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The Influence of Organized Physical Activity (including Gymnastics) on Young Adult Skeletal Traits: Is Maturity Phase Important?

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Abstract

We prospectively evaluated adolescent organized physical activity (PA) as a factor in adult female bone traits. Annual DXA scans accompanied semi-annual records of anthropometry, maturity and PA for 42 participants in this preliminary analysis (criteria: appropriately timed DXA scans at ~1 year pre-menarche [predictor] and ~5 years post-menarche [dependent variable]). Regression analysis evaluated total adolescent inter-scan PA and PA over 3 maturity sub-phases as predictors of young adult bone outcomes: 1) bone mineral content (BMC), geometry and strength indices at non-dominant distal radius and femoral neck; 2) sub-head BMC; 3) lumbar spine BMC. Analyses accounted for baseline gynecological age (years pre- or post-menarche), baseline bone status, adult body size and inter-scan body size change. Gymnastics training was evaluated as a potentially independent predictor, but did not improve models for any outcomes ($p < 0.07$). Pre-menarcheal bone traits were strong predictors of most adult outcomes (semi-partial $r^2 = 0.21-0.59$, $p = 0.001$). Adult 1/3 radius and sub-head BMC were predicted by both total PA and PA 1-3 years post-menarche ($p < 0.03$). PA 3-5 years post-menarche predicted femoral narrow neck width, endosteal diameter and buckling ratio ($p < 0.05$). Thus, participation in organized physical activity programs throughout middle and high school may reduce lifetime fracture risk in females.

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Keywords

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Introduction

Bone may be more sensitive to loading during growth than in adulthood, yielding greater benefits in bone mass, geometry and strength for future fracture prevention (4). However, it is unknown whether bone loading benefits are equivalent throughout all growth stages, or whether loading during specific key maturity phases yields superior long-term benefits (4). Furthermore, it is unknown whether key maturity phases for loading adaptation differ by skeletal site and tissue type (cortical vs. trabecular) (25). This information is critical to the development of targeted physical activity programming for reduction of long-term osteoporosis and fracture risk (25).

Baxter-Jones et al. published a unique study that specifically associated prospectively, longitudinally tracked adolescent physical activity exposure with both young adolescent and adult bone mass (2). One year after peak height velocity (PHV), girls in the highest adolescent activity quartile demonstrated significant BMC advantages compared to girls in the lowest (total body) and lowest and middle two quartiles (lumbar spine). At adult follow-up, after adjusting for adolescent bone status (1 yr post-PHV), women who had been in the highest quartile for adolescent activity exhibited significant BMC advantages compared to those in the lowest (femoral neck) and the lowest and middle two adolescent activity quartiles (total hip) (2). These results provide prospective evidence that adolescent activity improves adult bone health, but do not evaluate the relative influence of activity exposure during specific maturity phases.

As reported by our research group and many others, gymnastics exposure during growth is a useful loading model, associated with advantages in DXA and pQCT indices of bone mass, geometry and skeletal strength (10,14-17,19,20,22). In this context, gymnastics exposure is often treated as a discrete variable, classifying subjects as gymnasts based on training levels above a specified threshold. In contrast, physical activity exposure can be assessed quantitatively, and analyzed as a continuous variable. Our group has previously evaluated organized physical activity participation (hours per week, h/wk) as a factor in bone development, identifying positive associations between physical activity and pre-menarcheal bone traits (9,34). The current analysis was designed to examine organized physical activity during specific adolescent maturity phases as a factor in young adult female bone traits, using observational, prospective, longitudinal records to quantify physical activity as a continuous variable. Based upon our prior work, we purposefully included gymnasts to expand the range of loading exposures. We used statistical methods to examine the explanatory value of: 1) average non-aquatic, organized physical activity exposure (PA) (h/wk) from ~1 year pre-menarche through ~5 years post-menarche, and 2) average PA exposure during peri-menarcheal (PERI), early post-menarcheal (POST1) and late post-menarcheal (POST2) maturity phases. Young adult bone traits served as the dependent variables of interest.

Because growth in bone area and mass appear to plateau 5 to 7 years after peak height velocity (PHV) (1), young adult bone traits were assessed at 4 to 5.5 years post-menarche (menarche occurs ~1 to ~2 years after PHV (3)). This scan timing is likely to capture traits at 94%-100% of peak bone mass and 95%-99% of peak bone area (1). Based on comparatively elevated bone accrual rates circum-menarche (approximately 0-2 years post-PHV) (1,28), we hypothesized that peri-menarcheal PA (~1 yr pre-menarche to 1 yr post-menarche) would provide the largest contribution to young adult bone traits, whereas the influence of PA dose beyond 1 year post-menarche would decrease progressively with increasing gynecological age (years pre- or post-menarche). Based on our prior cross-sectional evaluation of maturity-specific loading associations in gymnasts versus non-gymnasts, we hypothesized that the key phase for PA exposure would vary by skeletal site (forearm, hip, spine) and by predominant tissue (cortical versus cancellous) (14). Specifically, we expected associations with loading exposure to be greater **pre-menarche** for the femoral neck and **post-menarche** for most distal radius traits (14). To address these hypotheses within-subject, across adolescence, we evaluated a mature subset of our longitudinal cohort who provided activity data before menarche, through adolescence and into young adulthood.

