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# Early characteristics of bone deficits in children with Fontan palliation

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## Abstract

*Background:* This is a cross-sectional study aiming to understand the early characteristics and background of bone health impairment in clinically well children with Fontan circulation. *Methods:* We enrolled 10 clinically well children with Fontan palliation (operated >5 years before study entrance, Tanner stage ≤3, age 12.1 ± 1.77 years, 7 males) and 11 healthy controls (age 12.0 ± 1.45 years, 9 males) at two children's hospitals. All patients underwent peripheral quantitative CT. For the Fontan group, we obtained clinical characteristics, NYHA class, cardiac index by MRI, dual x-ray absorptiometry, and biochemical studies. Linear regression was used to compare radius and tibia peripheral quantitative CT measures between Fontan patients and controls. *Results:* All Fontan patients were clinically well (NYHA class 1 or 2, cardiac index 4.85 ± 1.51 L/min/m$^2$) and without significant comorbidities. Adjusted trabecular bone mineral density, cortical thickness, and bone strength index at the radius were significantly decreased in Fontan patients compared to controls with mean differences −30.13 mg/cm$^3$ ($p = 0.041$), −0.31 mm ($p = 0.043$), and −6.65 mg$^2$/mm$^4$ ($p = 0.036$), respectively. No differences were found for tibial measures. In Fontan patients, the mean height-adjusted lumbar bone mineral density and total body less head z scores were −0.46 ± 1.1 and −0.63 ± 1.1, respectively, which are below the average, but within normal range for age and sex. *Conclusions:* In a clinically well Fontan cohort, we found significant bone deficits by peripheral quantitative CT in the radius but not the tibia, suggesting non-weight-bearing bones may be more vulnerable to the unique haemodynamics of the Fontan circulation.

Single ventricle CHDs occur in about 1350 newborns each year in the United States of America.[1] The management of most single ventricle patients culminates in the Fontan palliation that forms a passive systemic venous return to the lungs aiming to separate the pulmonary from the systemic venous return. This unique physiology allows the single ventricle patients to survive into adolescence and adulthood, but their course is complicated by multiple morbidities.[2–6] As the lifespan after Fontan procedure has increased, it is important to recognise and prevent long-term morbidities resulting from the unique Fontan physiology with elevated central venous pressure, and often diminished cardiac output. Post-Fontan sequelae include among others reduced bone density and quality.[7–9]

Data on quantitative analysis of bone health by dual x-ray absorptiometry or peripheral quantitative CT in children with Fontan palliation are scarce.[7,8,10] Small series of Fontan patients have shown decreased bone mineral density by dual x-ray absorptiometry or significant bone deficits by peripheral quantitative CT.[7–11] While peripheral quantitative CT studies showed reductions in bone quality at either the radius or the tibia individually, there is no study that examined bone mineral density by dual x-ray absorptiometry and bone microarchitecture by peripheral quantitative CT of the tibia and radius in the same individuals. With this study, we aimed to (1) determine whether a distal radius or a distal tibia is more sensitive to identify bone deficits and (2) examine the correlation between relevant dual x-ray absorptiometry and peripheral quantitative CT parameters.

## Methods

### Patients

Ten Fontan patients (7 males, 12.2 ± 1.69 years) were enrolled in this cross-sectional study from the outpatient paediatric cardiology clinics of two participating sites (Children's Hospital and Clinics of Minnesota and the Masonic Children's Hospital at the University of Minnesota, Minneapolis, Minnesota, United States of America). Inclusion criteria for Fontan patients were a history of Fontan palliation more than 5 years prior to enrolment, being ≥7 years of age with Tanner stage ≤ 3. Fontan patients were recruited from the cardiology services at the University of Minnesota and the Children's Hospitals and Clinics of Minnesota as part of an ongoing multi-centre study evaluating end-organ biomarkers in the Fontan circulation and coordinated by the Cincinnati Children's Hospital. Exclusion criteria were active protein-losing enteropathy, current steroid treatment for an independent disease process, bone fracture within the last 12 months, known endocrine disorders, autoimmune diseases, independent disease process leading to moderate or severe liver or kidney impairment, paraplegia or hemiplegia, patients with electronic pacemaker or persistent arrhythmias, patients on the transplantation list or having received cardiac transplantation, and participants unable to comply with study procedures. Control patients were identified from two separate cross-sectional studies at the University of Minnesota evaluating cardiometabolic risk factors in healthy children. These included 11 healthy controls (9 males, 12.0 ± 1.45 years) for the peripheral quantitative CT study and 4 additional (2 males, 11.1 ± 1.49 years) for the biomarker studies.

