The Muscle-Bone Unit in Children and Adolescents: Insights from the Canadian Health Measures Survey

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Data Availability Statement
This study is based on data from the Canadian Health Measures Survey cycles 5 and 6. Access to data from the Canadian Health Measures Survey is available by contacting Statistics Canada (https://www.statcan.gc.ca), an agency of the federal government of Canada.

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Competing interests
The authors declare no competing interests.

Ethics Approval
Ethics approval for the overall CHMS was provided by Health Canada's Research Ethics Board and the Office of the Privacy Commissioner of Canada. In addition, ethics approval for the individual-level analyses of pQCT scan images, jumping mechanography data, and serum markers of bone metabolism was obtained from the Institutional Review Board of McGill University (IRB study number A05-M31-15B).

Participant consent statement
All participants or their caregivers provided written informed consent to participate in the study. For children 6 to 13 years of age, a parent or legal guardian provided written consent.

Manuscript classification: Clinical

Unstructured Abstract (299/300 words):

Muscle and bone are closely related during growth and development. We sought to identify determinants of the muscle-bone relationship using peripheral quantitative computed tomography (pQCT) at the distal lower extremity and jumping mechanography among Canadian children. Data were obtained from the Canadian Health Measures Survey (CHMS), a national, cross-sectional, population-based study. 3,225 participants (aged 6 to 19.9 years) were included. Calf muscle cross-sectional area (CSA; 66% site) z-score was used as a surrogate measure of muscle strength, bone mineral content (BMC) z-score of the tibia shaft cross-section (38% site)

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was used as a surrogate measure of bone strength. Maximum jumping power (P_{max}) z-score from the single two-legged jump test was assessed as a mediator between muscle CSA and BMC z-scores using path analysis. Peripheral QCT and P_{max} z-scores were calculated using CHMS data. Height, weight, and body mass index (BMI) z-scores were calculated using Centers for Disease Control reference data. We found a strong positive association between muscle CSA z-score and tibia BMC z-score (β = 0.64, 95% confidence interval [CI]: 0.62 to 0.67). Calf subcutaneous fat CSA (β = -0.04, 95% CI: -0.07 to -0.01) and muscle density (β = 0.08, 95% CI: 0.03 to 0.13) z-scores were weak determinants, and weight (β = 0.10, 95% CI: 0.05 to 0.15) and BMI (β = 0.14, 95% CI: 0.07 to 0.21) z-scores were strong determinants of the association between muscle CSA z-score and BMC z-score. Self-reported moderate-to-vigorous physical activity and serum total calcium, 25-hydroxy-vitamin D_3, and parathyroid hormone were not determinants. P_{max} z-score was a partial mediator of the association between muscle CSA and BMC z-scores. In conclusion, we found that the effect of muscle size on bone mass was in part mediated by muscle power. Subcutaneous fat, muscle density, weight, and BMI were determinants of the muscle-bone relationship.

Lay Summary (100/100 words)

This study aimed to identify factors influencing the muscle-bone relationship in Canadians aged 6 to 19.9 years. We found a strong relationship between muscle size and bone mass, which was partially dependent on maximum jumping power. Calf fat cross-sectional area and muscle density, measures of fat under the skin and fat infiltration in the muscle, had small, negative effects on the relationship between muscle size and bone mass. Weight and body mass index had strong, positive effects on that same relationship. Physical activity and serum concentrations of

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calcium, 25-hydroxy-vitamin D_3 and parathyroid hormone had minimal effect on the muscle-bone relationship.

Key Words:

Canadian Health Measure Survey; Children; Jumping Mechanography; Muscle-Bone Interaction; Peripheral Quantitative Computed Tomography

Introduction

Muscle and bone are closely related.[1-3] Frost's mechanostat model proposes that the mechanical forces placed on bone by muscle contraction cause it to adapt through bone modeling and remodeling, driving bone strength as a function of muscle strength.[1] From this perspective, it is important to consider muscle and bone together as a single unit.[1,3] In addition to this mechanical muscle-bone relationship, recent evidence suggests that muscle and bone are endocrine organs that engage in crosstalk between each other through the release of myokines and osteokines, respectively.[4] Dual-energy x-ray absorptiometry (DXA) is the most commonly used imaging tool for the assessment of the muscle-bone unit due to its wide availability.[5] However, DXA is based on the analysis of two-dimensional projection images of three-dimensional structures, which leads to some loss of information. Devices using cross-sectional images, such as peripheral quantitative computed tomography (pQCT) provide additional information, and allow for the analysis of parameters such as bone and muscle cross-sectional area (CSA), as well as muscle density (reflecting the fat content of muscle).[6,7]

In addition to imaging modalities, muscle can be assessed by measuring its function. Dynamic muscle function measures obtained through jumping mechanography is an attractive approach in

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this context, as this method uses tests that are similar to real-world movements performed by children and adolescents, such as jumping and hopping.[8]

Many studies have demonstrated positive relationships between muscle and bone parameters in youth.[9,10] However, clinical muscle-bone interaction studies vary widely with regard to which muscle and bone parameters are considered, and it is often unclear on what basis specific muscle and bone parameters were selected to assess the muscle-bone unit.[11,12]

It is important to identify determinants of the muscle-bone unit, as this will show which factors make bone more sensitive to the effect of muscle size or function. Previous studies using DXA suggest that body fat mass has a detrimental effect on the muscle-bone unit, as children and adolescents with increased fat mass have lower bone mass than would be expected for their muscle mass or size.[13,14] However, these studies did not take muscle function into account and therefore it is unclear whether increased fat mass impairs the muscle-bone unit by decreasing muscle function or through other effects.