In summary, for this preliminary analysis, we examined PA exposures during specific maturity phases, as continuous predictors of young adult bone mass and DXA-derived indices of bone geometry and theoretical strength, aligning data by maturity status and adjusting for baseline bone status and metrics of maturity and body size.

Methods

Study Design and Participants

The study protocol was approved by the Institutional Review Board at SUNY Upstate Medical University (5332F) and conducted in accordance with the Declaration of Helsinki. At enrollment, study participants provided written, informed assent, and parents/guardians provided written, informed consent for child participation. Upon attainment of age 18 years, participants provided written, informed consent to continue study participation. Hereafter, we refer to study participants as “subjects”, to avoid confusion with issues of activity participation.

The 42 subjects included in the current analysis are a subset from an ongoing longitudinal study of bone growth in relation to activity-specific loading exposure (1997-present), recruited from local gymnastics clubs (_{GYM}), local grade schools and girl scout troops (_{NON}). Subjects were originally enrolled in one of 2 cohorts: Cohort 1 (n= 82, 18-month pilot study (2 excluded due to high BMI)) and Cohort 2 (n= 40, 3-year longitudinal study). At enrollment, _{NON} were matched to _{GYM} by group for chronologic age, size, and physical maturity. _{NON} were originally enrolled as inactive peers, for comparison against _{GYM}. However, many _{NON} initiated and continued participation in variable levels of physical activity across adolescence; they were not sedentary, allowing for evaluation of organized physical activity at low to moderate exposures, compared to _{GYM}. Subjects underwent annual whole body and regional DXA scans (forearm, hip, lumbar spine), as well as semi-annual records of anthropometrics, PA and maturity (34). Because subjects were not originally enrolled for

extended longitudinal observation, considerable sample attrition occurred after the original pilot study periods (18 months, 3 years).

For the present analysis, we evaluated the influence of organized physical activity exposure on young adult bone. Thus, we required only two appropriately timed DXA scans for inclusion: a baseline pre-menarcheal scan and a young adult scan. Additional scans, obtained between baseline and young adult follow-up, were not evaluated due to inconsistent alignment of scan dates with gynecological age-based intervals. For determination of gynecological age, self-reported menarche date was queried semi-annually until positive; gynecological age (GA) was calculated as years pre-menarche (GA = negative number) or post-menarche (GA = positive number); menarche was defined as $GA = 0$. Inclusion criteria were as follows: 1) Achievement of menarche; 2) DXA scans and complete accompanying data for a time-point falling between GA of -2.0 and -0.5 years (pre-menarche baseline scan= $DXA1$, $\sim ga-1$) and a time-point falling between GA of $+4.0$ and $+5.5$ years (post-menarcheal young adult scan= $DXA2$, $-GA5$); 3) semi-annual PA records available for the inter-DXA interval. Thus, many subjects were excluded from this analysis due to pre-menarcheal status, mistimed scans or missing data, and one subject was excluded due to thyroid cancer. This yielded a $TOTAL\ n = 42$ for the current analysis. Comparisons of baseline data for included subjects ($n = 42$) versus excluded study enrollees ($n=80$) from the same cohorts detected no systematic differences for Tanner breast stage, race, ethnicity, age, height, mass, percent body fat, whole body (or sub-head) BMC and whole body (or sub-head) lean mass (chi square and Mann Whitney U $p > 0.14$).

Although evaluation of gymnastic exposure was **not** the focus of this analysis, to evaluate the possibility that gymnastic PA may have a separate effect from non-gymnastic PA, we evaluated adolescent gymnastic training as a separate predictor. Based on observed skeletal advantages in our prior work (14), GYM were defined by mean gymnastics participation of 5.0 hours/week (h/wk) within any of the three maturity-based intervals; NON included all others. Accordingly, some girls originally recruited as GYM were re-categorized as NON for this analysis. due to inadequate gymnastics exposure during the inter-DXA interval. None of the NON group experienced sufficient gymnastic training exposure to require reclassification. This resulted in $GYM\ n=17$, $NON\ n=25$ for the current analysis.