### Clinical characteristics, anthropometry, and pubertal development

Patient's underlying diagnoses, type and timing of surgical procedures as well current medications were obtained from medical records. Functional assessment was performed by the NYHA classification score system at the time of the clinical evaluation. Standing height and weight were measured for all patients and their controls. Height and weight z scores were calculated using GenenCALC 3.0 Software (Genentech Inc., South San Francisco, California, United States of America), which uses the Centers for Disease Control and Prevention (CDC) 2000 growth data (available at www.cdc.gov/growthcharts). The data for the CDC 2000 growth curves incorporate data from white, black, Mexican American, and other ethnic groups but do not provide ethnic-specific data. White children were the majority of those children made to generate the CDC data and the population in our study.[12] Body mass index was calculated using height and weight and converted to z scores using standard growth charts from the CDC.[13] Although breast development was assessed in females, testicular examination was not performed in males; therefore, Tanner staging was recorded based only on pubic hair development in females and males for consistency.[14] Bone age was calculated by radiographs of the non-dominant hand and wrist by the Greulich–Pyle method and was available only for the Fontan population.[15] The Fontan patients (9 out of 10) underwent physical activity tracking with a GT3 accelerometer for 3–5 consecutive days including weekdays and weekend days. On average, the Fontan patients engaged in 94.5 ± 50.9 min/day of moderate–vigorous physical activity with 6 out of 9 patients averaging more than 60 min of moderate–vigorous physical activity per day over the assessment period, which is consistent with the American Heart Association recommended activity for children per day.[16]

### Dual x-ray absorptiometry

Height-adjusted[17] bone mineral density z scores for the posterior–anterior lumbar spine at L1–L4 and total body less head were measured in Fontan patients by dual x-ray absorptiometry (GE Healthcare Lunar Prodigy scanner; Madison, Wisconsin, United States of America) using enCORE software version 9.3. Sex- and age-specific bone mineral density z scores were calculated using enCORE reference data based on healthy, ambulatory patients from the general population. The enCORE reference database consists of data from children 5 to 18 years old of multiple ethnicities participating in the National Health and Nutrition Examination Survey Dual X-ray Absorptiometry Database (NHANES 1999–2004) (available at www.cdc.gov/nchs/nhanes/dxx/dxa.htm). The heights of individuals in NHANES 1999–2004 who received dual x-ray absorptiometry scans were representative of the US population and were incorporated into the CDC 2000 growth curves, so they should have a mean height z score of zero. Dual x-ray absorptiometry bone mineral density z scores were adjusted for height as suggested by Zemel et al.[17]

### Peripheral quantitative CT

Measures of cortical and trabecular bone and estimated bone strength were obtained using peripheral quantitative CT (XCT-3000; Stratec Medizintechnik GmbH, Pforzheim, Germany), taken at the 3 and 38% sites of the left tibia, and the 3 and 33% sites of the non-dominant radius as previously described.[18] Tibia and radial length were measured to adjust for bone architectural measures. The reference line for both tibia and radius was placed at the proximal end of the distal growth plate. Image processing and calculation of bone parameters were completed using the manufacturer's software (version 6.0). Phantom scanning was done daily for quality control.

Bone outcome measures[19] at metaphyseal sites included total and trabecular volumetric bone mineral density (mg/cm$^3$), total and trabecular cross-sectional area (mm$^2$), and total bone strength index (mg$^2$/mm$^4$). At diaphyseal sites, the measures included cortical bone mineral density, cortical cross-sectional area, cortical bone mineral content (mg/mm), cortical thickness (mm), periosteal and endosteal circumference (mm), non-weighted polar section modulus (mm$^3$), and strength–strain index (mm$^3$). Muscle and fat cross-sectional areas (mm$^2$) were measured at the 66% site of the left tibia to assess for possible sarcopenia.

### Laboratory studies

A number of laboratory studies were performed in the Fontan patients to rule out protein-losing enteropathy and liver, renal, endocrine, or nutritional abnormalities that may affect bone health. These included serum albumin, calcium, thyroid-stimulating hormone (TSH), serum and urine creatinine (measured at Fairview Diagnostics Laboratory, Minneapolis, Minnesota, United States of America by standard techniques), cystatin-C, 25-OH vitamin D, B-type natriuretic peptide and alkaline phosphatase, and intact parathormone (measured by the LabCorp laboratory services, Denver, Colorado, United States of America). Faecal alpha-1-antitrypsin was measured by ARUP Laboratories (Salt Lake City, Utah, United States of America) to detect subclinical protein-losing enteropathy.

