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Mediating Effect of Muscle on the Relationship of Physical Activity and Bone

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Abstract

Purpose: This study analyzed prospective associations between distinct trajectories of objectively measured physical activity (PA) and late adolescent bone parameters and explored the mediating effects of lean soft tissue (LST), a surrogate of muscle mass to associations.

Methods: PA was measured by accelerometry starting at age 5 and continuing at 8, 11, 13, 15 and 17 years in approximately 524 participants from the Iowa Bone Development Study. Gender-specific group-based trajectory modeling was used to construct developmental trajectories of moderate-and-vigorous intensity PA (MVPA) from childhood to late adolescence. At age 17, proximal femur bone mineral density (aBMD) was assessed by dual X-ray energy absorptiometry, and its distribution was calculated by aBMD ratios. Specific geometric measures of the proximal femur were assessed using Hip Structural Analysis.

Results: A significant portion of the total effect of MVPA from age 5 to 17 yr on bone parameters at age 17 was explained by an increase in leg LST in both sexes. For males and females, indirect effects were observed on the total and all regional proximal femur aBMDs, and on the ratio between the inferomedial and superolateral neck aBMD. The effect on the ratio between the trochanter and the total proximal femur was specific to females, while the effect on the hip axis length was specific to males. Direct effects of MVPA on aBMDs were identified only in males.

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Conflict of interest

No potential conflict of interest was reported by the authors. The authors have no conflicts of interest to disclose in relation to this study. The results of the present study do not constitute endorsement by American College of Sports Medicine. The results of this study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

Conclusions: Using robust mediation analysis, this is the first study addressing the indirect effect (through muscle) of PA across childhood and adolescence on proximal femur bone parameters. To improve bone health at the proximal femur, the results suggest PA interventions during growth that increase muscle mass, particularly in females.

Keywords

bone mineral density; lean soft tissue; femur; exercise; bone geometry; accelerometry

1. Introduction

Because it affects the material and structural properties of bone, physical activity is an important strategy to optimize bone strength during the growing years (1). Moreover, physical activity can improve skeletal muscle mass (2), which is a strong determinant of bone strength (3). Improvements in bone properties associated with physical activity and skeletal muscle are due to mechanical loading generated from gravitational load and muscular contractions (4, 5). This mechanism is described in the Frost's mechanostat theory. It proposes that bone strains, caused by mechanical loading that exceed a modeling threshold range, stimulates bone to increase its strength by changing its mass, density, and structure (6, 7). In addition, muscle may also regulate bone modeling via paracrine and endocrine signaling pathways (8).

The variation of lean mass (a surrogate of muscle mass) in boys during puberty seems to explain a significant percentage (~45%) of the bone mass accrual at the proximal femur in this period (9). Compared with other femoral regions, the association between lean mass and bone mass (expressed as bone mineral content, BMC or density, aBMD) does not seem to be so obvious (9) or may not even exist at the femoral neck (10). At this clinically significant region, there is also evidence that lean mass (or fat-free mass) may more determinant for bone mass differences among girls and that physical activity may be more important in boys accounting between 10% and 20% for these variations (11, 12).

Despite the relevant role that muscle plays in the skeletal response to mechanical loading during physical activity, most of the studies analyzing the associations/effects of physical activity on bone outcomes have not considered muscle and bone together as a functional unit. Too often, a single analysis of each of these factors (i.e., physical activity or muscle) is examined. In fact, a recent systematic review on youth, physical activity, and bone strength reported that only 46% of the reviewed studies considered the contribution of muscle to bone outcomes within studies using physical activity as the exposure (13).

Much remains to be learned concerning the indirect effect (through muscle) of physical activity on bone outcomes. Very few studies examined associations between physical activity and bone health across childhood and adolescence using multiple data points of objective measure of physical activity and bone outcomes (14, 15), and to the best of our knowledge, there are no studies that have analyzed the effect of muscle on this relationship, using mediation analysis. The mediation analysis allows quantifying the indirect effect of exposure on a given outcome through a specified intermediate variable providing a better understanding of the mechanism or process by which one variable influences another

variable (16). Thus, the main purpose of this study was to analyze the indirect (muscle-mediated) effects of distinct trajectories of physical activity across childhood and adolescence on late adolescent bone parameters. More specifically, we conceptualized physical activity as a behavioral process where levels of physical activity were likely to change across the growing years; we captured this concept using group-based trajectory modeling which enables the data themselves to divide a study population into subgroups and to fit trajectory models for each of those subgroups. We then examined the associations of the physical activity trajectories at age 17 1. bone mineral density (aBMD) of the total proximal femur and its regions (trochanter, inter-trochanter, and neck (including its inferomedial and the superolateral sub-regions)), 2. aBMD distribution, and 3. specific proximal femur geometric measures. Mediation analysis was used to explore the underlying mechanism of how physical activity indirectly influences bone parameters via muscle.