Physical Activity (PA) Quantification for Total and Maturity-Specific Time Periods

Activity records were used to generate monthly means for organized activity (hours per week, h/wk); aquatic activity was excluded based on studies indicating that aquatic activities are not osteogenic (5,7,18,23,27,29,30,32). For all subjects, semi-annual queries were made, generating activity-specific records of organized PA (h/wk) over the entire inter-DXA interval. These records documented training for sports and other organized PA (e.g. dance), as well as periods of illness/injury/vacation 1 week and associated activity modifications. Habitual non-organized activity was not quantified, nor was the vigorousness of individual activity bouts. Similar methodology was validated in this cohort by comparison to gymnastics coach daily training logs ($r > 0.97$, $p < 0.001$) (16). Subsequently, monthly means for total, organized, non-aquatic PA (hours/week, including gymnastics) were used to calculate means across the complete adolescent inter-scan interval ($TOTALPA$, $\sim GA-1$ to $GA5$), and

for specific phases of interest: peri-menarcheal ($PERI, \sim GA-1$ to $GA1$), early post-menarcheal ($POST1, GA1$ to $GA3$) and late post-menarcheal ($POST2, GA3$ to 'adult' $DXA2 (-GA5)$). Each specific maturity phase PA variable covered approximately two years of activity, yielding approximately 6 years of total adolescent exposure.

Densitometry

Postero-anterior DXA scans were performed for whole body, lumbar spine, left proximal femur and non-dominant forearm. Most scans used a Hologic QDR4500W DXA scanner (92%); a few $DXA2$ used a cross-calibrated Discovery A scanner (7/42 $DXA2$). All scans were performed per study protocol by one of two certified DXA technologists and re-analyzed by a single investigator (JD) using Apex software (Hologic Discovery A, software v.12.7.3, Waltham, MA, USA). Bone outcomes were chosen based upon previous studies, in which gymnastic loading associations were demonstrated for these outcomes at these sites (12,14,15), and/or based on strong agreement with pQCT indices of bone strength in this cohort (11).

Bone mineral content of sub-head (subBMC, g), PA lumbar spine vertebra 3 (L3BMC, g), and femoral neck (FNBMC, g) regions of interest were assessed using standard protocols. Hip structural analysis (HSA, Hologic) assessed the narrow neck of the proximal femur (NN), yielding section modulus (NNZ, cm^3), width (NNWidth, cm), endosteal diameter (NNED, cm), cortical thickness (NNCT, cm), and buckling ratio (NNBR). For the non-dominant forearm, the most distal reference line of the analysis box was positioned distal to the ulnar border of the radial articular surface; radius-specific positioning ensures consistent scan analyses within and between subjects across development (35). Ultradistal (UD) and 1/3 radius BMC (g) were evaluated. Indices of bone geometry and theoretical strength were derived using formulae adapted from Sievänen et al. (36), validated and refined by our group (11,13), yielding UD and 1/3 width (cm), 1/3 Z (cm^3) and UD axial compression strength index (UDIBS, g^2/cm^4).

DXA coefficients of variation (CVs) were calculated using duplicate scans of approximately 30 middle-aged females (Discovery A scanner). For whole body, 1/3 and UD radius BMC, and radius widths, CVs were <1%. For PA lumbar spine BMC, CVs were <2.0%. Femoral neck BMC and NN HSA CVs were <3.5%, with two exceptions: NN BR (4.1%) and NN Z (7.2%). Aside from NN Z, our CVs were lower than or similar to those reported in other studies using FN and HSA data (24,31).

Statistical Analysis

Continuous data were evaluated for normality. Appropriate transformations were performed in cases of non-normality (weight, sub-head fat mass, $DXA1$ L3BMC), with ln-transformation performed in pre/post pairs as needed (repeated measures ANOVA). Although not normally distributed, $POST1$ PA and $POST2$ PA were analyzed in raw form (distributions were not improved by ln-transformation). A 2-tailed alpha of < 0.05 was considered statistically significant. We used repeated measures analysis of variance (RM ANOVA) to calculate descriptive statistics for unadjusted non-bone characteristics and bone outcomes for the two scan dates ($DXA1$, $DXA2$). To evaluate potential self-selection bias for gymnastic participation, we also evaluated

univariate results for raw differences between activity groups (RMANOVA_{GROUP} effect: GYM versus NON, maturity phases pooled), change across time in the total sample (RMANOVA_{MATURITY} effect: activity groups pooled) and variability in “growth” rate between activity groups (RMANOVA interaction: GROUP × MATURITY). To evaluate group differences in physical activity dose, we performed univariate ANOVA comparing activity group means across the total adolescent period and for each maturity phase (TOTPA; PERI, POST1, POST2), as the repeated measures design (DXA1, DXA2) did not apply.

