.06 ± 0.77.

3.3 | Bone densitometry

Bone densitometry results are provided in Figure 1. Subgroup analysis was performed according to regular medications that have been associated with reduced bone density (warfarin and loop diuretics[6,7]) with no significant difference found between these groups. There was no difference in BMD between total cavopulmonary connection (89%) and atriopulmonary connection (11%).

Nine (32%) participants had prior fractures. None of these were pathological and there were no significant differences in clinical characteristics between participants who had a prior fracture compared with those who did not.

3.4 | Exercise & strength testing

Average resting oxygen saturation of our cohort was 94% ± 3%. This fell by an average of 3.3% ± 3.4% at peak exercise. Average peak oxygen uptake (VO_2) was 24 ± 7 mL/kg/min (66% predicted).

TABLE 1 Participant characteristics

Participant characteristics	(*n*, %) or mean ± SD
Sex (female)	15 (54)
Age (years)	26 ± 7
BMI (kg/m^2)	23.7 ± 4.8
BSA (m^2)	1.73 ± 0.20
Predominant ventricular morphology	
Left	16 (57)
Right	8 (29)
Uni-ventricular	2 (7)
Indeterminate	2 (7)
Type of Fontan procedure	
Extracardiac conduit	14 (50)
Intracardiac total cavopulmonary connection	11 (39)
Atriopulmonary	3 (11)
Fenestration	9 (32)
Systolic function	
Normal	22 (78)
Mildly impaired	5 (18)
Moderately impaired	1 (4)
Severely impaired	0 (0)
AV valve regurgitation	
None	10 (36)
Mild	13 (46)
Moderate	4 (14)
Severe	1 (4)
Systemic valve regurgitation	
None	23 (82)
Mild	4 (14)
Moderate	1 (4)
Severe	0 (0)
Medications	
Antiplatelet agent	16 (57)
Warfarin	9 (32)
DOACs	1 (4)
ACE-I/A2RB	6 (21)
Beta-blocker	2 (7)
Digoxin	1 (4)
Loop diuretics	5 (18)
Anti-arrhythmic	4 (14)
Resting SpO_2 (%)	94 ± 3
Peak VO_2 (mL/kg/min)	24.02 ± 6.89
Predicted peak VO_2 (%)	66 ± 15
NYHA class	
NYHA I	21 (75)
NYHA II	7 (25)
NYHA III	0 (0)
NYHA IV	0 (0)
Pacemaker	6 (21)
Previous fracture	
Traumatic	9 (32)
Pathological/Low impact	0 (0)

Abbreviations: ACE-I, angiotensin converting enzyme inhibitor; AV, atrio-ventricular; A2RB, angiotensin II receptor blocker; BMI, body mass index; BSA, body surface area; DOAC, direct acting oral anticoagulant; NYHA, New York Heart Association; SpO_2, room air oxygen saturation; VO_2, volume oxygen consumption.

3.5 | Correlation analysis

Important correlations between *t* scores (*n* = 21), clinical and biochemical data are shown in Table 3. Apart from those described in Table 3, there were no other important correlations between *t* scores and any other biochemical or endocrine markers (Table 2), BMI, type of Fontan anatomy, ventricular systolic function, New York Heart Association (NYHA) functional class, exercise, and strength results. Aside from a negative correlation with alkaline phosphatase (ALP; see Table 3), there were no other associations between BMD and other liver function tests or platelet count.

There were important correlations between *z* scores (*n* = 28) and AVVSD ratio (hip *z* score: $\rho = -0.63$, $P < .01$; spine *z* score: $\rho = -0.39$, $P = .06$). Apart from this, there were no other important correlations between *z* scores or any other indices.

We performed a post hoc correlation analysis to characterize associations between biochemical parameters and parathyroid hormone (PTH) levels. There were significant inverse correlations between PTH and 25-OH vitamin D ($\rho = -0.53$, $P = .01$) and corrected calcium ($\rho = -0.46$, $P = .02$). PTH was positively correlated with aldosterone ($\rho = 0.65$, $P = .01$) and exhibited a similar trend with N-terminal pro b-type natriuretic peptide (NT-proBNP; $\rho = 0.40$, $P = .05$).