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**Table 1.** Demographic and anthropometric characteristics of Fontan patients and control patients*

| Covariate | Fontan patients (n = 10) | Controls (n = 11) | p-Value** |
|---|---|---|---|
| Male sex | 7 (70.0) | 9 (81.8) | 0.635 |
| Race/ethnicity | | | |
| Black | 1 (10.0) | 0 (0.0) | 0.672 |
| Hispanic | 1 (10.0) | 2 (18.2) | |
| Native American | 1 (10.0) | 0 (0.0) | |
| White (non-Hispanic) | 7 (70.0) | 9 (81.8) | |
| Age (years) | 12.1 ± 1.77 | 12.0 ± 1.45 | 0.919 |
| Height (cm) | 149 ± 10.2 | 146 ± 9.72 | 0.512 |
| Height z score | −0.09 ± 1.26 | −0.59 ± 0.96 | 0.317 |
| Weight (kg) | 41.2 ± 9.55 | 37.4 ± 7.72 | 0.335 |
| Weight z score | −0.07 ± 0.93 | −0.6 ± 0.91 | 0.203 |
| BMI (kg/m$^2$) | 18.4 ± 2.98 | 17.4 ± 1.67 | 0.352 |
| BMI z score | −0.004 ± 0.97 | −0.28 ± 0.75 | 0.464 |
| Tanner stage | | | |
| 1 | 4 (40.0) | 7 (63.6) | 0.607 |
| 2 | 3 (30.0) | 2 (18.2) | |
| 3 | 3 (30.0) | 2 (18.2) | |

*Values presented are mean ± standard deviation or n (%) where indicated.
**p-Values were derived from two sample t-tests for continuous variables or Fisher's exact tests for categorical variables

In addition, we performed a panel of serum and urine bone biomarkers for both patients and a group of control patients to assess for potential differences in bone formation, calcification, or bone resorption. The serum biomarkers included the bone formation marker osteocalcin, the mineralization inhibitor osteopontin, the inhibitor of osteoclastogenesis osteoprotegerin, and intact parathormone that were measured in plasma using the commercially available Human Bone Panel Luminex 4-plex immunoassay (Millipore Corporation, Billerica, Massachusetts, United States of America). The bone resorption marker tartrate-resistant acid phosphatase was measured by a commercially available ELISA assay (QuidelTRAP5b; Quidel Corporation, Bilerica, Massachusetts, United States of America). The urine biomarkers, urinary pyridinoline and deoxypridinoline were measured as markers of bone resorption using the MicroVue pyridinoline and deoxypridinoline EIA kit, respectively (Quidel Corporation) and are expressed as ratios to creatinine to adjust for urinary concentration. All bone metabolic markers were measured at the cytokine reference lab of the University of Minnesota. Not all biomarkers were measured in each patient and control patients due to insufficient sample.

### Cardiac MRI

Cardiac MRI was performed on the Fontan group of patients on a 1.5 Tesla Siemens Symphony scanner (Munich, Germany) to estimate indexed systemic cardiac output. Patients were free breathing for acquisition of localising, cine steady-state free precession, and flow quantification sequences. The ventricular short-axis sequence was performed with a cine steady-state free precession sequence with 7 mm slices with no gap for quantification of ventricular size, systolic function, and cardiac output. Flow quantification in the ascending aorta, inferior and superior caval veins, and branch pulmonary arteries was performed to assess cardiac output and flow patterns. MRI scans were performed on the same day with the laboratory and physical examinations.

### Statistical analysis

Descriptive statistics were tabulated separately for Fontan and control groups. These included the mean and standard deviation for continuous variables and frequency for categorical variables. p-Values for differences in means were evaluated using a t-test with unequal variance and Welch's degrees of freedom, while differences in categorical variables were evaluated using a chi-square test. Peripheral quantitative CT bone density and strength measures were adjusted for Tanner stage and sex. In addition, geometric bone measures were also adjusted for either radius or tibia length. Sensitivity analysis was performed by adjusting for age instead of Tanner stage. Adjusted differences between Fontan patients and controls were evaluated using linear regression and the t-distribution with corresponding model degrees of freedom for confidence intervals and p-values. Since this is a pilot exploratory study, no adjustments were made for multiple comparisons. Analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, North Carolina, United States of America) and R v2.15.2 (Vienna, Austria).[20]

## Results

### Clinical characteristics

Most patients with Fontan physiology were classified as NYHA class 1 (80%) except two who met criteria for NYHA class 2 (20%). Table 1 summarises demographic characteristics of 10 Fontan patients and 11 controls with Tanner stage ≤3. There were no significant differences with regard to sex, race, age, pubic hair development, or anthropometrics. Clinical characteristics and main laboratory findings in Fontan patients are presented in Tables 2 and 3, respectively. None of the patients had symptoms of protein-losing enteropathy or faecal leakage of alpha-1-antitrypsin. Three of the Fontan patients received medications such as coumadin and/or furosemide that can interfere with bone metabolism when used long term.