Our main purpose is to examine associations between adolescent PA exposure, analyzed as a continuous variable, and young adult skeletal status. We accounted for baseline pre-menarcheal bone status (DXA1) to evaluate whether bone traits simply tracked during growth, or whether adolescent PA contributed to young adult bone status (bone outcomes observed at DXA2). This adjustment accounted statistically for unmeasured factors in early childhood bone growth (e.g. genetics, diet and prior loading), allowing us to focus on the inter-DXA, adolescent PA period. As defined earlier, PA exposure dose was evaluated as: 1) TOTALPA, mean adolescent PA over the entire study period (DXA1 to DXA2, GA−1 to GA+5); 2) and as three distinct, maturity-specific phases within the study period. Maturity phases lasted ~2 yrs: PERI= DXA1 to GA+1 (baseline pre-menarcheal DXA to 1 yr post-menarche); POST1= GA+1 to GA+3; POST2= GA+3 to DXA2 (young adult). In contrast, bone outcomes were evaluated at a single time point (DXA2: adult ~5 years post-menarche), using pre-menarcheal baseline status (DXA1) as a predictor of adult bone status. As noted earlier, intervening scan data were not evaluated due to maturational timing issues. All 42 subjects contributed both bone outcome and physical activity information for the entire six year period of this analysis.

Total Adolescent PA Exposure, TOTALPA (mean for adolescent inter-scan interval)

Regression models were constructed to evaluate associations between TOTALPA and bone outcomes. In addition to the variable for TOTALPA, each bone outcome model included: 1) pre-menarcheal baseline gynecological age (DXA1 GA); 2) the baseline bone outcome (DXA1 bone status); 3) young adult height or forearm length (DXA2 body size index) and 4) change in height or forearm length from DXA1 to DXA2 (change in body size index). For body size index, forearm length was used for radius outcomes; height was used for all others. Whole-group analyses were performed, but potential gymnast selection bias and specificity to gymnastic training were also evaluated by adding gymnast status to each of these models as a dichotomous variable (no/yes). For models with significant TOTALPA associations, we report adjusted model r^2 and significance, also reporting beta coefficients with 95% confidence intervals and accompanying p values. For adjusted relationships between bone outcomes and predictors of interest, individual variable significance and semi-partial correlation coefficients (SPCC) were evaluated; SPCC were used as a measure of independent effect size r and squared to yield independent % variance explained.

Maturity-specific PA exposure- PERI, POST1, POST2

We used a partial correlation matrix to independently evaluate the association between young adult bone status (the dependent variables for DXA2) and PA dose during each of the three maturity-specific activity phases; each continuous maturity phase PA variable was assessed separately in this preliminary screening step. As in the TOTALPA regression analyses,

partial correlations were adjusted for: baseline GA , baseline bone outcome, $DXA2$ body size index and change in body size index from $DXA1$ to $DXA2$. We selected the activity phase with the greatest, significant partial correlation coefficient for regression modeling, entering the same predictors as noted for $TOTALPA$ followed by the maturity-specific PA dose for the most predictive maturity phase ($PERI$, $POST1$, $POST2$). Again, potential specificity to gymnast status and/or gymnastic training exposure was evaluated by adding gymnast status as a dichotomous variable to each of the constructed regression models.

Results

Unadjusted subject characteristics are presented in Table 1 for data collected at pre-menarcheal baseline ($DXA1$) and young adult ($DXA2$) scan sessions. Overall, at baseline, the mean (sd) gynecological age was -1.3 (0.3) yrs, with a range from -2.0 to -0.8 yrs; at $DXA2$, the mean (sd) gynecological age was $+4.9$ (0.3) yrs, with a range from $+4.2$ to $+5.5$ yrs. No significant gynecological age differences were observed based on gymnastic exposure. Subjects participated in a wide variety of organized physical activities at a wide range of exposures (h/wk); the range of *maximum* h/wk participation for each activity is depicted in Figure 1. For all three maturity phases, PA was higher for GYM than NON ($p < 0.001$) (Table 2). In general, PA decreased with increasing maturity ($p < 0.001$), although this was primarily a result of decreasing PA in the GYM group, with NON PA remaining fairly stable across adolescence ($GROUP \times MATURITY$ interaction, $p < 0.001$).

Subject characteristics for unadjusted non-bone traits

As expected due to growth, all non-bone traits increased from $DXA1$ to $DXA2$ ($p < 0.001$) (Table 1). With the exception of sub-head fat mass, there were no group differences between GYM and NON for unadjusted non-bone traits, and no significant $GROUP \times MATURITY$ interactions. Sub-head fat mass tended to be lower in GYM than NON ($GROUP$ main effect $p < 0.06$), and increased faster in GYM than NON ($p < 0.05$, $GROUP \times MATURITY$ interaction).

Subject characteristics for unadjusted bone measures

All bone measures increased with $MATURITY$ ($p < 0.001$), except $NNBR$, which decreased, indicating increasing bone strength, ($p < 0.001$) and $NNED$, which showed a positive trend, ($p < 0.07$) (Table 1). Compared to NON , GYM demonstrated greater $1/3BMC$, $1/3width$, $1/3Z$, $UDBMC$, $UDwidth$, $UDIBS$ and $NNCT$, but lower $NNwidth$, $NNED$ and $NNBR$ ($p < 0.04$). No differences were detected for $FNBMC$, NNZ , $subBMC$ or $L3BMC$ GYM status ($p < 0.28$). Growth rates were higher in GYM than NON for $1/3Z$ only, and $NNBR$ decreased more rapidly in NON than GYM ($p < 0.03$, $GROUP \times MATURITY$ interactions). There was a trend toward a higher GYM growth rate for $1/3BMC$ ($p = 0.06$).