2. Methods

2.1. Sample Design and Data Collection

The Iowa Bone Development Study (IBDS) is an ongoing longitudinal study of bone health during childhood, adolescence, and young adulthood. Participants are a subset of the Iowa Fluoride Study birth cohort; 1,882 families from eight Iowa hospital postpartum wards who were recruited between 1993 and 1997 (17). The IBDS cohort was recruited between 1998 and 2002 when Iowa Fluoride Study participants were approximately 5 years of (child) age. The IBDS continues to use a rolling admission to allow Iowa Fluoride Study members to participate in any follow-up examinations. This report focused on data collected from 1998 to 2013. We did not exclude participants based on medical conditions, mostly we have healthy participants. The following conditions that could be related to bone development were reported for analysis sample in concurrent health questionnaire: musculoskeletal diseases (scoliosis: 2, kyphosis: 1), diabetes (3), and arthritis (2). Current asthma was reported by a relatively high number of participants (22). Adjusting for body size and sex, proximal femur bone outcomes were slightly lower for participants with current asthma, but differences were not statistically significant. The study was approved by the University of Iowa Institutional Review Board (Human Subjects).

At approximately age 5, 8, 11, 13, 15, and 17 yr, accelerometer measures of physical activity were obtained, and a clinical examination that included anthropometry and dual-energy X-ray absorptiometry (DXA) imaging was conducted. Each measurement wave was conducted over a three-year period, and the age standard deviation for each wave was ~ 0.4 yr resulting in 4 and 6 yr olds within the 5 yr old wave, 7 and 9 yr olds within 8 yr old wave, etc. Since the anatomical landmarks for a proper identification of the specific regions of the proximal femur (such as the greater and lesser trochanter) are fully formed by late adolescence (18), only age 17 yr was used to derive bone parameters from DXA.

2.2. Accelerometry

At each clinical examination, participants and their parents were given instructions on accelerometer wear. ActGraph accelerometers (Pensacola, FL) were mailed to participants during the autumn season (September to November). Due to availability, model 7164 was

used for age 5, 8, 11, 13 yr; GT1M for age 15 yr; and GT3X for age 17 yr. At age 5 and 8 yr, participants were asked to wear the monitor during all waking hours for four consecutive days, including one weekend day. At the other examination ages, they were asked to wear for five consecutive days, including both weekend days. Accelerometry movement counts were collected in a one-minute epoch at ages 5, 8, 11, and 13 yr. Accelerometry data at age 15 were collected in a five-second epoch, and raw acceleration data were collected at age 17 yr. Age 15 yr and age 17 yr accelerometry data were re-integrated to one-minute epochs.

Accelerometers were considered as having not been worn if a period of 60 consecutive minutes of zero accelerometry counts (with allowance for two non-zero interruptions) was encountered in the accelerometry data array. Accelerometry data were only used from participants who wore an accelerometer for a minimum of 10 hours per day and three days at each examination. Moderate-and-vigorous intensity physical activity (MVPA) was defined as 2,296 or greater accelerometry counts per minute (19, 20).

2.3. Dual-energy X-ray absorptiometry (DXA)

At age 17 yr, DXA measures were conducted using the Hologic QDR 4500A DXA (Delphi upgrade) with software version 12.3, in the fan-beam mode. A spine phantom was scanned daily to maintain quality assurance. To minimize operator-related variability, all measurements were conducted by one of two experienced technicians. The precision error for BMC measurements was low in our laboratory (coefficient of variation of < 1% for quality control scans performed daily using the Hologic phantom).

2.3.1. aBMD and Bone Mass Distribution—aBMD measures used in this study included aBMD of the total proximal femur and its regions: inter-trochanter, trochanter, and neck. The analysis of the scans was performed by software-specific Global Regions of Interest (ROI), which designate the general boundaries of the hip images. A review of the bone within the ROI box was confirmed by the operator and edited to ensure appropriate bone-edge detection. A specific femoral neck analysis was also performed to differentiate the aBMD of the neck between the superolateral and inferomedial sub-regions. This approach was published in a previous work (21). aBMD ratios among proximal femur regions were used as surrogates of bone mass distribution and calculated as follows:

$$TR|PF \text{ aBMD} = \frac{\text{Trochanter aBMD}}{\text{Proximal femur aBMD}}, \quad N|PF \text{ aBMD} = \frac{\text{Neck aBMD}}{\text{Proximal femur aBMD}},$$

$$IT|PF \text{ aBMD} = \frac{\text{Intertrochanter aBMD}}{\text{Proximal femur aBMD}}, \quad IM|SL \text{ aBMD} = \frac{\text{Inferomedial Neck aBMD}}{\text{Superolateral Neck aBMD}}$$

This approach provides measures of intra-individual relative difference of mineralization at the proximal femur regions (21).