### Bone measurements

#### Dual x-ray absorptiometry measures

In Fontan patients, the mean height-adjusted L1–L4 bone mineral density z score was −0.46 ± 1.1 and total body less head z score was −0.63 ± 1.1, which are below the average (32 and 26%, respectively). These values reflect a downwards shift from the general population, but they are still within normal range for age and sex.

#### Peripheral quantitative CT results

Among Fontan patients, reduction in bone measurements was seen at the radius compared to controls after adjustment for sex and Tanner stage (Table 4). The adjusted differences in means for Fontan versus control patients were −30.13 mg/cm$^3$ (95% confidence interval −58.82, −1.44; p = 0.041) for trabecular bone mineral density, −0.31 mm (95% confidence interval −0.61, −0.01; p = 0.043) for cortical thickness, and −6.65 (95% confidence interval −12.78, −0.52; p = 0.036) for bone strength index with a Cohen's effect size value d of 1.05, 0.93, and 0.95, respectively, suggesting

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**Table 2.** Clinical characteristics of Fontan patients*

| | Fontan patients (n = 10) |
|---|---|
| Diagnosis | |
| Hypoplastic left heart syndrome | 3 (30) |
| Unbalanced atrioventricular canal | 3 (30) |
| Double inlet left ventricle | 1 (10) |
| Double outlet right ventricle | 1 (10) |
| Pulmonary atresia/intact ventricular septum | 1 (10) |
| Hypoplastic right ventricle | 1 (10) |
| Systemic ventricular morphology | |
| Left | 3 (30) |
| Right | 4 (40) |
| Unclassifiable | 3 (30) |
| Chronological age (years) | 12.2 ± 1.69 |
| Bone age (years) | 12.4 ± 1.46 |
| Oxygen saturation (%) | 93.8 ± 5.2 |
| DXA** | |
| Lumbar bone mineral density z score | −0.46 ± 1.1 |
| Total body less head z score | −0.63 ± 1.1 |
| MRI cardiac index*** (L/min/m$^2$) | 4.85 ± 1.51 |
| NYHA functional classification | |
| NYHA 1 | 8 (80.0) |
| NYHA 2 | 2 (20.0) |
| Medications**** | |
| Coumadin | 2 (20) |
| Furosemide | 2 (20) |
| Spironolactone | 1 (10) |
| Aspirin | 7 (70) |
| Angiotensin converting enzyme inhibitor/ angiotensin receptor blocker | 9 (90) |
| $PDE_5$ inhibitors | 1 (10) |

DXA = dual x-ray absorptiometry
*Values presented are mean ± standard deviation or n (%) where indicated.
**DXA-based cardiac index is available for n = 8 patients.
***MRI-based cardiac index is available for n = 9 patients.
****Some patients are exposed to more than one medication; three patients (30%) were exposed to bone metabolism affecting medications (warfarin and/or furosemide)

high clinical significance.[21] However, no significant differences between Fontan patients and controls were seen for the tibial peripheral quantitative CT parameters (Table 5). There were no significant differences in tibial muscle and fat cross-sectional area, muscular density, intermuscular adipose tissue between Fontan and control patients, although we noted a downward shift in tibial muscle density in the Fontan patients (Table 5, Supplementary Table 2). Sensitivity analysis of bone peripheral quantitative CT measures with adjustment for age (instead of Tanner stage) and sex revealed similar findings with mild variations in the levels of statistical significance (Supplementary Tables 1 and 2). No significant associations were detected between peripheral quantitative CT bone measurements and MRI-based cardiac output, NYHA class, or use of medications (data not shown).