Primary Analyses, PA Exposure Regression Models, Partial and Semi-Partial Correlations

Total Adolescent PA Exposure, $TOTALPA$ (Table 3a)—Regression models were constructed for prediction of all “adult” bone outcomes at $DXA2$, but results are only presented for models with significant $TOTALPA$ explanatory value (Table 3a). After adjusting for $DXA1$ GA , $DXA1$ bone status, $DXA2$ body size and change in size, $TOTALPA$ predicted only $1/3$ radius BMC and $subBMC$ (Table 3a), explaining approximately 10% (medium effect, $p = 0.002$) and 4%

of variance (small effect, $p < 0.03$), respectively (squared semi-partial correlation coefficients). TOTAL PA associations with DXA2 bone status were not detected for any other bone outcomes ($p = 0.10$). Overall, DXA1 bone status was a significant predictor of all adult bone traits, explaining 21% to 35% of variance for most outcomes ($p = 0.001$) [higher: 1/3 Z (48%, $p = 0.001$) and 1/3 width (59%, $p = 0.001$); lower: subBMC (3%, $p < 0.05$), NNwidth (8%, $p < 0.03$), NNED (14%, $p = 0.003$), NNZ (17%, $p = 0.001$). In TOTAL PA regressions, adolescent gymnastic status (no/yes) did not predict DXA2 bone outcomes (SPCC $r = 0.11$, $p = 0.23$).

Maturity-Specific PA Models: PERI, POST1, POST2 (Table 3b, Table 4)—Inter-correlation was detected for maturity-specific PA between most phases (PERI VS. POST1 Spearman's $\sigma = 0.67$, $p < 0.0001$; PERI VS. POST2 $\sigma = 0.18$, $p = 0.268$; POST1 VS. POST2 $\sigma = 0.41$, $p = 0.006$). After adjusting for influential covariates and baseline status, each maturity-specific PA phase was associated with at least one adult bone index (Table 4): PERI (1/3 radius BMC, $p = 0.005$), POST1 (1/3 BMC, $p = 0.002$; subBMC, $p < 0.05$) and POST2 (NNwidth $r = -0.34$, NNED $r = -0.35$, NNBR $r = -0.32$, $p < 0.05$). Trends ($p = 0.08$) were observed for associations between POST1 PA and 1/3Z ($r = 0.28$) and POST2 PA vs. both 1/3 and subBMC (respectively: $r = 0.30, 0.31$).

Regression results are only reported for significant PA associations ($p < 0.10$) and for the strongest PA association at each site (Table 3b). Thus, L3BMC results are not reported in the table (height change and DXA1 L3BMC explained 6% and 30% of variance, $p = 0.029$, $p < 0.0001$; no significant PA associations). For select 1/3, sub-head and narrow neck bone outcomes, post-menarcheal activity exposure explained 3-10% of variance in young adult bone status (small to medium effect sizes, SPCC r). Similar to TOTAL PA models, DXA1 bone status predicted DXA2 bone at the narrow neck and 1/3 radius (NNwidth (8%), NNED (14%), NNBR (33%), 1/3 BMC (34%), $p = 0.03$). However, DXA1 bone status explained little variance for subBMC (5%, $p < 0.02$), as DXA1 gynecological age (10%, $p = 0.001$) and adult (DXA2) height (14%, $p < 0.001$) claimed considerable explanatory value. Entry of maturity-specific gymnast status did not improve models for any outcomes ($p > 0.07$, negligible or negative change in model adjusted r^2).

Discussion

This preliminary analysis suggests that higher doses of non-aquatic organized activity participation may provide substantial skeletal benefits to females during adolescence. Both long-term activity associations and maturity-specific activity trends suggest that greater participation in non-aquatic, organized activity may yield young adult bone mass benefits for 1/3 radius and sub-head BMC, across all maturity phases evaluated (peri, early post- and late post-menarche). Furthermore, maturity-specific activity participation was associated with site-specific adult bone advantages: early post-menarcheal PA with 1/3 BMC and sub-head BMC advantages; late post-menarcheal PA with NNwidth, NNED and NNBR advantages. Strong trends were observed for maturity-specific PA advantages for other site-specific bone traits. Our hypothesis that high earlier activity exposure doses (peri-menarche) would yield greater bone benefits than high doses during later maturation was **not** supported. However, our hypothesis that phases of greatest influence would vary by site and outcome **was** supported by partial correlation results. POST1 PA may be most influential for outcomes that primarily reflect cortical bone mass (1/3 radius BMC, sub-head BMC). In

contrast, $_{POST2}$ PA may be more influential at the femoral narrow neck, indicating lower periosteal and endosteal expansion with greater loading exposure. Alternatively, it is possible that waning gymnastics participation during $_{POST2}$, with associated reduction in upper extremity and total body loading, explains the lack of an association between $_{POST2}$ PA and bone outcomes at other sites (1/3 radius and sub-head). Nonetheless, despite greater levels of gymnastics participation in early adolescence than late adolescence, we did not observe significant associations between early adolescent PA and femoral neck outcomes; these findings support our conclusion that promotion of femoral neck skeletal strength via PA exposure may be most effective during late adolescence.