2.3.2. Proximal Femur Geometry—Linear geometric measures were derived from hip DXA images using the Hip Structure Analysis program (Hologic Apex 3.0 software). The geometric measures used in this study included the hip axis length (HAL, cm), the narrow

neck width (NNW, cm), and the neck-shaft angle (NSA, degree). The HAL is the linear distance from the base of the greater trochanter to the edge of the pelvic inlet. The NNW is the narrowest distance across the femoral neck. The NSA is the angle between derived axes of neck and shaft. The HAL is calculated by the system from the lateral bone map edge of the greater trochanter to the edge of the pelvic inlet or to the Global ROI edge, whichever point is intersected first. Therefore, a strict protocol was followed for positioning the Global ROI edge in the same point of the edge of the pelvic inlet.

2.3.3. Leg LST—Leg LST (kg), was estimated from a total body scan by DXA and used as a surrogate of leg muscle mass. NHANES body composition method was used to analyze age 17 whole body scans obtained on Hologic QDR 4500 DXA (Delphi upgrade with software version 13.4).

2.4. Other measures

Research nurses trained in anthropometry measured the participant's height (cm) and weight (kg). Participant's height (cm) and weight (kg) were measured by a Healthometer physician's scale (Continental, Bridgeview, IL) and Harpenden stadiometer (Holtain, UK). Both devices were routinely checked for accuracy and precision. Children were measured while wearing indoor clothes but without shoes. Leg length (cm) was calculated as standing height minus sitting height. Peak height velocity (PHV) age was estimated using predictive equations established by Mirwald et al. (22) where height, weight, age, sex, sitting height, leg length, and weight by height ratio were predictors of somatic maturity. Since we had estimates at age 11, 13, and 15, as advised by Malina and colleagues, the PHV age estimates closest to a chronological age when measurements were done was used as the best estimate (23).

2.5. Statistics Analysis

Data were analyzed using SPSS statistical software package (version 24.0 for Windows; SPSS, Chicago, IL, USA). Distribution properties were examined using the Kolmogorov-Smirnov test. Differences between groups (females and males) for age, PHV age, body size, and composition, aBMD, aBMD distribution and specific geometric measures of proximal femur were analyzed by independent-samples t-test when normally distributed with equal variance and by independent-samples t-test with Welch correction when there was normality, but variances were not equal. Mann-Whitney nonparametric test was used when there was no normality.

To characterise patterns of physical activity over the study period, each participant's MVPA (approximate age 5, 8, 11, 13, 15, and 17 yr) was grouped within a pattern of conditional probabilities based on structural equation modelling theory that assumes individuals differ qualitatively as members of homogeneous (latent) subgroups (24). Individual-specific probabilities of belonging to each subgroup allowed assignment to a subgroup based on the highest probability. The relationship between MVPA and age was fitted up to a third-degree polynomial model that included the latent subgroup variable. All available data for physical activity were used. Under the assumption that data were missing at random, individuals with incomplete data were included with the requirements of at least three measurements per

participant over the study period. The best fitting polynomial model was determined by comparing the Bayesian Information Criterion (BIC) for models with different numbers of subgroups. SAS procedure TRAJ developed by Jones BL et al. was used for analysis.

Analyses of covariance (ANCOVA) were utilized for comparison of bone variables (aBMD, aBMD distribution, and specific geometric measures) between groups defined by MVPA trajectories for females and males. To reduce the confounding effects of body size and maturity on bone variables, the lower limb length, body weight, and PHV age were included as covariates. Normality and homoscedasticity of the residuals were checked, and there were no severe departures from these assumptions.

To examine whether leg LST mediated the relationship between MVPA trajectories and bone variables for females and males, a model for each bone outcome included MVPA trajectory group as an independent variable, and with leg LST as mediator. Lower limb length and body weight were added as covariates. Figure 1 depicts the overall mediation model used in the analysis. Mediation analyses were performed according to MacKinnon et al. approach (25), which advises that mediation can occur even with a lack of a significant relationship between the independent (X) and the dependent variable (Y). Briefly, these authors postulate that statistical test of the overall relation between X and Y can have less power than the test of the links in the mediation model in several circumstances. Before testing mediation, the *path a* (the effect of independent variable on the mediator) and *path b* (the effect of mediator on outcomes partialling out the effect of the independent variable) were quantified with unstandardized regression coefficients. Indirect effects through the mediator were analyzed only when both paths were significant. SPSS macro developed by Preacher and Hayes were used to test mediation (16). This tool tests the significance of total and specific indirect effects using the product-of-coefficients approach or by bootstrap procedures. Bootstrap procedures are favored since they do not require assumptions of normality of the sampling, improve power and reduce Type I error rates when compared with the product-of-coefficients approach (16, 26). We report results for both, product-of-coefficients approach and bootstrap procedures, with a resampling of 5,000 bootstrap samples (95% confidence intervals, 95% CIs). Finally, the mediated effects were described by calculating the effect ratios which express the amount of the total effect that is explained by the indirect effect via the mediator (26).

3. Results

Accelerometry data from ages 5 to 17 years were available for 524 participants (264 females) with at least 3 accelerometry assessments over time. The number of accelerometry assessments at each wave was 396, 498, 506, 460, 380 and 358 for wave 1 to 6, respectively. Combining proximal femur DXA data with physical activity latent groups we have 349 participants (191 females).