**Table 3.** Laboratory values in Fontan patients

| Serum biomarker | Mean ± SD | Reference values |
|---|---|---|
| Serum | | |
| Albumin (g/dl) | 4.5 ± 0.49 | 3.5–5.5 |
| Creatinine (mg/dl) | 0.61 ± 0.11 | 0.39–1.27 |
| Cystatin-C (mg/L) | 0.71 ± 0.08 | 0.53–0.95 |
| TSH (mIU/L) (n = 8) | 4.17 ± 1.58 | 0.27–4.20 |
| 25-OH vitamin D (ng/ml) | 29.9 ± 17.4 | 30–100 |
| Parathyroid hormone (pg/ml) | 45.6 ± 18.3 | 15–65 |
| Calcium (mg/dl) (n = 5) | 9.94 ± 0.75 | 8.5–10.4 |
| Total alkaline phosphatase (IU/L) | 237.3 ± 101.8 | 86–362 |
| BNP (pg/ml) | 43.3 ± 57.6 | <100 |

### Bone biomarkers

Fontan patients had borderline low 25-OH VitD (29.9 ± 17.4 ng/ml) and borderline elevated TSH levels (4.17 ± 1.58 mIU/L). A selected list of available serum and urine biomarker measures for comparison between Fontan and the second group of control patients is presented in Table 6. Comparison of bone biomarkers between Fontan and the control patients demonstrated increased levels of osteopontin in the Fontan patients (47.18 ± 15.66 versus 19.69 ± 10.30, p = 0.013) (Table 6). In addition, there was a trend towards higher osteoprotegerin and parathormone levels in Fontan patients that did not reach statistical significance.

## Discussion

Bone has long been known to be an exquisitely mechanosensitive organ, and its homoeostasis depends on the ability of bone cells to sense and respond to mechanical stimuli including blood flow alterations.[22] In addition, hypoxia has been shown in cell models *in vitro* and in hypoxemic patients *in vivo* to be an important stimulator of osteoclast differentiation and bone resorption.[23–25] Therefore, survivors with Fontan palliation may have decreased bone formation and/or increased bone resorption resulting in bone loss and ultimately osteoporosis due to low cardiac output and chronic, albeit, mild hypoxia.[11] In addition, protein-losing enteropathy, vitamin D deficiency and other nutritional deficiencies,[9] anticoagulant therapy,[8,26] chronic use of diuretics,[27] decreased exercise capacity,[28] muscle wasting with decreased weight-bearing activity,[29] and chronic kidney dysfunction[30] may impose additional risks to bone health. The overall incidence of fractures among patients with Fontan physiology is not known, but a small series from Australia recently reported a 32% incidence of non-pathologic fractures in their cohort.[11] Another small series of critically ill patients with CHDs that suffered bone fractures while hospitalised identified that 40% of them had single ventricle physiology.[31] Published data suggest that patients with Fontan palliation or single ventricle have low bone mineral density, and this is considered to be the best non-invasive predictor available for bone fracture risk.[7–11,32] As survival after the Fontan palliation is improving, it is important to identify patients with decreased bone density to initiate early therapy and prevent fractures.

Our study examined bone health in a homogeneous age group of a clinically well cohort of active young Fontan patients using

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**Table 4.** Tanner stage and age-adjusted comparison of peripheral quantitative CT radial measurements between Fontan and control patients**

| Radial outcome | Fontan (mean ± SD) | Control (mean ± SD) | Fontan–control (95% CIs) | p-Value | Effect size*** |
|---|---|---|---|---|---|
| Trabecular BMD (mg/cm$^3$) | 214.39 ± 23.48 | 241.70 ± 27.68 | −30.13 (−58.82, −1.44) | 0.041* | 1.05 |
| Trabecular CSA (mm$^2$) | 207.46 ± 48.29 | 168.82 ± 78.02 | 30.94 (−30.45, 92.32) | 0.294 | 0.46 |
| Total BMD (mg/cm$^3$) | 277.36 ± 25.63 | 323.41 ± 42.56 | −41.76 (−87.42, 3.90) | 0.070 | 1 |
| Total CSA (mm$^2$) | 207.46 ± 48.29 | 224.87 ± 87.95 | −22.76 (−90.40, 44.87) | 0.477 | 0.32 |
| Cortical BMD**** (mg/cm$^3$) | 1098.34 ± 39.41 | 1112.84 ± 34.32 | −14.52 (−64.17, 35.13) | 0.533 | 0.4 |
| Cortical CSA**** (mm$^2$) | 57.96 ± 11.71 | 60.87 ± 11.32 | −3.57 (−13.07, 5.94) | 0.426 | 0.32 |
| Cortical bone mineral content**** (mg/mm) | 63.72 ± 13.47 | 67.54 ± 11.41 | −4.54 (−15.32, 6.25) | 0.374 | 0.37 |
| Cortical thickness**** (mm) | 2.49 ± 0.35 | 2.73 ± 0.30 | −0.31 (−0.61, −0.01) | 0.043* | 0.92 |
| Periosteal circ**** (mm) | 31.05 ± 3.18 | 30.84 ± 3.06 | 0.27 (−2.61, 3.15) | 0.842 | 0.09 |
| Endosteal circ**** (mm) | 15.40 ± 2.94 | 13.70 ± 2.72 | 2.21 (−1.09, 5.51) | 0.169 | 0.77 |
| Total bone strength index (mg$^2$/mm$^4$) | 16.21 ± 4.85 | 22.81 ± 7.38 | −6.65 (−12.78, −0.52) | 0.036 | 0.95 |
| Polar section modulus (mm$^3$) | 169.91 ± 49.76 | 165.20 ± 42.81 | 5.54 (53.23, 42.15) | 0.806 | 0.12 |
| Strength strain index (mm$^3$) | 139.25 ± 39.33 | 140.46 ± 36.23 | −9.84 (−48.23, 28.73) | 0.591 | 0.27 |