4 | DISCUSSION

This study characterizes BMD abnormalities specifically in a population of young adults with a Fontan circulation and identifies a significant inverse relationship between BMD and ventricular diastolic dysfunction as well as possible associations between reduced BMD and reduced oxygen saturations, secondary hyperparathyroidism, and neurohormonal activation.

In our cohort of clinically stable adolescents and adults living with a Fontan circulation we found significant abnormalities of BMD. Compared with reference populations, both hip and spine *t* and *z* scores were significantly reduced with 29% in the osteopenic

range and one patient in the osteoporotic range, using conventional definitions. Fourteen percent of participants had hip and spine *z* scores < −2.0, which is below the expected range for age.[20] These findings align with recent data that describe substantial defects in trabecular volumetric BMD (using peripheral quantative-computed tomography), cortical structure and muscle area in children and young adults living with a Fontan circulation.[5] Previous data suggest abnormal BMD occurs independent of accepted risk factors such as protein losing enteropathy (PLE) and corticosteroid use.[21] None of the participants in our cohort had a diagnosis of PLE and none were taking corticosteroids. On subgroup analysis of our participants, medication with warfarin and/or loop diuretic therapy did not appear to impact BMD.

In an important study of 26 children (5-12 years age) with single ventricle physiology, Bendaly et al[22] demonstrated that BMD is significantly reduced in patients with a single ventricle compared with age and sex-matched norms. Twenty-five of the participants were post-Fontan. This work supports our findings of abnormal BMD in patients with single ventricle physiology (after palliation with the Fontan operation) and highlights that decreased BMD in these patients starts in childhood and should be evaluated and treated at an early stage in life, not only in adulthood.

TABLE 2 Biochemical results

Biochemical result	Mean ± SD	Lab reference range	Reference mean or median	*P*
Hb (g/L)				
Male	162.38 ± 9.70	130-170	150[a]	**<.01**
Female	147.74 ± 13.02	120-150	135[a]	**<.01**
WCC ($\times 10^9$/L)	6.58 ± 2.31	4.0-10.0	7	.35
Plt ($\times 10^9$/L)	177.46 ± 60.58	150-400	275[a]	**<.01**
eGFR (ml/min/1.73m^2)	108.04 ± 19.69	>60	–	–
Albumin (g/L)	47.96 ± 3.20	38-48	43	**<.01**
ALP (U/L)	77.32 ± 31.17	30-110	[b]	.66
NT-proBNP (pmol/L)	24.36 ± 28.74	<13.0	2.4[a]	**<.01**
INR	1.46 ± 0.59	0.9-1.2	1.05[a]	**<.01**
25-OH D (nmol/L)	66.04 ± 26.21	>50	69.2[ζ]	.54
PTH (pmol/L)	6.07 ± 3.45	2.0-6.0	4[a]	**.01**
Ca (mmol/L)	2.30 ± 0.09	2.10-2.60	2.35	**.01**
Mg (mmol/L)	0.82 ± 0.06	0.70-1.10	[b]	**<.01**
PO_4 (mmol/L)	1.15 ± 0.19	0.75-1.50	[b]	.79
hsCRP (mg/L)	1.95 ± 1.77	<5.0	–	–
TSH (mIU/L)	3.58 ± 2.13	0.270-4.200	1.16[a]	**<.01**
IGF-1 (nmol/L)				
Male	26.92 ± 11.92	14.3-39.2	[b]	.92
Female	33.42 ± 18.17	11.8-38.6	[b]	.38
SHBG (nmol/L)				
Male	42.97 ± 19.06	14.0-75.0	33.2[a]	.06
Female	92.00 ± 39.06	19.0-120.0	67.8[a]	.06
Testosterone (nmol/L)				
Male	16.21 ± 4.16	10.0-30.0	18.6[a]	.08
Female	0.53 ± 0.43	0.0-1.8	0.94[a]	**.02**
Estradiol (pmol/L)				
Male	102.62 ± 36.00	<220	72.6[a]	**.02**
Female	341.50 ± 352.53	<700	228[a]	.64
FSH (IU/L)				
Male	4.35 ± 2.07	1.5-12.4	4.6[a]	.55
Female	9.05 ± 7.09	3.5-12.5	6.9[a]	.58
LH (IU/L)				
Male	5.78 ± 2.13	1.7-8.6	4[a]	**.01**
Female	13.08 ± 19.93	2.4-12.6	5.9[a]	.14