It is also important to understand how muscle function impacts the muscle-bone unit. In adults, it is known that muscle size is a key determinant of maximum muscle strength.[15] Additionally, previous studies suggest that both muscle size and muscle function are strong predictors of bone strength in children and adolescents.[9] However, it is unclear to what extent, if any, muscle function contributes to or mediates the established, positive relationship between muscle size and bone strength.

In the present study, we used the mechanostat model as a basis to investigate the muscle-bone unit in a large cohort of children, adolescents and young adults. To operationalize the muscle-bone unit concept in this study, the present analysis used BMC z-score of the tibia shaft as a surrogate measure of bone strength, given that tibia BMC strongly correlates with failure load.[16]

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In contrast, volumetric BMD, as determined by pQCT, is not a logical bone parameter for muscle-bone interaction studies during growth, as volumetric BMD does not capture the changes in bone size that are a key aspect of bone adaptation to mechanical loads during growth.[17,18] Calf muscle CSA z-score was used as a surrogate indicator of muscle strength, as muscle CSA is a strong predictor of the maximum force generated by a muscle.[19] Maximal power (P_{max}) during a single two-legged jump (S2LJ) was used as the parameter reflecting muscle function as P_{max} in this test had been identified as a determinant of tibia cortical parameters in previous studies.[9,20] Based on these considerations, the aims of the present study were (1) to identify determinants of the relationship between muscle and bone structure, as assessed by calf muscle CSA and tibia BMC at the tibial diaphysis; and (2) to evaluate the extent to which muscle function, as reflected by peak jump power, mediates the association between muscle mass and bone structural outcomes.

Materials and Methods

Study Population and Study Design

The Canadian Health Measures Survey (CHMS) is a nationally representative, cross-sectional survey conducted across Canada.[21-23] The objective of the CHMS is to gather data to inform the prevention, diagnosis, and treatment of health-related issues amongst Canadians. CHMS data collection is conducted in two-year cycles on a sample of approximately 5,000 participants per cycle, ages 3-79 years. This sample is demographically representative of approximately 96% of the Canadian population, excluding only full-time members of the Canadian Armed Forces, institutionalized individuals, and people living in the three territories, on reserves or other Aboriginal settlements in the provinces, or in remote regions.

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The general CHMS study design includes a household health interview followed by a visit to a mobile clinic where direct health measures are taken, such as anthropometry, physical performance tests as well as blood and urine sampling. The specific test program varies between the 2-year CHMS study cycles. During cycles 5 (2016-2017) and 6 (2018-2019), the data collection included pQCT scans of the distal lower extremity and jumping mechanography to address the theme of bone health in Canadians. Pubertal staging was not part of the test program. Ethics approval for the overall CHMS was provided by Health Canada's Research Ethics Board and the Office of the Privacy Commissioner of Canada. In addition, ethics approval for the analyses of pQCT scan images and jumping mechanography force plate raw data, performed at Shriners Hospitals for Children-Canada, was obtained from the Institutional Review Board of McGill University (IRB study number A05-M31-15B). All participants or their caregivers provided written informed consent to participate in the study.

In the present analysis, we included individuals from CHMS cycles 5 and 6 who were (1) aged 6 to 19.9 years and (2) had usable pQCT measures available. Thus, data obtained from 3,225 CHMS participants were included in this study.

Peripheral Quantitative Computed Tomography

Peripheral QCT (XCT 3000, Stratec Inc., Pforzheim, Germany) was performed on the non-dominant lower leg as described.[24] If the non-dominant leg had a metal implant or a fracture within the past 12 months, the other leg was scanned. The lower leg was scanned at three sites along the tibia, 4% (distal tibia, for trabecular bone measures), 38% (diaphyseal tibia, for cortical bone measures), and 66% (the site of the largest calf diameter, for muscle and fat measures) of

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tibial length measured from the distal end of the bone.[25] Children under 6 years of age did not undergo pQCT or jumping mechanography.

The following pQCT measures were analyzed: calf muscle CSA (mm^2, 66% site), subcutaneous fat CSA (mm^2, 66% site), muscle density (mg/cm^3, 66% site), and tibia BMC (mg/mm, 38% site). Tibia length (mm) was measured prior to the pQCT assessment using a rigid ruler. The measurement was taken from the inferior margin of the medial malleolus to the superior margin of the tibial plateau. pQCT scans were analyzed at Shriners Hospital for Children-Canada using XCT software (version 6.20C) to generate quantitative results. Z-scores for each pQCT measure were then calculated based on reference data that were derived from the same study cohort (manuscript in preparation). Muscle CSA, fat CSA, and BMC z-scores were adjusted for age, sex, and tibia length. Muscle density z-score was adjusted for age and sex only.