Adult lumbar spine vertebra 3 BMC did not exhibit significant associations with activity dose during any maturity phase; it tracked from pre-menarche, increasing as a function of statural growth. Although not specifically evaluated here, in a prior analysis, we reported significantly greater vertebral height in pre- and post-menarcheal non-gymnasts versus ex/gymnasts, using post-menarcheal paired postero-anterior and supine lateral (PALAT) DXA data from many of the current subjects (15). Similarly, other researchers have reported shorter growth in sitting height in gymnasts than non-gymnasts (6). Thus, in the current analysis, it is possible that greater adolescent growth in vertebral height among $_{NON}$ versus $_{GYM}$ masked activity dose associations in postero-anterior L3BMC, although no significant effect of gymnast status was observed ($p>0.40$). Unfortunately, paired PALAT scans were not available at both time points to address this question in the current analysis. Lack of significant findings could also be related to low statistical power in this preliminary analysis. Future analyses of our younger cohort of maturing subjects ($n>70$) will increase available sample size for L3BMC analyses and use paired PALAT DXA data to detail associations between adolescent loading and vertebral bone mass, geometry, density and theoretical strength.

Strong tracking was observed for most radius, femoral neck and lumbar spine outcomes from pre-menarche to adulthood, suggesting that ~20% to 60% of variance in peak adult bone properties may be established before menarche, at clinically relevant sites. Although tracking may indicate a strong genetic component to lifetime skeletal status for some outcomes, only 5% of variance in sub-head BMC and <20% of variance in narrow neck outcomes were explained by $_{DXA1}$ status. Alternatively, high $_{DXA1}$ status explanatory value may indicate that pre-menarcheal dietary factors and/or loading build the critical foundation for later bone development. The latter concept has been supported by evidence of significant residual benefits attributed to pre-menarcheal loading, 4 to 14 years after training cessation, for indices of radius bone mass, geometry, density (35) and radius/tibia bone mass, geometry, density and theoretical strength (21). Although not focal to this analysis, overall $_{GYM}$ advantages were indicated by $_{RMM}$ ANOVA for unadjusted bone status (most outcomes and sites); however, $_{GYM}$ status was not a significant predictor in regression models. This indicates that $_{GYM}$ advantages were already well-established before menarche, and additional advantages were associated with total adolescent activity rather than specifically with adolescent $_{GYM}$ exposure. These findings may also be related to low participation in gymnastic training (few subjects) throughout adolescence into later maturity phases (Table 2).

Femoral narrow neck periosteal and endosteal expansion did not appear to track strongly from pre-menarche to post-menarche, and may remain malleable late in adolescent development for reduction of fracture risk (lower buckling ratio). Interestingly, PA associations with sub-head BMC also suggest that overall bone mass can still be modified after menarche. It is possible that these outcomes reflect relative estrogen exposure or luteal phase sufficiency (progesterone exposure), which were not measured in this prospective longitudinal study. Alternatively, low predictive value of $_{DXA1}$ narrow neck section modulus (Z) for adult NNZ may reflect relatively low reproducibility for this outcome (CV= 7.0%, adult repeated measures). However, NNED and NNwidth CVs were <3.0%, and they followed a similar pattern to NNZ.

Our results indicate that inclusion of non-elite gymnasts in studies of adolescent physical activity dose, as a factor in bone growth, extends the range of activity exposure without incurring a significant bias based on group membership or activity type. In addition, because gymnastic training is often discontinued peri-menarche, inclusion of gymnasts in physical activity analyses provides a means to evaluate physical activity exposure that is concentrated in pre- and peri-menarcheal phases of development, contrasting with other organized activities which appear to yield steady, or even increasing, levels of participation throughout adolescence.

Limitations

This preliminary analysis evaluated a small portion of our existing cohort, including only girls for whom appropriate data was available; thus statistical power was limited. Furthermore, because a large number of predictors and outcomes were evaluated, 2.8/56 significant associations would be expected due to chance alone ($p= 0.05$); overall, 8/56 significant associations were detected. In site-specific terms, due to a greater number of tests per site, femoral neck and radius associations are most likely to be due to chance alone (1.2/24 spurious associations predicted due to chance for each bone). Accordingly, our preliminary findings should be confirmed in a larger sample using more stringent alpha criteria.