(Continues)

TABLE 2 (Continued)

Biochemical result	Mean ± SD	Lab reference range	Reference mean or median	*P*
Prolactin (ng/mL)				
Male	9.0 ± 4.20	2.0-16.0	6.2[a]	.05
Female	9.04 ± 4.50	2.0-20.0	9.4[a]	.64
Renin activity (fmol/L/s)	426.37 ± 344.94	130-2350	471[a]	.42
Aldosterone (pmol/L)	582.21 ± 390.05	60-980	247[a]	**<.01**
DHEAS (umol/L)				
Male	3.90 ± 1.46	3.0-18.0	[b]	**<.01**
Female	2.76 ± 1.56	2.0-14.0	[b]	**<.01**

Notes: Table adapted from: Tran et al (2019). Body composition in young adults living with a Fontan circulation: the myopenic obesity profile. Currently submitted for consideration of publication. Bold values indicates $P < .05$

Abbreviations: 25-OH D, 25-hydroxy vitamin D ([ξ]mean of reported 25-OH D levels in an Australian population[13]); ALP, alkaline phosphatase; Ca, corrected calcium level calculated from the serum calcium and serum albumin levels (0.8 × (serum albumin) + serum calcium); DHEAS, dehydroepiandrosterone sulfate; eGFR, estimate glomerular filtration rate; FSH, follicle-stimulating hormone; Hb, hemoglobin; hsCRP, high sensitive C-reactive protein; IGF-1, insulin-like growth factor 1; INR, international normalized ratio (reported for all participants; $n = 26$); LH, luteinizing hormone; Mg, magnesium; NT-proBNP, N-terminal pro b-type natriuretic peptide; Plt, platelets; PO_4, phosphate; PTH, parathyroid hormone; SHBG, sex hormone binding globulin; TSH, thyroid-stimulating hormone; WCC, white cell count.

[a]Median.

[b]Reference value differs for age and/or sex. P values reflect patient cohort compared with kit or lab reference.

In our cohort, we found a significant and novel association between abnormal BMD and diastolic dysfunction, measured with echocardiography using the AVVSD ratio. This is a parameter that our group has recently shown to reflect invasively measured ventricular end-diastolic pressure[14] and to be an independent predictor of mortality in patients living with a Fontan circulation.[23] It is possible that the association between BMD and diastolic dysfunction reflects raised venous pressure and/or reduced cardiac output, which has been shown to affect bone blood and interstitial fluid flow in other settings.[10,11] We also found that neurohormonal activation, reflected by elevated NT-proBNP levels, tended to negatively correlate with hip *t* scores which is not surprising in view of the known association between bone loss and cardiac cachexia in the setting of biventricular heart failure.[24]

Interestingly, in our cohort, NT-proBNP and aldosterone also correlated with PTH levels although PTH did not correlate with any measured echocardiographic indices, including AVVSD ratio. In the setting of biventricular heart failure, inappropriate renin-angiotensin-aldosterone system (RAAS) activation has been shown to increase PTH levels as aldosterone can bind directly to mineralocorticoid receptors on the PTH gland.[25,26] Thus, in addition to our description of a novel association between BMD and cardiac diastolic dysfunction, the above findings support reported associations between inappropriate RAAS activation and hyperparathyroidism.