Cortical bone measures at the 38% measurement site were judged usable (not affected by movement artefact) based on the automated quantitative method described by Blew et al.[26] For the muscle and fat measures at the 66% measurement site, all scan images were visually assessed by two independent analysts. In case of disagreement between readers whether a scan was of sufficient quality to be usable, consensus was reached through discussion between readers.

Of the 4,060 individuals aged 6 to 19.9 years, 25 (0.6%) did not undergo pQCT assessment (due to acute/chronic conditions or pregnancy). The percentage of study participants who had scans that were not usable due to movement artefacts was 29.0% in 6- to 9.9-year-old children and was 10.2% in study participants from 10 to 19.9 years of age. Thus, data obtained from 3,225 CHMS participants were included in this study.

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Jumping Mechanography

The Leonardo Mechanograph® Ground Reaction Force Plate (Novotec Medical GmbH, Pforzheim, Germany) was used to assess dynamic muscle function as described.[8] The reliability and reproducibility of jumping mechanography assessment have been confirmed in previous studies.[8,27] Participants were ineligible if they had elevated risk of injury, assessed through physical activity readiness questionnaire, eye injury, history of surgery, history of acute or chronic conditions. Participants performed three valid trials (maximum of five attempts) of the single two-legged jump (S2LJ) test. The S2LJ test required each participant, initially at a standstill, to jump as high as possible and land on the force plate with both feet. After three valid trials, the trial with the highest maximum jump height was used to calculate results for the participant. Age- and sex-adjusted Z-scores for P_{max} from the S2LJ were calculated using Canadian reference data derived from the same study.[28]

Laboratory Analyses

Blood samples were collected during a participant's visit to the mobile examination center. Participants and/or caregivers provided separate written informed consent for blood and urine samples. Blood was drawn from either the left or right arm and sent to the Nutrition Research Division, Health Canada, on dry ice and stored at -80°C until analysis. CHMS data regarding the following laboratory measures obtained from blood samples were used in this study: serum 25-hydroxyvitamin D_3 ($25OHD_3$), serum calcium, and serum parathyroid hormone (PTH). Serum $25OHD_3$ was quantified using liquid chromatography tandem mass spectrometry as described.[29] The standard threshold for $25OHD_3$ sufficiency is 50 nmol/L.[30] Serum calcium (pediatric reference interval: 2.31 to 3.64 mmol/L) was quantified at a commercial laboratory (Dynacare)

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using a colorimetric assay (Roche Cobas c501). Plasma concentrations of PTH (pediatric reference interval: 1.2 to 6.3 pmol/L) were determined at the same laboratory by electrochemiluminescence immunoassay (Roche Cobas e601). Reference intervals were obtained from the Canadian Laboratory Initiative on Pediatric Reference Intervals database.[31]

Self-Reported Physical Activity

Self-reported moderate-to-vigorous physical activity (MVPA) was obtained through the Physical Activity Adult Questionnaire and the Physical Activity Youth Questionnaire. Within the household health interview portion of the CHMS, participants were asked about their amount of physical activity in hours or minutes within the last 7 days prior to the interview. Further details about the questionnaires and their validation are described elsewhere.[32,33] Age-specific versions of the questions were asked based on the following age groups: 6 to 11, 12 to 17, and ≥18 years. Thus, to compare responses from different questions, we created age- and sex-adjusted MVPA categories by estimating age-specific percentiles within each age group and by sex using quantile regression with MVPA as the outcome and age as the explanatory variable. We fit models to obtain the 25th and 75th percentile curve across age. Because the age span within each age group is narrow, a linear age term provided adequate fit (i.e. good calibration/coverage at 25th and 75th percentiles). Each participant's MVPA score was then compared with the age- and sex-specific cut-points predicted by these quantile curves and classified as: Low MVPA (< 25th percentile), Medium MVPA (25th to 75th percentile), and High MVPA (> 75th percentile). Medium MVPA was treated as the reference group for all calculations.

Body Mass Index (BMI)

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Height and weight were measured during the mobile clinic examination. Age- and sex-specific height, weight, and BMI z-scores were determined using Centers for Disease Control and Prevention 2000 reference data.[34]

Statistical Analysis

This study was a secondary analysis of data from cycles 5 and 6 of the CHMS. Therefore, the study sample was determined by the number of eligible participants with available data rather than by a prospectively specified sample size or power calculation. All participants aged 6 to 19.9 years with usable pQCT measures were included in the primary analyses (n = 3,225). This approach maximized the use of the available population-based sample and the precision of the estimated associations. Analyses requiring additional measures, including P_{max}, were based on participants with available data, with multiple imputation performed as a sensitivity analysis to assess the potential influence of missing data.

All analysis was conducted using R (version 3.4.2) and SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Participant characteristics were summarized using mean and standard deviation (SD) for continuous variables and frequencies and percentages for categorical variables (Table 1). Variable normality was assessed using normal probability (Q-Q) plots, boxplots and histograms. In the set of 3,225 individuals with complete pQCT scan data, the following variables had missing data: serum calcium (8.1%), serum $25OHD_3$ (9.3%), serum PTH (10.7%), and P_{max} (24.6%).