3.1. Participant characteristics

Table 1 describes the maturity status, body size and bone outcomes of the participants by sex at age 17 yrs.

3.2. Physical activity trajectories

The MVPA trajectories are presented in Figure 2-A (females) and Figure 2-B (males). For females, two latent group trajectories were identified: (1) relatively low MVPA over all study period with some decrease over time and (2) relatively high MVPA with some decreasing activity over time. The proportion of individuals in each group was 66.7% and 33.3%, respectively. On average, group 1 females participated in approximately 38 min/day of MVPA at age 5 which steadily declined to 20 min by age 17. Group 2 females participated in 55 min/day of MVPA at age 5, 61 min/day of MVPA at age 8, which declined to 31 min/day by age 17.

For males, three latent group trajectories were identified: (1) relatively low MVPA with decreasing MVPA levels over time; (2) moderately active with increasing MVPA levels during middle childhood followed by decreasing levels over time and (3) active with increasing MVPA levels during middle childhood followed by decreasing levels over time. The proportion of individuals in each group was 45.2%, 44.1%, and 10.7%, respectively. On average, group 1 males participated in approximately 46 min/day of MVPA at age 5, which declined steadily to 33 min/day by age 17. Group 2 males participated in 61 min/day of MVPA at age 5 and increased at age 8 to 76 min/day, before steadily declining to 35 min/day by age 17. Finally, group 3 males participated in 79 min/day of MVPA at age 5 and increased at age 8 to 105 min/day, before declining to 50 min/day by age 17. Due to a small sample size for males' group 3, group 2 and 3 were combined for further analyses and are referred as group 2 in tables and discussion.

3.3. Analysis of covariance

Leg LST and bone outcomes were compared for MVPA trajectory groups 1 (less active) and 2 (more active) by sex, adjusting for lower limb length, body weight and PHV age (Table 2) as estimated marginal means from ANCOVA models. Females and males in group 2 showed greater leg LST than group 1. Females in the group 2 had significantly greater aBMD at the total and all proximal femur regions than females in the group 1. Females in the group 2 had lower IM|SL neck aBMD (i.e., a more homogeneous aBMD distribution between the superolateral and inferomedial regions of the femoral neck) than females in the group 1. Similarly to females, males in the group 2 had significantly greater aBMD at the total and all proximal femur regions than males in the group 1. Males in the group 2 had longer HAL than males in group 1.

3.4. Mediation Analyses

The mediation analyses (depicted in Figure 1) are summarized in Table 3 for females and in Table 4 for males. There was a significant indirect effect of MVPA trajectory groups on aBMD of total and all proximal femur regions via leg LST in both females and males. The directions of the *path a* (see Table, Supplemental Digital Content, path a—females and males unstandardized regression coefficients of physical activity to leg LST) and *path b* (see Table, Supplemental Digital Content, path b—females and males unstandardized regression coefficients of leg LST to bone outcomes) were positive. The effect ratios show that leg LST explained 43–49% of the total effects of MVPA on proximal femur aBMD in females and

27–32% in males. Significant direct effects of MVPA trajectory groups on aBMD of total and all proximal femur regions were also observed, but only in males.

For aBMD distribution, an indirect effect of MVPA trajectory groups on TR|PF aBMD through leg LST was observed in females, although the total effect was not significant ($p=0.073$). The directions of the *path a* and *path b* were positive. In addition, an indirect effect of MVPA trajectory groups on IM|SL through leg LST was observed in both females and males, however, in males, the total effect was not significant ($p=0.105$). The direction of the *path a* was positive, while the direction of the *path b* was negative.

Finally, for specific geometric measures, we found an indirect effect of MVPA trajectory groups on HAL via leg LST in both, females and males, and on NNW only in males. The directions of the *path a* and *path b* were positive. However, for females HAL and males NNW, the mediation model was inconsistent as the direct effect of MVPA on the geometric measures was negative but not significant ($p=0.797$ and $p=0.823$ respectively). Mediation analyses were not performed for N|PF aBMD, IT|PF aBMD, NNW, and NSA in females and for N|PF aBMD, TR|PF aBMD, IT|PF aBMD and NSA in males, since *path b* was not significant for these variables.

4. Discussion

The purpose of this study was to examine the effect of MVPA trajectories on adolescent bone parameters including an analysis of the indirect (i.e., muscle-mediated) effects.