In addition, we found that resting oxygen saturations tended to positively correlate with hip *t* scores. Relative hypoxia in cell models is an important stimulator of osteoclast formation and bone absorption[27] and disease processes characterized by lower oxygen saturations, longer duration of hypoxemia and higher number of operations have been associated with reduced radial bone mass.[28] This reflects another possible mechanism underlying abnormal BMD in patients living with a Fontan circulation.

In a population of children and young adults living with a Fontan circulation, Avitabile et al[5] did not report any association between bone and muscle deficits and age, cardiac anatomy, Fontan characteristics, PTH or 25-OH vitamin D levels. We similarly found no association between abnormal BMD and age, cardiac anatomy, Fontan characteristics or 25-OH vitamin D levels but there was the suggestion of a negative correlation between PTH and hip *t* scores ($P = .07$). Whilst overall 25-OH vitamin D levels in our cohort were not significantly reduced, 27% of our cohort had readings below 50 nmol/L, the level generally recognized as vitamin D deficiency.[13] PTH levels were significantly elevated and were negatively correlated with 25-OH vitamin D levels, which is highly suggestive of secondary hyperparathyroidism related to subclinical vitamin D deficiency. Recently, Holler and colleagues[29] focused on the prevalence of vitamin D deficiency in patients living with a Fontan circulation ($n = 27$); 70% were vitamin D deficient and 24% had hyperparathyroidism. Lack of exercise and sun exposure during childhood and early adult life may underlie this deficiency although our patient population were relatively well (NYHA class I-II symptoms). Other possible mechanisms have been described, including reduced gut absorption (gut inflammation, restricted intestinal blood flow and vascular congestion) and impaired vitamin D synthesis (liver dysfunction). Holler et al[29] proposed additional mechanisms including inappropriate RAAS activation and low-grade systemic inflammation, which have been associated with abnormal BMD in other disease states.[30] In our cohort, we did not find any evidence of association between low-grade systemic inflammation and abnormal BMD.

Total serum alkaline phosphatase (ALP) may be another important biomarker in these patients. While classically an indicator of osteoblastic function and increased in the setting of secondary hyperparathyroidism, case series of patients who have had Fontan procedures have identified important correlations between cardiac