In this study, we defined a determinant as any clinically relevant factor that substantially alters the association between muscle and bone. Potential determinants were identified using the change-in-estimate criterion method.[35] In short, we assessed the percentage change between (1)

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the linear regression coefficient of the association between muscle CSA z-score and BMC z-score and (2) each linear regression coefficient of the same model but adjusted for one of the potential determinants. Based on the percentage change, we categorized them as follows: ≥5%, strong determinant; 1% to <5%, weak determinant; <1%, not a determinant. These cut-off values were arbitrarily chosen as common thresholds (e.g., >10%) were deemed too large, and calculating a threshold using a method proposed by Lee yielded a cut-off of >0.25%, which was deemed too small for clinical application.[35,36] After this, we ran a multivariable linear regression model of the association between muscle CSA z-score and BMC z-score, adjusted for all identified determinants (>1%), to assess their combined influence on the muscle-bone relationship.

Dynamic muscle function (P_{max} z-score from the S2LJ test) as a potential mediator of the association between muscle CSA z-score and BMC z-score was assessed via path analysis using the R lavaan package (version 0.6-19).[37,38] We fit a path analysis model that included the direct effect of muscle CSA z-score (independent of the effect of P_{max} z-score) on BMC z-score, and the indirect effect of muscle CSA z-score on BMC z-score via P_{max} z-score (calculated as the product of the effect of the muscle CSA z-score on P_{max} z-score and the effect of P_{max} z-score on the outcome). P_{max} z-score mediated the muscle-bone relationship if its indirect effect was statistically significant. The total effect was the sum of the direct and indirect effects. The proportion mediated by P_{max} z-score was calculated as the indirect effect divided by the total effect and then multiplied by 100%. Model fit was assessed using root mean square error of approximation (RMSEA), Comparative Fit index (CFI), and Tucker-Lewis index (TLI). RMSEA assesses approximate model misfit per degree of freedom, with values ≤ 0.08 generally indicating acceptable fit. CFI and TLI compare the hypothesized model with an independence

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(null) model, with values ≥ 0.90 generally indicating acceptable fit. All results were reported with corresponding 95% confidence intervals (CI) and p-values. P-values less than 0.05 were considered statistically significant. CHMS sampling weights were applied to all analyses. We repeated this analysis with adjustment for one of fat CSA z-score, muscle density z-score, or BMI z-score to adjust for the effects of fat-related variables. Multiple imputation using the 'Multivariate Imputation by Chained Equations' R package (version 3.18.0) was performed as a sensitivity analysis to assess the robustness of the findings in the presence of missing data (Supplementary Appendix).[39]

Results

Participant Characteristics (Table 1)

The analysis included 3,225 individuals aged 6.0 to 19.9 years. Among these, 146 (5%) were classified as underweight, 2,269 (70%) were normal weight, 472 (15%) were overweight, and 338 (11%) had obesity. Of note, 1,316 (40.8%) individuals had serum $25OHD_3$ levels less than 50 nmol/L, and 540 (16.7%) study participants had serum $25OHD_3$ below 30 nmol/L. 2,713 (84.1%) of individuals had serum calcium levels within the reference range, whereas 2,733 (84.7%) of individuals had serum PTH concentrations in the reference range.

Determinants of the Relationship Between Calf Muscle CSA z-score and Tibia BMC z-score (Table 2)

The unadjusted linear regression coefficient between muscle CSA z-score and BMC z-score was 0.644 (95% CI = 0.618 to 0.669, $p < 0.0001$). Of the variables assessed as potential determinants of this relationship, serum calcium, serum $25OHD_3$, serum PTH, and MVPA did not change the

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regression coefficient by more than 1%. Including fat CSA, muscle density, or height z-scores into the regression model changed the association by more than 1% but less than 5% and therefore these parameters were considered weak determinants of the association between muscle CSA z-score and BMC z-score. However, height z-score was not statistically significant. Weight and BMI z-scores changed the association between muscle CSA z-score and BMC z-score by more than 5% and therefore were interpreted as strong determinants.

In the multivariable linear regression model including all identified determinants, the regression coefficient between muscle CSA z-score and BMC z-score was 0.579 (95% CI = 0.507 to 0.652, $p < 0.0001$) after adjusting for fat CSA z-score ($\beta = -0.140$, 95% CI = -0.211 to -0.069 , $p < 0.0001$), muscle density z-score ($\beta = 0.117$, 95% CI = 0.066 to 0.168, $p < 0.0001$), weight z-score ($\beta = 0.103$, 95% CI = 0.014 to 0.191, $p < 0.05$) and BMI z-score ($\beta = 0.111$, 95% CI = 0.007 to 0.215, $p < 0.05$) (adjusted $R^2 = 0.468$). Height z-score was not included because it was not statistically significant, z-scores were adjusted for tibia length, and it had multicollinearity with weight and BMI z-scores (Variance Inflation Factor > 10).

Dynamic Muscle Function as a Mediator of the Relationship Between Calf Muscle CSA z-score and Tibia BMC z-score (Figure 1)

We assessed to what extent the relationship between muscle CSA z-score and tibia BMC z-score was mediated by dynamic muscle function, as reflected by P_{max} from the S2LJ test. Additional analyses were conducted adjusting for measures of body fat (fat CSA as a measure of subcutaneous fat at the distal lower extremity; muscle density as a measure of intramuscular fat; BMI as a measure of total body fat), based on the analyses shown in the previous section. Mediation analysis showed that the direct effect of muscle CSA z-score on BMC z-score was

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0.500, whereas the indirect effect, mediated through P_{max} z-score, was 0.155 (proportion mediated = 23.6%) (Figure 1A).