Accelerometry was not used to objectively quantify activity or vigorousness of individual exercise bouts, because extended periods (>6 years) of year-round accelerometry are not feasible (participant burden, logistics). Because there is substantial seasonal variability in activity exposure among females in this maturity range, year-round activity quantification is important; thus, annual “snapshots” of weekly accelerometry are unlikely to reflect total annual activity dose (8). Accordingly, we specifically evaluated reported organized, non-aquatic physical activity in order to: 1) reflect long-term loading exposures; 2) account for seasonal loading variability; 3) quantify exposure to vigorous activities likely to confer skeletal advantages. We believe that this practice is superior to the use of single annual accelerometry sessions to quantify osteogenic activity exposure. Future research is necessary to evaluate this premise.

Although activity data were accumulated prospectively with bone data, DXA scans were not timed by gynecological age (menarche date unknown at enrollment). Thus, variation

between subjects in interval length and timing may have influenced results, as scans were only roughly aligned by gynecologic age. Use of scans timed between 4 and 5.5 years post-menarche was intended to represent young “adult” bone outcomes, but it is possible that some subjects were still experiencing growth in bone area and accrual of bone mass when these scans were obtained.

Most of our models left substantial variance unexplained (30-60%); this may reflect hormonal, nutritional and other factors that were not evaluated in this analysis. Recent evidence suggests that bone traits are linked to blood levels of vitamin D, phosphorus and calcium in adolescent females (33), while vitamin C and zinc intakes have been linked to bone traits in younger girls (26). Future analyses should investigate the potential influence of habitual intakes of key macro- and micro-nutrients (e.g. protein and calcium) on bone characteristics.

Finally, this study was observational in design. Activity varied spontaneously, and exposure was not specifically maturity-limited. Many subjects participated in gymnastics and other organized, non-aquatic activities for years prior to the baseline pre-menarcheal scan. Thus, benefits may have already been attained before baseline, with continued high loading building on this pre-existing platform. We also cannot discriminate between pre-existing benefits of childhood loading exposure, childhood nutritional status and subjects’ innate genetic potential. In the current analysis, adolescent activity may add to adult bone traits, and/or prevent deterioration of existing pediatric activity advantages. In the latter scenario, all girls would gain bone at a similar rate throughout adolescent growth, but low post-menarcheal activity would cause loss of pre-existing benefits, while high activity would maintain pre-existing benefits. Regardless of the precise mechanism, our findings suggest that organized, non-aquatic physical activity participation beyond menarche yields dose-dependent adult bone benefits at clinically relevant sites.

Conclusions

Although adult bone properties are well-explained by factors present before menarche, late adolescent, organized non-aquatic activity is associated with measurable advantages in whole body and radius bone mass, as well as indices of femoral neck structure and strength. These preliminary findings suggest that for females, participation in organized physical activity programs throughout middle school and high school may optimize adult bone health, reducing lifetime fracture risk.

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Figure 1. Organized Physical Activity: Range of Maximum Hours per Week Participation

Bars depict a range of participation in various organized physical activities, reported in maximum hours per week (h/wk). Maximum h/wk were calculated as mean participation over specific periods during the 6 years of observation, such as seasons, which had variable lengths, ranging from several weeks to many months. Many subjects participated in more than one activity during a given season, year and/or over the 6 year period. Participant numbers for each activity are noted in parentheses, activity (#).

Table 1

Subject Characteristics, ANOVA Results (Repeated Measures Where Applicable)

NON-BONE VARIABLES	DXA1	DXA2	BONE VARIABLES	DXA1	DXA2
Age at Menarche (yrs)	13.3 (1.2)		Sub-head BMC (g)	1002.8 (201.2)	1666.8 (212.4) ^C
Chronologic Age (yrs)	12.0 (1.3)	18.3 (1.3) ^C	1/3 Radius BMC (g)	1.25 (0.22)	1.81 (0.27) ^C
Gynecologic Age (yrs)	−1.3 (0.3)	4.9 (0.3) ^C	1/3 Radius Width (cm)	0.111 (0.013)	0.130 (0.015) ^C
Height (cm)	148.5 (7.8)	163.9 (7.6) ^C	1/3 Radius Z (cm ³)	0.287 (0.109)	0.506 (0.210) ^C
Forearm Length (cm)	22.4 (1.5)	25.1 (1.3) ^C	UD Radius BMC (g)	0.96 (0.25)	1.55 (0.26) ^C
*Ln Weight (kg)	39.0 (1.2)	57.7 (1.2) ^C	UD Radius Width (cm)	0.184 (0.020)	0.215 (0.020) ^C
Body Mass Index (kg/m ²)	17.8 (1.8)	21.7 (2.6) ^C	UD Radius IBS (g ² /cm ⁴)	0.152 (0.056)	0.290 (0.071) ^C
*Ln Sub-Head Fat Mass (kg)	7.4 (1.5)	13.5 (1.4) ^C	Femoral Neck BMC (g)	3.06 (0.42)	4.41 (0.46) ^C
Sub-Head Lean Mass (kg)	26.3 (4.0)	36.8 (4.7) ^C	NN width (cm)	2.56 (0.19)	2.74 (0.22) ^C
TOTAL PA (h/wk)	6.8 (4.2)		NN ED (cm)	2.21 (0.19)	2.26 (0.24) ^(0.069)
PERI PA (h/wk)	8.3 (6.4)		NN CT (cm)	0.18 (0.02)	0.24 (0.03) ^C
POST1 PA (h/wk)	7.2 (4.9)		NN Z (cm ³)	0.85 (0.18)	1.33 (0.21) ^C
POST2 PA (h/wk)	5.6 (4.8)		NN BR	7.68 (1.28)	5.99 (0.96) ^C
			*Ln L3 BMC (g)	8.34 (1.24)	15.54 (1.15) ^C