BMD = bone mineral density; circ = circumference; CIs = confidence intervals; CSA = cross-sectional area; SD = standard deviation.
*Statistical significance at $p < 0.05$.
**Differences, 95% CIs, and p-values are calculated from linear regression adjusting for Tanner stage and sex.
***Effect size is calculated as absolute difference in means divided by pooled SD at baseline (Cohen's d).
****Geometrical parameters are also adjusted for radial length.

**Table 5.** Tanner stage and sex adjusted comparison of peripheral quantitative CT tibial measurements between Fontan and control patients*

| Tibial outcome | Fontan (mean ± SD) | Control (mean ± SD) | Fontan–control (95% CIs) | p-Value | Effect size** |
|---|---|---|---|---|---|
| Trabecular BMD (mg/cm$^3$) | 224.74 ± 19.42 | 253.55 ± 40.85 | −26.37 (−62.35, 9.60) | 0.139 | 0.73 |
| Trabecular CSA (mm$^2$) | 637.38 ± 85.67 | 644.13 ± 170.48 | −30.85 (−172.57, 110.87) | 0.649 | 0.22 |
| Total BMD (mg/cm$^3$) | 264.54 ± 18.71 | 300.70 ± 48.27 | −30.28 (−59.99, 9.43) | 0.125 | 0.72 |
| Total CSA (mm$^2$) | 772.08 ± 100.20 | 791.90 ± 191.13 | −45.88 (−206.93, 115.17) | 0.553 | 0.29 |
| Cortical BMD*** (mg/cm$^3$) | 1081.45 ± 43.09 | 1084.44 ± 33.02 | −6.29 (−49.34, 36.76) | 0.757 | 0.17 |
| Cortical CSA*** (mm$^2$) | 202.36 ± 31.03 | 201.88 ± 46.33 | −0.08 (−37.97, 37.81) | 0.996 | 0 |
| Cortical bone mineral content*** (mg/mm) | 218.81 ± 34.91 | 218.50 ± 47.96 | −1.00 (−40.00, 37.99) | 0.956 | 0.02 |
| Cortical thickness*** (mm) | 4.40 ± 0.49 | 4.49 ± 0.80 | −0.08 (−0.70, 0.53) | 0.770 | 0.12 |
| Periosteal circ*** (mm) | 59.77 ± 4.79 | 58.89 ± 5.52 | 0.69 (−4.94, 6.31) | 0.796 | 0.14 |
| Endosteal circ*** (mm) | 32.14 ± 4.41 | 30.66 ± 4.52 | 1.22 (−3.96, 6.40) | 0.619 | 0.28 |
| Total bone strength index (mg$^2$/mm$^4$) | 54.51 ± 12.07 | 72.50 ± 27.46 | −17.15 (−41.21, 6.91) | 0.150 | 0.73 |
| Polar section modulus (mm$^3$) | 1181.94 ± 275.87 | 1122.38 ± 299.27 | 30.62 (−260.12, 321.37) | 0.825 | 0.11 |
| Strength strain index (mm$^3$) | 996.03 ± 219.57 | 945.61 ± 238.65 | 21.27 (−210.15, 252.68) | 0.847 | 0.09 |
| Muscle CSA (mm$^2$) | 4443.10 ± 871.30 | 4028.90 ± 666.29 | 255.88 (−381.17, 892.94) | 0.405 | 0.33 |
| Muscle CSA/BSA | 3381.07 ± 346.31 | 3257.99 ± 371.62 | 118.26 (−249.77, 486.30) | 0.504 | 0.33 |
| Muscle density (mg/cm$^3$) | 82.64 ± 1.59 | 83.51 ± 1.20 | −1.16 (−2.48, 0.15) | 0.079 | 0.82 |
| Intermuscular adipose tissue CSA (mm$^2$) | 1225.88 ± 460.63 | 919.68 ± 214.37 | 244.88 (−67.80, 557.55) | 0.116 | 0.67 |
| Fat CSA (mm$^2$) | 1162.12 ± 529.08 | 1240.97 ± 391.90 | −137.06 (−604.71, 330.58) | 0.542 | 0.31 |