4.1. aBMD

Females and males who accumulated higher levels of MVPA across childhood and adolescence had greater aBMD at the total proximal femur and its regions at age 17 years when compared to less active peers. A significant indirect effect of MVPA on aBMD of the proximal femur through leg LST was observed in both females and males. The causal paths suggest that high levels of MVPA across childhood and adolescence increase muscle mass, which in turn increases the mechanical loading imposed on bone resulting in positive adaptations. However, muscles may also influence healthy adaptations via by secreting local growth factors that can stimulate osteogenesis (27). The leg LST explained a higher percentage of the total effects of MVPA on proximal femur aBMD in females than in males (43–49% vs. 27–32%). Moreover, a significant direct effect of MVPA on aBMD was observed in males but not in females. These findings emphasize the importance of muscle loading for bone health, particularly in females, since muscle appears to mediate a greater proportion of physical activity and bone relationship in females than males, despite their lower lean mass. This may be a possible explanation for girls having a more bone per unit of lean body mass compared to boys (11, 28). We do not know to what extent this better adaptation of the bone to the muscular forces in female sex with extra storage of calcium is a factor preventing the need for gestation and lactation (29). In practice, these findings provide an additional intervention avenue, focus on physical activities that increase muscle mass. Frost, in its Mechanostat theory, already highlighted the bone adaptation to muscle forces (6, 7). In accordance, the increase of momentary muscle forces as it occurs in physical activities with strong (des)accelerations of the body or with maximal-force muscle contractions, are

the ones with greater potential to augment muscle mass and bone strength (30). The results also suggest that in males, the muscle may not matter as much for health since they are getting more vigorous physical activity (i.e., more impact forces) than females (31). So muscle loading/strength training may be particularly important for youth who do not engage in impact activities.

4.2. aBMD distribution

In relation to aBMD distribution, an indirect effect of MVPA on the ratio between the inferomedial and the superolateral neck regions through leg LST was observed in both, females and males. Thus, the effect of physical activity via muscle may also contribute to more homogenous bone mass distribution between the inferomedial and the superolateral neck regions. This finding is noteworthy since a greater imbalance between these two regions can compromise the femoral neck structural integrity and, with aging, increases the risk for local buckling, particularly at the superolateral neck region (32). In fact, this latter region normally has a lower BMD and/or cortical thickness when compared with inferomedial neck region. To sum up, our findings indicate that a more uniform mineral distribution between the inferomedial and superolateral neck associated with higher levels of physical activity may contribute to the femoral neck resistance to fracture. Furthermore, the BMD at trochanter region has a critical role in the risk of intertrochanteric fractures later in life (33). The proportion of intertrochanteric fractures increases with age in women but not in men (34, 35) and appear to reflect a bigger bone loss at the trochanteric region with age in women (33). Therefore, the indirect effect of physical activity on the ratio between the trochanter and the total proximal femur through leg LST which we report for females may be important to prevent this specific type of fracture in older age.

4.3. Geometric measures

We report an indirect effect of physical activity on HAL via leg LST in females and males and on NNW only in males. The causal paths suggest that high levels of physical activity across childhood and adolescence increase muscle mass which in turn increase HAL and NNW. However, in females HAL and males NNW, the mediation models were inconsistent since the direct effect of physical activity on these geometric measures was negative, although insignificant. This finding suggests that in determining HAL for females and NNW for males, the absolute amount of muscle may be more important than its use (via physical activity). Nevertheless, physical activity appears to be determinant to increase muscle mass. Associations between leg LST and geometric measures were reported previously (36) but the information is scarce. Future studies should use computational approaches (e.g., finite element models) to understand better how mechanic loading from physical activity and muscle could explain the variation of HAL and NNW.

4.4. Limitations and strengths

This study has some limitations that should be acknowledged. First, our study includes cross-sectional (age 17 yr) DXA measures; it is possible that participants in the most active groups had greater bone parameters due to genetics, hormonal factors, or other factors that were not measured. Also, the participants of this study were drawn from a regional sample of children born in Iowa. Thus, caution should be used to the generalization of study results.

Second, we used leg LST as a surrogate of muscle mass with the assumption that muscle mass (size) indicates muscle strength (function). Although studies indicate positive associations between muscle mass and muscle strength (37), it is not clear whether increases in muscle mass necessarily parallel increases in muscle strength or vice versa. Third, the evaluation of the physical activity through accelerometry does not allow inferring about the muscular activity requested. However, the greater the volume or intensity of physical activity, the greater the muscle activity involved (38). There is evidence that more than the volume may be the pattern of accumulation of physical activity that presents greater osteogenic potential (39). Future studies should investigate the total volume versus bouts of physical activity in muscle health, particularly in girls, since muscle-bone interaction appears to be more determinant in girls' bone health than in boys. Considering the negative associations between sedentary behavior and bone health (40), it is also important to analyze whether this association is mediated by muscle mass/strength. Finally, although the point of our study was not to precisely estimate time spent in different physical activity categories, the necessary use of a one-minute epoch, due to the long (12-year) follow-up, would suggest an underestimate of MVPA particularly when our participants were children. Future research will limit analysis to ages 15 and older to exploit the advancement of accelerometry technology including the use of g as the unit (raw data) of analysis. An additional limitation associated with our long follow-up included the necessary use of three models of Actigraph.