When fat CSA z-score was adjusted for in the mediation analysis (Figure 1B), the direct effect of muscle CSA z-score on BMC z-score was 0.507. The indirect effect between muscle CSA z-score and BMC z-score, adjusted for fat CSA z-score and mediated through P_{max} z-score, was 0.157 (proportion mediated = 23.7%). Similarly, in the path analysis model adjusted for muscle density z-score (Figure 1C), the direct effect was 0.507 and the indirect effect was 0.157 (proportion mediated = 23.7%).

When the mediation analysis was repeated using BMI z-score as an indicator of body fat (Figure 1D), the direct effect between muscle CSA z-score and BMC z-score was 0.433, whereas the indirect effect, adjusted for the effects of BMI z-score and mediated through P_{max} z-score, was 0.084 (proportion mediated = 16.3%).

Age- and Sex-Subgroup Analyses of the Relationship Between Calf Muscle CSA z-score and Tibia BMC z-score (Figure 2)

Weight and BMI z-scores were strong determinants in all age- and sex-subgroups except for pubertal females. However, in pre-pubertal males, after adjustment for either weight z-score or BMI z-score, the muscle CSA z-score regression coefficient decreased in both pre-pubertal and pubertal males but increased in the post-pubertal group. In pre-pubertal females, after adjustment for the same determinants, the muscle CSA z-score regression coefficient decreased to varying degrees in all subgroups. Fat CSA and muscle density z-scores were strong determinants in post-pubertal males and weak determinants in pre-pubertal and pubertal females. Fat CSA, but not muscle density, was a weak determinant in pre-pubertal males.

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Age- and Sex-Subgroup Analysis of Dynamic Muscle Function as a Mediator of the Relationship Between Calf Muscle CSA z-score and Tibia BMC z-score (Figure 3)

P_{max} was a consistent mediator of the relationship between calf muscle CSA z-score and tibia BMC z-score across all age groups in males. In females, P_{max} mediated the muscle-bone relationship primarily within the post-pubertal subgroup, with significant indirect effects within the pre-pubertal subgroup after adjustment for fat CSA z-score or muscle density z-score. After adjustment for BMI z-score (Model 4), P_{max} was not a mediator of the muscle-bone unit in post-pubertal males or females.

Results from Multiple Imputation Analysis

The results from multiple imputation analysis indicate that there was no effect of missing data on our findings (Supplementary Appendix, Tables S1-S2, Figure S1).

Discussion

In this study, we assessed determinants of the muscle-bone unit in 3,225 children, adolescents and young adults aged 6 to 19.9 years who participated in the CHMS from 2016 to 2019. Calf fat CSA and calf muscle density were identified as weak determinants of the association between calf muscle CSA and tibia BMC. Weight and BMI were strong determinants. Mediation analysis demonstrated that maximal jumping power was a partial mediator of the association between calf muscle CSA and tibia BMC.

Our results confirm that both muscle and fat size influence bone mass. However, the model identifying subcutaneous calf fat CSA as a weak determinant indicates the effect of muscle size

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on bone mass was much larger than the effect of fat. For example, our data indicate that an increase in muscle CSA z-score by 1.00, adjusted for fat CSA z-score, is associated with an increase in tibia BMC z-score by 0.660. In contrast, when fat CSA z-score, adjusted for muscle CSA z-score, increases by 1.00, the regression equation predicts a *decrease* in tibia BMC z-score of 0.039. Thus, the effect of a given change in muscle CSA z-score on tibia BMC z-score is about 17 times (0.660/0.039) larger than the effect of an equivalent change in fat CSA z-score. This finding seemingly conflicts with common knowledge that individuals with greater fat mass generally have larger muscles and stronger bones.[14] However, as the inverse effect of subcutaneous fat on bone mass is only detectable after muscle mass is taken into account, future research assessing the relationship between fat and bone should be adjusted for the effects of muscle.

Beyond measures of subcutaneous fat, pQCT also provides information about muscle density, which is inversely related to intramuscular fat content.[40] The positive association between muscle density z-score and tibia BMC z-score therefore indicates a negative effect of intramuscular fat on bone, in accordance with previous studies in both children and adults.[41,42] We also evaluated BMI as a measure of fat mass, as BMI is widely used in epidemiological studies as an indicator of obesity. However, we observed that BMI z-score, adjusted for muscle CSA z-score, was *positively* associated with tibia BMC z-score and thus had the opposite effect of fat CSA z-score on bone mass. As BMI is calculated using weight and height, it varies not only with fat mass but also with muscle mass. As a result, these observations indicate that there are limitations with using BMI as an indicator of body fat mass in the general pediatric population, compared to site-specific measures like subcutaneous and intramuscular fat, when analyzing the muscle-bone unit.[43]

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Height and weight were also tested as potential determinants. Although height met the criteria for a weak determinant, it was not statistically significant, likely due to the use of tibia length adjusted z-scores in our analysis. On the other hand, weight was a strong determinant, which makes sense as greater body mass would require stronger muscles when performing everyday tasks. However, weight falls under the same concern as with BMI, where greater muscle mass also contributes to increased body weight.