BMD = bone mineral density; circ = circumference; CIs = confidence intervals; CSA = cross-sectional area; SD = standard deviation.
*Differences, 95% CIs, and p-values are calculated from linear regression adjusting for Tanner stage and sex.
**Effect size is calculated as absolute difference in means divided by pooled SD at baseline (Cohen's d).
***Geometrical parameters are also adjusted for tibial length.

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**Table 6.** Comparison of selected bone biomarkers between Fontan and control patients

| Biomarker | Fontan (n = 10) | Control (n = 4) | p-Value** |
|---|---|---|---|
| Serum | | | |
| Bone alkaline phosphatase (IU/L) | 105.1 ± 31.2 | 124.5 ± 35.9 | 0.269 |
| Parathyroid hormone (pg/ml)*** | 67.9 ± 38.0 | 39.3 ± 34.8 | 0.134 |
| Osteopontin (ng/ml) | 47.18 ± 15.66 | 19.69 ± 10.30 | 0.013* |
| Osteoprotegerin (pg/ml) | 177.5 ± 70.9 | 90.0 ± 32.8 | 0.064 |
| Osteocalcin (ng/ml) | 42.17 ± 22.47 | 31.44 ± 29.50 | 0.573 |
| Tartrate-resistant acid phosphatise (U/L) | 19.51 ± 7.08 | 18.75 ± 7.84 | 0.567 |
| Urine | | | |
| Deoxypridinoline (nml/L)**** | 197.4 ± 109.2 | 160.1 ± 138.2 | 0.740 |
| Pyridinoline (nml/L)**** | 381.6 ± 135.7 | 312.3 ± 138.5 | 0.530 |

Values presented are mean ± standard deviation.
*Statistical significance at $p < 0.05$.
**p-Values are calculated from linear regression adjusting for Tanner stage and sex
***Parathyroid hormone was measured by the Human Bone Panel Luminex 4-plex immunoassay for comparison with controls and not by LabCorp as in Table 3.
****Two patients in Fontan group are missing their urine markers.

both dual x-ray absorptiometry and peripheral quantitative CT, the latter at both radius and tibia. The data showed potentially clinically significant reductions in total and trabecular bone mineral density, cortical thickness, and bone strength index at distal radius. The study also showed that dual x-ray absorptiometry may not be as sensitive as peripheral quantitative CT in detecting changes in bone density in Fontan patients because mean height-adjusted L1–L4 and total body less head bone mineral density z scores were within normal range.

Only five studies examined bone parameters in Fontan patients with three of them using dual x-ray absorptiometry to evaluate bone density, and two using peripheral quantitative CT to evaluate bone mineral density and geometry.[7–11] The dual x-ray absorptiometry studies by Bendaly et al and Goldberg et al reported lower bone mineral density z scores in Fontan patients compared to our study.[8,9] Bendaly et al studied 26 Fontan patients aged 5–12 years and showed reduced lumbar bone mineral density z score ($-1.0 + 0.2$ standard deviations) and total body less head z score ($-0.8 + 0.2$ standard deviations) compared to the normal population[9]. Sixteen out of 26 children who were on warfarin tended to have lower bone mineral density z-scores compared to the ones who were not on warfarin. The D'Ambrosio study from Australia with predominantly young adults described osteoporotic findings in trabecular bones (spine and hip) in a third of their cohort.[11] The investigators of this older cohort found correlations between the osteoporotic findings with lower oxygen saturation, echocardiographic indices of diastolic dysfunction and biochemical evidence of secondary hyperparathyroidism, but no correlation with medications affecting bone metabolism such as diuretics and anticoagulants. Goldberg et al studied 12 Fontan patients with protein-losing enteropathy, aged 7.2–25.2 years and showed decreased mean bone mineral density z scores of −1.73 standard deviations; 5 out of the 12 patients had bone mineral density z score below −2 standard deviations[8]. The lower bone mineral density z scores seen in this study are most likely due to the presence of protein-losing enteropathy in these patients, which none of our patients had.