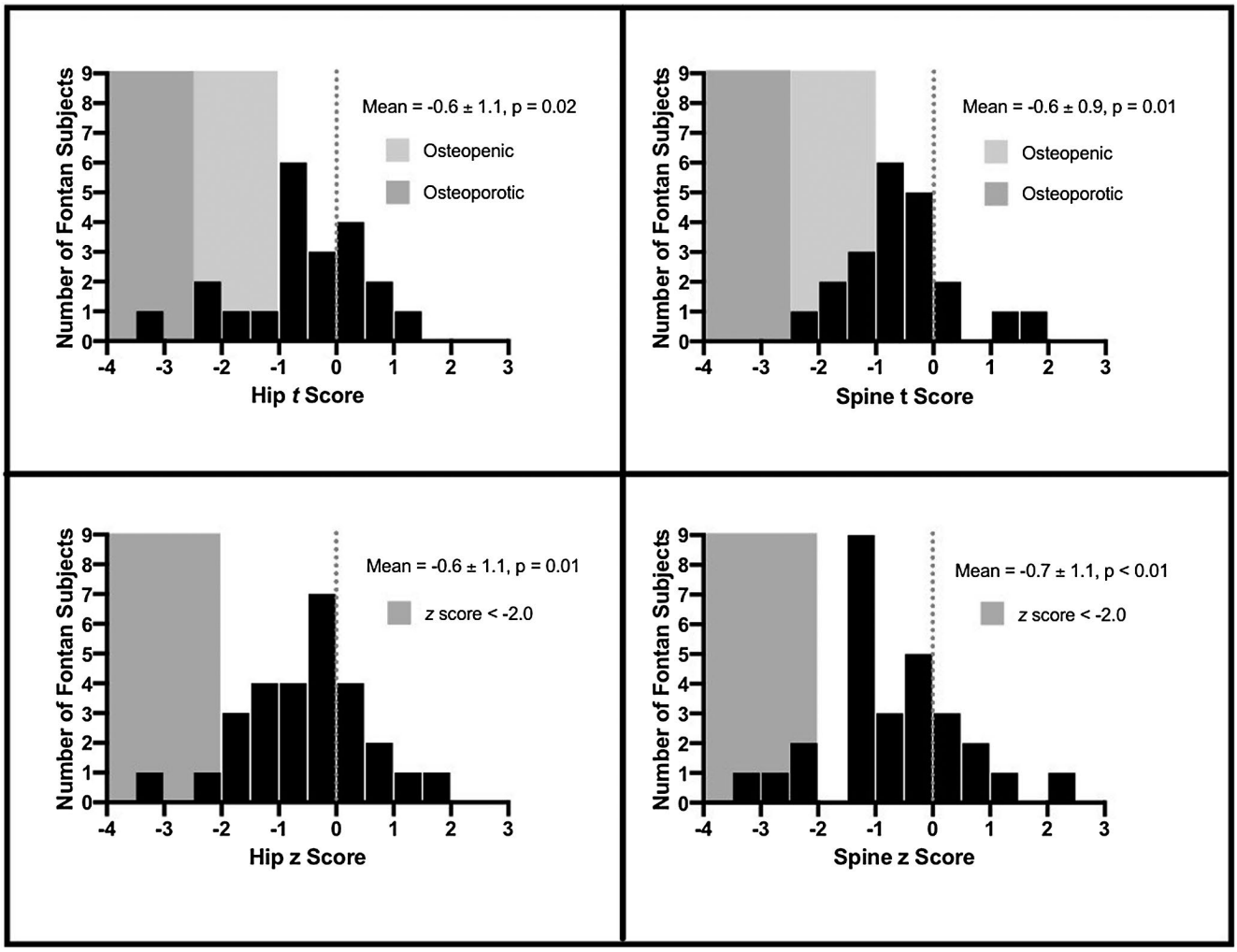

FIGURE 1 Bone density was reduced compared to a young healthy reference population (*t* score, *n* = 21, adults ≥20 yrs only) and compared to a healthy age-matched reference population (*z* score, *n* = 28).

TABLE 3 *T* score correlations

	Variable	Spearman ρ	*P* value
Hip *t* score			
	Resting SpO_2	0.45	.05
	NT-proBNP	−0.42	.08
	AVVSD ratio	−0.71	**<.01**
	PTH	−0.44	.07
Spine *t* score			
	AVVSD ratio	−0.45	.06
	ALP	−0.42	.06

Note: Bold value indicates *P* > .05.
Abbreviations: ALP, alkaline phosphatase ; AVVSD ratio, atrioventricular valve systolic to diastolic duration ratio measured by echocardiography; SpO_2, room air oxygen saturation; NT-proBNP, N-terminal pro b-type natriuretic peptide; PTH, parathyroid hormone.

output and total ALP levels.[31,32] However, it is probable that specific isoenzymes of ALP likely have different effect on total ALP levels. For example, animal models suggest heart-specific ALP probably contributes significantly to total ALP and may explain marked and rapid variations in this enzyme accompanying cardiac output variations.[33] In addition, bone-specific ALP has been hypothesized as a marker of oxygen delivery in this cohort[34] and liver-specific ALP reflects hepatic congestion, which is common in these patients. In our participants, total ALP levels were not overtly deranged but there was an important negative correlation between ALP and spine *t* scores. There were no additional correlations between BMD and other measures of hepatic function or portal hypertension. While this finding might suggest a possible link between hepatic congestion and reduced BMD, it is difficult to interpret as we did not measure specific ALP isoenzymes.