We assessed self-reported MVPA as a potential determinant of the association between muscle CSA z-score and BMC z-score. Our results indicate that MVPA was not a determinant of the muscle-bone relationship. Although many studies highlight the effects of physical activity (e.g., resistance training) on muscle hypertrophy and bone mineral accretion, we hypothesize that physical activity influences bone health mostly through muscle and therefore does not change the muscle-bone relationship.[44,45]

We also tested maximum power (P_{max}) from the S2LJ test as a mediator of the association between muscle size and bone mass. To our knowledge, no previous literature has assessed muscle strength as a mediator between muscle size and bone mass in a general pediatric population. Our results indicate that P_{max} z-score is a partial mediator of the relationship between muscle CSA and BMC z-scores even after adjusting for the effects of fat CSA, muscle density, or BMI z-scores. This is expected, as measures of muscle size or mass alone are not accurate representations of an individual's muscle strength. Other factors like muscle density and motor unit recruitment may also play a role in determining physical performance, and thus muscle's effect on bone.[46]

Our subgroup analyses reflecting prepubertal, pubertal and post-pubertal age groups indicated differences between males and females and between developmental stages. In post-pubertal

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males, fat CSA, muscle density, weight, and BMI were strong determinants of the muscle-bone unit, and addition of these parameters to the regression model was associated with a positive increase in the regression coefficient between muscle CSA z-score and tibia BMC z-score. This contrasts with the findings in younger males or in females, where fat CSA, muscle density, weight, and BMI tended to change the association between muscle CSA z-score and BMC z-score in the opposite direction. It thus appears from these data that pubertal changes in males either make the bone more sensitive to the effects of muscle or have an independent additional effect on bone mass. For example, both clinical and animal studies have shown that sex steroids have a direct effect on periosteal bone formation during male puberty.[47,48]

This study has some limitations. About 20% of individuals did not have valid data for P_{max}, which was most often due to movement artefacts and other technical issues during jumping mechanography. However, results from multiple imputation analysis indicate that missingness did not have a substantial effect on our results. Additionally, self-reported MVPA may have been impacted by recall bias, as participants were asked about the amount of time spent performing specific activities within the past week. Our analysis of fat mass was limited to subcutaneous fat at the distal lower extremity, and thus we could not analyze the effect of visceral fat mass, which may have a larger effect on bone than subcutaneous fat.[49] To perform subgroup analysis, we separated males and females into different age groups based on approximate pubertal ages, as pubertal stages were not assessed within the CHMS. Also, all subgroup analyses were performed using z-scores adjusted for age and sex. Thus, our findings may not accurately reflect sex differences in the muscle-bone unit before, during, and after pubertal development. Lastly, results from mediation analysis should be considered with caution, as we used cross-sectional data.

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In conclusion, we found that fat CSA and muscle density were weak determinants and weight and BMI were strong determinants of the muscle-bone relationship, which was in part mediated by muscle power. However, the effect of these determinants was much smaller than the direct effect of muscle size on bone mass.

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Table 1. Characteristics of children and adolescents aged 6 to 19.9 years who participated in cycles 5 and 6 of the CHMS with usable pQCT measures, separated by sex

	Males (n = 1580)	**Females (n = 1645)**
Age (years)	13.0 (3.7)	12.8 (3.7)
Obesity Status		
Underweight	74 (5%)	72 (4%)
Normal weight	1093 (69%)	1176 (72%)
Overweight	242 (15%)	230 (14%)
Obesity	171 (11%)	167 (10%)
Serum calcium (mmol/L)	2.42 (0.08)	2.41 (0.08)
Serum $25OHD_3$ (nmol/L)	60 (22)	60 (24)
≥50 nmol/L	950 (60%)	959 (58%)
<50 nmol/L	630 (40%)	686 (42%)

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Serum PTH (pmol/L)	3.67 (1.37)	3.54 (1.21)
Moderate-to-vigorous physical activity (min/week)		
Low (<25th percentile)	417 (26%)	466 (28%)
Med (25th to 75th percentile)	772 (49%)	802 (49%)
High (>75th percentile)	391 (25%)	377 (23%)

Results are shown as mean (SD) or N (percentage).
Abbreviations:, 25-hydroxy-vitamin D_3; PTH, parathyroid hormone

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Table 2. Assessment of potential determinants of the relationship between calf muscle CSA z-score and tibia BMC z-score