A peripheral quantitative CT study by Witzel et al of 29 postpubertal patients with various forms of CHD found adjusted bone mineral content at distal radius significantly reduced in 6 patients with Fontan palliation compared to the reference values.[10] Another peripheral quantitative CT study by Avitabile et al. focused on the distal tibia in 43 Fontan patients between 5 and 33 years of age.[7] The study reported decreased tibial cortical area, periosteal circumference, trabecular bone mineral density, and muscle area in Fontan patients when compared to healthy reference participants.[7] The investigators in this mixed-age cohort did not find any association with age, Fontan characteristics, or biochemical markers of hyperparathyroidism.

In contrast to the Avitabile study, we did not identify any significant bone or muscle deficits in the tibia, probably a reflection of the younger age and "healthier" background of our cohort. The data for our fundamentally different cohort suggest that the radius, a non-weight bearing bone, may be a more vulnerable bone site to be impacted by the unique haemodynamics in the Fontan circulation. Therefore, radius and not tibia may be the most sensitive bone to screen for the early detection of reduced bone density and quality in Fontan patients even in those considered clinically well and without associated significant morbidities. In accordance with the concept of "Muscle-Bone Unit," bone modelling and strength are achieved in relation to mechanical needs.[33] We speculate that the tibial exposure to weight-bearing is able to compensate up to some degree for the adverse bone remodelling effects of the Fontan circulation. As a result, in states of compromised bone health, the first areas in which changes can be appreciated are unloaded bones such as radius. These findings are in congruence with previous studies documenting greater impairment of trabecular parameters at the distal radius as compared to tibia in post-menopausal women and mineral loss from unloaded bones in amenorrhoeic athletes and anorexic individuals.[32,34–36] Of importance, the radial trabecular parameters were shown to be better predictors of fracture at distant sites in both osteoporotic men and women.[35,36]

Although the small number of patients does not allow a comprehensive analysis of the impaired bone pathophysiology in the Fontan circulation, several potential leading points emerge. Of particular interest are the elevated osteopontin and osteoprotegerin in the Fontan patients. Osteopontin is not only a non-collagenous component of bone matrix but also a multifunctional protein that has been implicated in a number of physiological and pathological events related to the regulation of inflammation, vascular tone, and both bone and cardiac remodelling.[37–39] In addition, osteopontin has been found to have predictive value for poor outcomes in heart failure with preserved ejection fraction,[40] which in some aspects may be more suitable for the model of circulatory failure in the Fontan population.[41] The osteoprotegerin is a potent inhibitor of osteoclast differentiation and activity, and the observed increase in the Fontan group may represent a reactive process aiming to protect against bone loss from osteoclast remodelling associated with increased parathormone and borderline low vitamin D, found to be at the upper and lowest normal ranges, respectively, in our study.[42,43]

This study has several limitations, one of them being the small sample size that did not allow us to detect differences between Fontan and controls, but this does not change the fact that the osteopenia was more pronounced in the same sample of patients. Tanner stage in males was not fully assessed because the testicular volume was not measured, but use of age instead of Tanner stage yielded similar findings. Another limitation is the use of a second set of controls for some parts of the study that limits

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the interpretation of the findings related to the measured biomarkers. Additional research will be required to understand the long-term consequences of these findings and potential relevance for other CHDs. Most importantly, further research is warranted to assess whether based on these findings, early preventive measures and therapeutic interventions with vitamin D/calcium supplementation[44,45] and prescribed physical activity[32] will improve relevant clinical outcomes in the growing population of survivors with complex CHD.

**Supplementary Material.** To view supplementary material for this article, please visit https://doi.org/10.1017/S1047951120000293

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**Conflicts of Interest.** None.

**Ethical Standards.** The study was approved by the Institutional Review Board at the University of Minnesota, Children's Hospitals and Clinics of Minnesota, and the Cincinnati Children's Hospital. Informed consent was obtained from a legal guardian, and assent was taken from all study participants.

**Authors' Contributions.** K.S. contributed to study design, interpreted data, and wrote the manuscript. A.P. contributed to study design, interpreted peripheral quantitative CT, and dual x-ray absorptiometry studies. P.E.M. drafted the manuscript. L.E. P. contributed to study design and funding, recruited patients, acquired data, and interpreted data. A.P.-M. performed biochemical analysis and interpreted data. R.B. performed statistical analysis. B.S.M. contributed to study design and funding, acquired data, and recruited patients. D.G. recruited patients and acquired data. C.S. performed and interpreted MRI. A.S.K. recruited study patients, acquired data, and contributed funding. B.S.M. recruited study patients and contributed to study design. K.R. contributed to statistical analysis. C.M. contributed to statistical analysis. L.K.K. conceived the study, contributed funding, interpreted results, and edited final manuscript. All authors reviewed and approved the submitted manuscript.

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