Study strengths include our 12-year comprehensive assessment of physical activity using accelerometry and group-based trajectory modeling that provided an analytic method to effectively utilize multiple points of data assessed longitudinally and avoid pre-defined subgroups, e.g., increase/decrease/no change groups. An additional strength was our focus on the indirect effects of physical activity through muscle rather than inferring the muscle-bone unit. Our chosen bootstrapping approach tends to have good statistical power and is seen as a useful tool to avoid Type I error (26). In addition, bone mass distribution was estimated through aBMD ratios among proximal femur regions, rather than trying to estimate the bone mass distribution of complex three-dimensional bone cross section from 2D-DXA images. Our approach provided measures of intra-individual relative difference of mineralization at the proximal femur in the same plane of 2D-DXA images. Finally, the distinction between the inferomedial and the superolateral aspects of neck aBMD supplied additional insight.

5. Conclusions

To the best of our knowledge, this is the first study addressing the indirect effect (through muscle) of physical activity during growth on proximal femur bone outcomes. Our study highlights the importance to consider muscle and bone together as a functional unit to better understand the mechanisms of bone adaptation. More studies need to be conducted to clarify the mediator role of muscle in the relationship between physical activity and bone outcomes, not only as a mechanical factor but as an endocrine organ.

In conclusion, this study indicates that across childhood and adolescence a higher level of physical activity is associated with greater bone health of the clinically significant proximal femur. We also show that a significant portion of the effects of physical activity on the

proximal femur is mediated by muscle, specifically, a higher aBMD and a better aBMD distribution in both, females and males and a longer HAL in males. These results suggest that interventions of physical activity to improve bone health during childhood and adolescence should include activities that improve muscle mass and that these types of interventions may be particularly efficacious in females.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Overall mediation model. The model includes one independent variable (objectively measured moderate-and-vigorous intensity physical activity trajectories across childhood and adolescence), one mediating variable (leg LST), and dependent variables, (aBMD, aBMD distribution, and specific geometric measures of proximal femur)

Figure 2 -

Trajectories of moderate-and-vigorous intensity physical activity (minutes/day) across childhood and adolescence females (**A**), and males (**B**), based on latent group membership. Each line represents a percentage of the Iowa Bone Development Cohort which clustered within a discrete physical activity pattern.

Table 1 -

Description of maturity, body size and bone outcomes of participants at age 17.

	Females (n=191)		Males (n=158)		<i>p</i> -Value
	Mean	SD	Mean	SD	
Age (years)	17.5	0.4	17.5	0.4	0.387
PHV age (years)	11.7	0.6	13.7	0.7	<0.001
Body weight (kg)	67.1	17.1	78.4	17.1	<0.001
Height (cm)	165.6	6.6	178.7	7.5	<0.001
Lower limb length (cm)	79.6	4.4	87.1	5.1	<0.001
Leg lean soft tissue (kg)	7.0	1.3	9.9	1.8	<0.001
Proximal femur aBMD (g/cm ²)	1.020	0.138	1.132	0.158	<0.001
Trochanter aBMD (g/cm ²)	0.792	0.128	0.898	0.140	<0.001
Inter-trochanter aBMD (g/cm ²)	1.180	0.154	1.291	0.177	<0.001
Neck aBMD (g/cm ²)	0.922	0.134	1.028	0.160	<0.001
Superolateral neck aBMD (g/cm ²)	0.844	0.157	0.936	0.189	<0.001
Inferomedial neck aBMD (g/cm ²)	0.987	0.126	1.105	0.152	<0.001
N PF aBMD	0.904	0.050	0.908	0.057	0.897
TR PF aBMD	0.775	0.039	0.792	0.036	<0.001
IT PF aBMD	1.158	0.032	1.141	0.031	<0.001
IM SL aBMD	1.186	0.117	1.201	0.138	0.473
HAL (cm)	10.7	0.6	12.1	0.7	<0.001
NNW (cm)	3.2	0.3	3.6	0.3	<0.001
NSA (°)	131	5	133	4	0.002

N, Neck; PF, Proximal femur; TR, Trochanter; IT, Inter-trochanter; IM, Inferomedial; SL, Superolateral; HAL, Hip axis length; NNW, Narrow neck width; NSA, Neck shaft angle.

Table 2 -

Estimated Marginal Means (EMM) and Standard Error (SE) for leg lean soft tissue and bone outcomes (adjusted for lower limb length, body weight and PHV age) by MVPA trajectory group membership.