Variable	N	Regression Equation	Percentage change	Adjusted r^2
Unadjusted model				
Muscle CSA z-score	3,225	0.015 + 0.644 × *Muscle CSA z-score*****	0%	0.430
Adjusted models				
Fat CSA z-score	**3,225**	**0.013 + 0.660 × *Muscle CSA z-score***** – 0.039 × *Fat CSA z-score****	**2.47%**	**0.431**
Muscle density z-score	**3,225**	**0.018 + 0.653 × *Muscle CSA z-score***** + 0.080 × *Muscle density z-score*******	**1.42%**	**0.437**
Height z-score	**3,225**	**0.010 + 0.652 × *Muscle CSA z-score***** + 0.018 × *Height z-score***	**1.20%**	**0.443**
Weight z-score	**3,225**	**-0.014 + 0.581 × *Muscle CSA z-score***** + 0.098 × *Weight z-score*******	**-9.74%**	**0.434**
BMI z-score	**3,225**	**-0.018 + 0.538 × *Muscle CSA z-score***** + 0.142 × *BMI z-score*******	**-16.42%**	**0.442**
Serum PTH	2,880	0.070 + 0.648 × *Muscle CSA z-score***** – 0.012 × *Serum PTH*	0.69%	0.440
Serum calcium	2,964	-0.596 + 0.643 × *Muscle CSA z-score***** + 0.255 × *Serum Calcium*	-0.20%	0.436
Serum $25OHD_3$	2,925	-0.129 + 0.645 × *Muscle CSA z-score***** + 0.003 × *Serum 25OH-vitamin* D_3^{****}	0.16%	0.437
MVPA	2,432	-0.008 + 0.641 × *Muscle CSA z-score***** – 0.091 × *Low MPVA** + 0.219 × *High MVPA*****	-0.43%	0.442

Abbreviations: CSA, cross-sectional area; BMI, body mass index; PTH, parathyroid hormone; MVPA, self-reported moderate-to-vigorous physical activity. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$
BMC z-score was the outcome variable for each model. 'Percentage change' refers to the percent difference in the regression coefficient for each 'adjusted model' compared to the 'unadjusted model'. Parameters highlighted in bold print were considered significant determinants.

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Figure 1

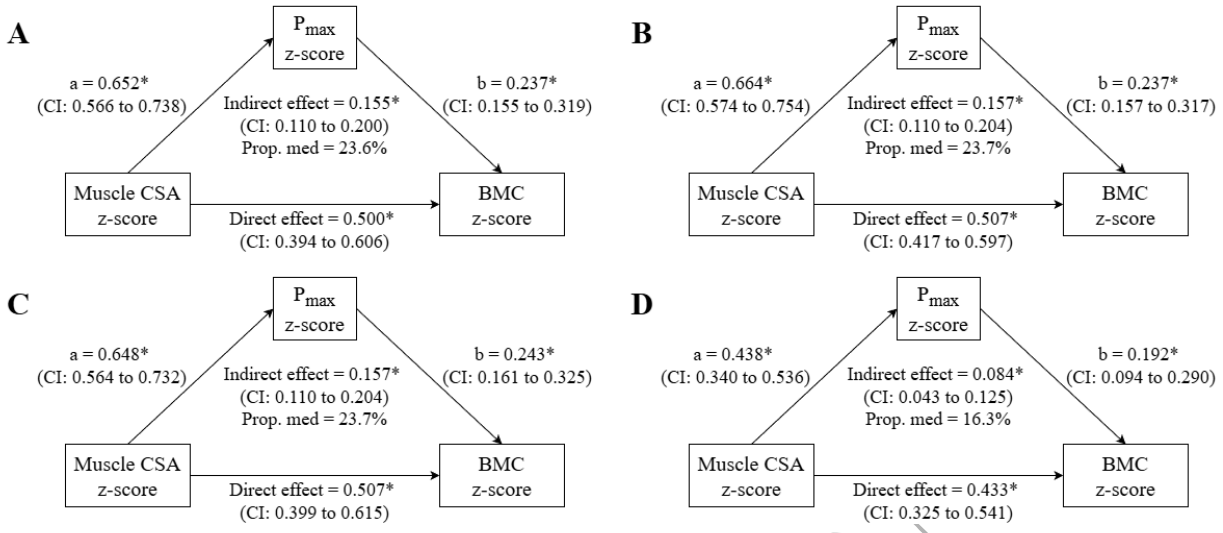

Figure 1. Summary of mediation analyses that assess dynamic muscle function (P_{max} z-score of the S2LJ) as the mediator of the effect of calf muscle (muscle CSA z-score) on tibia bone mass (BMC z-score). Results are displayed as regression coefficients and 95% confidence intervals (CI). Lines with single arrow heads indicate direct linear associations. Statistical significance ($p < 0.05$) was denoted with an asterisk (*). **A.** Unadjusted model. The indirect effect was calculated as the product of a (0.652) and b (0.237). The proportion mediated (Prop. med) was calculated as the indirect effect (a × b = 0.155) divided by the total effect (indirect effect + direct effect = 0.155 + 0.500) and then multiplied by 100%. Model fit indices: Comparative Fit Index (CFI), 0.993; Tucker-Lewis Index (TLI), 0.989; Root Mean Square Error of Approximation (RMSEA), 0.031. **B.** Adjusted for fat CSA z-score. Model fit indices: CFI, 0.994; TLI, 0.985; RMSEA, 0.029. **C.** Adjusted for muscle density z-score. Model fit indices: CFI, 0.992; TLI, 0.980; RMSEA, 0.030. **D.** Adjusted for BMI z-score. Model fit indices: CFI, >0.999; TLI, >0.999; RMSEA, <0.001.