Despite recent reports of specific endocrinopathies related to abnormal cardiovascular physiology in this cohort,[9] we could not find any important correlations between sex hormone or metabolic profile and abnormal BMD. In contrast to previously reported data in children and young adults with Fontan physiology,[8] insulin-like growth factor-1 (IGF-1) levels in our cohort of young adults were not significantly reduced and did not correlate with abnormal BMD.

4.1 | Limitations

The main limitation of this study was our small sample size and thus we may have been underpowered to detect important relationships. The study's cross-sectional design meant we could report association but not causation. We aimed to analyze a relatively well Fontan cohort and thus participants with more severe impairment (eg, NYHA class III-IV) and ventricular systolic dysfunction were not represented in this cohort. We lacked data on time spent outdoors and physical activity levels which, though difficult to quantify, has an important influence on bone density.[35] We also did not collect data on dietary calcium intake. Our analysis of BMD was limited to hip and spine BMD which have a relatively high proportion of trabecular bone. Measurement of a site with a higher proportion of cortical bone may have helped our interpretation of BMD abnormalities. While including a wide variety of biochemical and endocrine indices, laboratory analysis of sex hormones was limited by lack of data on menstrual cycles. We did not perform direct hemodynamic venous pressure measurement, which may have helped further clarify underlying pathophysiological mechanisms for abnormal BMD.

4.2 | Conclusions

This exploratory study demonstrated that clinically relevant abnormalities of BMD exist in relatively well adolescent and adult participants living with a Fontan circulation. Analysis of biochemical and endocrine markers coupled with echocardiographic parameters, strength, and exercise testing have uncovered a variety of complex interactions that may contribute to abnormal BMD and require further investigation. These include secondary hyperparathyroidism, reduced oxygen saturations, ventricular diastolic dysfunction, and neurohormonal activation. Regularly assessing physical activity levels, sun exposure, BMD, 25-OH vitamin D and PTH levels, and treating vitamin D deficiency, when present, should be considered standard of care for this group to optimize bone health and reduce the risk for fragility fractures.

ACKNOWLEDGMENTS

We are grateful for the assistance of Amy Coy, Subha Nasir Ahmad, and Calum Nicholson for their assistance in data acquisition. We are also grateful to Phillip Munoz, Clair Lake, and John Camps from the Department of Respiratory Medicine, RPAH for their assistance and expertise in collecting and interpreting spirometry and CPET data. We thank Mario D'Souza for his help with statistical analysis. Finally, we are grateful to Julie Hetherington and her team from the Metabolic Unit, Department of Endocrinology, RPAH for their assistance in performing and interpreting DXA.

CONFLICTS OF INTEREST

Professor Yves d'Udekem is a Clinician Practitioner Fellow of the NHMRC (1082186). Murdoch Children's Research Institute is supported by the Victorian Government's Operational Infrastructure Support Program.

AUTHOR CONTRIBUTIONS

Paolo D'Ambrosio: Primary author - concept/design, ethics approval, and development of study protocol, data collection, data analysis and interpretation, drafting article, statistics, preparation, and submission of manuscript.
Derek Tran: Data collection, data analysis and interpretation of data, drafting of article, assistance with development of study protocol, statistics, critical revision of the manuscript, and approval of article for submission.
Charlotte Verrall: Participant recruitment and data collection, critical revision of article and approval of article for submission.
Chantal Attard: Critical revision of article and approval of article for submission.
Maria Fiatarone Singh: initial study design contributions, data analysis and interpretation, critical revision of article, and approval of article for submission.
Julian Ayer: data analysis & interpretation, critical revision of article, and approval of article for submission.
Yves d'Udekem: assistance with patient recruitment, assistance with concept design, critical revision of article, and approval for submission.
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How to cite this article: D'Ambrosio P, Tran D, Verrall CE, et al. Prevalence and risk factors for low bone density in adults with a Fontan circulation. *Congenital Heart Disease*. 2019;14:987–995. https://doi.org/10.1111/chd.12836