Females (n=191)	Group 1 (n=129)		Group 2 (n=61)		p-Value
	EMM	SE	EMM	SE	
Leg lean soft tissue (kg)	6.8	0.047	7.2	0.068	<0.001
Proximal femur aBMD (g/cm ²)	1.001	0.010	1.056	0.015	0.002
Trochanter aBMD (g/cm ²)	0.774	0.010	0.828	0.014	0.002
Inter-trochanter aBMD (g/cm ²)	1.160	0.011	1.218	0.017	0.005
Neck BMD (g/cm ²)	0.905	0.009	0.953	0.014	0.005
Superolateral neck aBMD (g/cm ²)	0.824	0.012	0.881	0.017	0.007
Inferomedial neck aBMD (g/cm ²)	0.972	0.009	1.013	0.013	0.011
N PF aBMD	0.905	0.004	0.903	0.006	0.869
TR PF aBMD	0.772	0.003	0.782	0.005	0.084
IT PF aBMD	1.160	0.003	1.155	0.004	0.300
IM SL neck aBMD	1.198	0.010	1.161	0.015	0.042
HAL (cm)	10.7	0.047	10.8	0.068	0.306
NNW (cm)	3.2	0.027	3.2	0.039	0.435
NSA(°)	132	0.399	130	0.583	0.070
Males (n=158)	Group 1 (n=68)		Group 2 (n=90)		p-Value
	EMM	SE	EMM	SE	
Leg lean soft tissue (kg)	9.7	0.092	10.0	0.080	0.027
Proximal femur aBMD (g/cm ²)	1.087	0.016	1.166	0.014	<0.001
Trochanter aBMD (g/cm ²)	0.859	0.015	0.928	0.013	0.001
Inter-trochanter aBMD (g/cm ²)	1.239	0.018	1.330	0.016	<0.001
Neck BMD (g/cm ²)	0.989	0.016	1.058	0.014	0.002
Superolateral neck aBMD (g/cm ²)	0.894	0.020	0.969	0.017	0.005
Inferomedial neck aBMD (g/cm ²)	1.068	0.016	1.134	0.014	0.002
N PF aBMD	0.910	0.007	0.906	0.006	0.636
TR PF aBMD	0.789	0.004	0.794	0.004	0.377
IT PF aBMD	1.141	0.004	1.141	0.003	0.972
IM SL neck aBMD	1.221	0.016	1.186	0.014	0.105
HAL (cm)	12.0	0.071	12.2	0.061	0.007
NNW (cm)	3.6	0.025	3.6	0.022	0.847
NSA (°)	133	0.582	133	0.504	0.756

Females Group 1: relatively low MVPA over all study period. Females Group 2: relatively high MVPA with some decreasing over time. Males Group 1: relatively low MVPA over the study period. Males Group 2: relatively high MVPA with increasing levels during middle childhood followed by decreasing levels. **N**, Neck; **PF**, Proximal femur; **TR**, Trochanter; **IT**, Inter-trochanter; **IM**, Inferomedial; **SL**, Superolateral, **HAL**, Hip axis length; **NNW**, Narrow neck width; **NSA**, Neck shaft angle.

Table 3.Females mediation analysis^a

	Coefficient	SE	Normal theory p	Bootstrap 95% CI	Effect Ratio	Model R ² (p)
Proximal femur aBMD						0.423 (<0.001)
Total effect (path c)	0.057	0.018	0.002	(0.021; 0.092)		
Direct effect (path c')	0.029	0.018	0.100	(-0.006; 0.064)		
Indirect effect (via mediator)	0.028	0.009	0.001	(0.013; 0.049)	0.490	
Trochanter aBMD						0.365 (<0.001)
Total effect (path c)	0.055	0.018	0.002	(0.020; 0.090)		
Direct effect (path c')	0.028	0.017	0.098	(-0.005; 0.062)		
Indirect effect (via mediator)	0.027	0.008	0.001	(0.013; 0.047)	0.484	
Inter-Trochanter aBMD						0.390 (<0.001)
Total effect (path c)	0.060	0.021	0.004	(0.019; 0.100)		
Direct effect (path c')	0.031	0.020	0.129	(-0.009; 0.071)		
Indirect effect (via mediator)	0.029	0.009	0.002	(0.014; 0.053)	0.484	
Neck aBMD						0.416 (<0.001)
Total effect (path c)	0.050	0.017	0.004	(0.016; 0.084)		
Direct effect (path c')	0.028	0.017	0.109	(-0.006; 0.061)		
Indirect effect (via mediator)	0.022	0.007	0.003	(0.010; 0.041)	0.447	
Superolateral Neck aBMD						0.339 (<0.001)
Total effect (path c)	0.059	0.021	0.007	(0.017; 0.101)		
Direct effect (path c')	0.034	0.021	0.118	(-0.009; 0.076)		
Indirect effect (via mediator)	0.025	0.009	0.005	(0.011; 0.047)	0.427	
Inferomedial Neck aBMD						0.439 (<0.001)
Total effect (path c)	0.042	0.016	0.009	(0.011; 0.073)		
Direct effect (path c')	0.021	0.016	0.173	(-0.009; 0.052)		
Indirect effect (via mediator)	0.020	0.007	0.003	(0.009; 0.036)	0.486	
TRIPF aBMD						0.094 (0.001)
Total effect (path c)	0.011	0.006	0.073	(-0.001; 0.023)		
Direct effect (path c')	0.006	0.006	0.333	(-0.006; 0.018)		
Indirect effect (via mediator)	0.005	0.002	0.026	(0.001; 0.011)	0.448	