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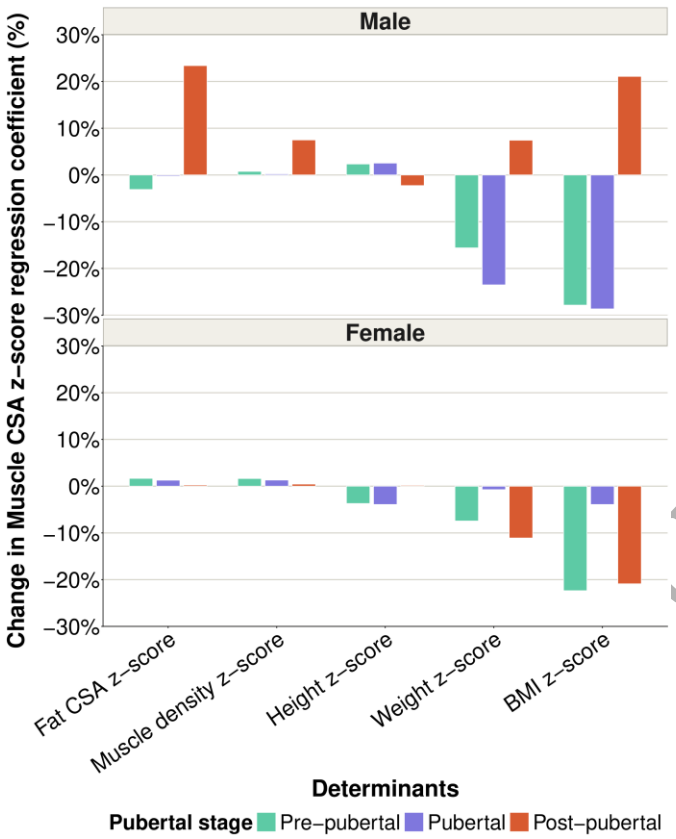

Figure 2. Percentage changes in the muscle CSA z-score regression coefficient regarding the linear association between muscle CSA z-score and bone mineral content z-score across different age and sex subgroups. Age ranges (in years): pre-pubertal (males 6 to 11.9, n = 691; females 6 to 9.9, n = 424), pubertal (males 12 to 16.9, n = 599; females 10 to 14.9, n = 716), and post-pubertal (males 17 to 19.9, n = 287; females 15 to 19.9, n = 508)

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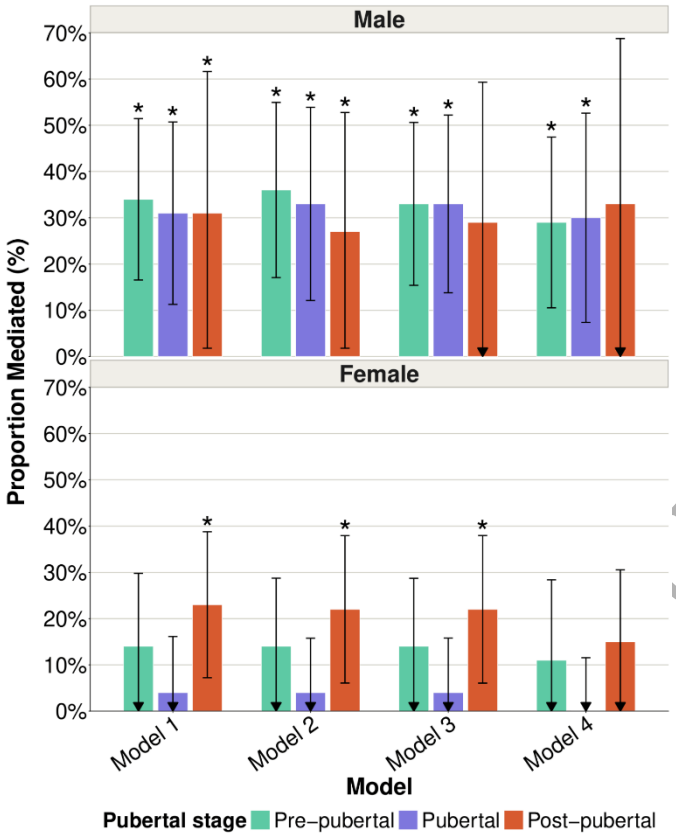

Figure 3. Proportion of mediation (and 95% CIs) of the association between muscle CSA z-score and bone mineral content z-score by maximal jumping power across different age and sex subgroups. Statistical significance ($p < 0.05$) indicated by an asterisk (*). Model 1: unadjusted. Model 2: adjusted for fat CSA z-score. Model 3: adjusted for muscle density z-score. Model 4: adjusted for BMI z-score. Age ranges (in years): pre-pubertal (males 6 to 11.9, n = 691; females 6 to 9.9, n = 424), pubertal (males 12 to 16.9, n = 599; females 10 to 14.9, n = 716), and post-pubertal (males 17 to 19.9, n = 287; females 15 to 19.9, n = 508)

Author contributions

FR, JA, AW, LMW, and JM were responsible for conceptualization, data curation, methodology, and supervision of procedures undertaken during CHMS cycles 5 and 6. FR, LMW, and JM provided supervision throughout the project. JM, LA, and LG advised the statistical analysis plan. OL contributed to the analysis plan, conducted all analysis, drafted the manuscript, and made all tables and figures. All authors contributed to the final version of the manuscript.

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