	Coefficient	SE	Normal theory p	Bootstrap 95% CI	Effect Ratio	Model R ² (p)
IM/SL Neck aBMD						0.081 (0.004)
Total effect (path c)	-0.038	0.018	0.040	(-0.074; -0.002)		
Direct effect (path c')	-0.026	0.019	0.161	(-0.064; 0.011)		
Indirect effect (via mediator)	-0.011	0.006	0.073	(-0.027; -0.001)	0.297	
HAL						0.399 (<0.001)
Total effect (path c)	0.080	0.083	0.336	(-0.084; 0.244)		
Direct effect (path c')	-0.021	0.083	0.797	(-0.185; 0.142)		
Indirect effect (via mediator)	0.101	0.035	0.004	(0.046; 0.184)		

Coefficients are presented in the unstandardized form.

^aFor consistency with coefficients (and p values) for total and direct effects, coefficients and standard errors shown for indirect effects are relative to normal theory calculations. However, bootstrap 95% CIs are preferably interpreted in the text (see Material and Methods for more details). All models were controlled to lower limb length and body weight. **TR**, Trochanter; **PF**, Proximal femur; **IM**, Inferomedial; **SL**, Superolateral; **HAL**, Hip axis length.

Table 4.Males mediation analysis^a

	Coefficient	SE	Normal theory p	Bootstrap 95% CI	Effect Ratio	Model R ² (p)
Proximal femur aBMD						
Total effect (path c)	0.078	0.022	<0.001	(0.035; 0.121)		0.485 (<0.001)
Direct effect (path c')	0.055	0.019	0.005	(0.017; 0.093)		
Indirect effect (via mediator)	0.023	0.011	0.042	(0.003; 0.044)	0.291	
Trochanter aBMD						
Total effect (path c)	0.068	0.020	0.001	(0.029; 0.108)		0.435 (<0.001)
Direct effect (path c')	0.049	0.018	0.007	(0.013; 0.084)		
Indirect effect (via mediator)	0.020	0.010	0.044	(0.003; 0.040)	0.289	
Inter-Trochanter aBMD						
Total effect (path c)	0.089	0.025	<0.001	(0.040; 0.138)		0.459 (<0.001)
Direct effect (path c')	0.064	0.022	0.004	(0.020; 0.107)		
Indirect effect (via mediator)	0.025	0.012	0.043	(0.003; 0.050)	0.283	
Neck aBMD						
Total effect (path c)	0.067	0.022	0.003	(0.023; 0.110)		0.442 (<0.001)
Direct effect (path c')	0.047	0.020	0.023	(0.007; 0.087)		
Indirect effect (via mediator)	0.020	0.010	0.047	(0.003; 0.039)	0.301	
Superolateral Neck aBMD						
Total effect (path c)	0.074	0.026	0.006	(0.022; 0.126)		0.427 (<0.001)
Direct effect (path c')	0.050	0.024	0.042	(0.002; 0.098)		
Indirect effect (via mediator)	0.024	0.012	0.047	(0.004; 0.048)	0.326	
Inferomedial Neck aBMD						
Total effect (path c)	0.064	0.021	0.003	(0.022; 0.106)		0.396 (<0.001)
Direct effect (path c')	0.047	0.020	0.020	(0.007; 0.086)		
Indirect effect (via mediator)	0.018	0.009	0.051	(0.003; 0.034)	0.273	
IM/SL Neck aBMD						
Total effect (path c)	-0.034	0.021	0.105	(-0.076; 0.007)		0.222 (<0.001)
Direct effect (path c')	-0.022	0.021	0.294	(-0.062; 0.019)		
Indirect effect (via mediator)	-0.013	0.007	0.073	(-0.029; -0.002)	0.368	

	Coefficient	SE	Normal theory p	Bootstrap 95% CI	Effect Ratio	Model R ² (p)
HAL						0.409 (<0.001)
Total effect (path c)	0.248	0.097	0.011	(0.058; 0.439)		
Direct effect (path c')	0.165	0.091	0.073	(-0.015; 0.346)		
Indirect effect (via mediator)	0.083	0.041	0.040	(0.016; 0.172)	0.335	
NNW						0.358(<0.001)
Total effect (path c)	0.013	0.034	0.705	(-0.054; 0.080)		
Direct effect (path c')	-0.007	0.033	0.823	(-0.073; 0.058)		
Indirect effect (via mediator)	0.020	0.011	0.066	(0.004; 0.049)		

Coefficients are presented in the unstandardized form.

^aFor consistency with coefficients (and p values) for total and direct effects, coefficients and standard errors shown for indirect effects are relative to normal theory calculations. However, bootstrap 95% CIs are preferably interpreted in the text (see Material and Methods for more details). All models were controlled to lower limb length and body weight. **IM**, Inferomedial; **SL**, Superolateral; **HAL**, Hip axis length, **NNW**, Narrow neck width.