Mean (sd).

a = p<0.05;

b = p 0.01;

PA= reported, non-aquatic, organized physical activity exposure (h/wk);

TOTALPA= mean PA over DXA1 to dxa2 interval (~1 yr pre-menarche to ~5years post-menarche);

PERI= ~1 yr pre-menarche to 1 yr post-menarche; POST1= 1 to 3 yrs post-menarche; POST2= 3 to ~5 yrs post-menarche;

BMC= bone mineral content; Z= section modulus; IJD= ultradistal; IBS= index of structural strength in axial compression;

NN= femoral narrow neck, ED=endosteal diameter; CT= cortical thickness, BR= buckling ratio, L3= lumbar spine Vertebra 3

* Ln= Ln-transformed, analyzed and back-transformed.

^C p<0.001.

Table 2

Physical Activity Summary

Maturity Range (by GA)	Non-Aquatic, Organized Physical Activity (h/wk)			Gymnastics Training (h/wk) *	
	ALL	GYM [n=17]	NON [n=25]	GYM	NON/EXGYM
PERI PA (DXA1 to <GA1)	8.3 (6.4)	14.4 (4.0)	4.2 (3.8)	12.6 (4.5) [n=17]	0.7 (0.3) [n=2]
POST1 PA (GA1 to <GA3)	7.2 (4.9)	9.4 (5.7)	5.6 (3.7)	12.6 (5.7) [n=7]	3.4 (1.3) [n=6]
POST2 PA (GA3 to DXA2)	5.6 (4.8)	6.8 (6.5)	4.8 (3.1)	17.4 (4.5) [n=3]	0.6 (0.5) [n=5]
TOTAL PA (DXA1 TO DXA2)	6.8 (4.2)	9.7 (4.3)	4.8 (2.7)	7.7 (5.2) [n=17]	0.4 (0.3) [n=2]

Mean (sd). GYM trained 5 h/wk for at least one maturity phase.

GA= Gynecological Age (years relative to menarche (menarche= GA0)); DXA1= Baseline pre-menarcheal DXA, ~1 year pre-menarche (ga -2.0 to -0.8 yrs); DXA2= Adult post-menarcheal DXA, ~5 years post-menarche (ga +4.2 to +5.5 yrs).

For TOTALPA, GYM > NON, ANOVA $p < 0.001$. **BOLD type indicates greater group means.**

Repeated Measures ANOVA indicated:

- 1) GROUP main effect ($p = 0.001$), indicating greater activity in GYM than NON;
- 2) MATURITY main effect ($p = 0.001$), indicating decreasing activity level across time;
- 3) However, GROUP X MATURITY interaction ($p = 0.001$), thus activity decrease limited to GYM.

For Gymnastics hours, although groups differed by design, statistical tests were not run due to cell size discrepancies for most comparisons.

* **Note decreasing “n” for Gymnastics Training with increasing Maturity Range.**

Table 3

Partial Correlation Results for Maturity-specific PA Phases vs. Adult Bone Outcomes

Partials Adjusted For:	DXA2 BONE OUTCOME	PERI PA	POST1 PA	POST2 PA
DXA1 GYNAGE DXA1 Bone Status DXA2 Forearm Length Change in Forearm Length	1/3 Width (cm)	0.16	0.22	0.21
	1/3 BMC (g)	<i>0.45^b</i>	0.49^b	0.30 (p<0.07)
	1/3 Z (cm³)	0.20	0.28 (p<0.09)	0.19
	UD Width (cm)	0.09	0.19	0.22
	UD BMC (g)	0.24	0.19	0.22
	UD IBS (g²/cm⁴)	0.23	0.12	0.10
DXA1 GYNAGE DXA1 Bone Status DXA2 Stature Change in Stature	Sub-Head BMC (g)	0.27	0.32^a	0.31 (p<0.06)
	FN BMC (g)	0.04	0.18	0.19
	NN Width (cm)	-0.13	-0.13	-0.34^a
	NN ED (cm)	-0.08	-0.12	-0.35^a
	NN CT (cm)	-0.15	0.05	0.22
	NN Z (cm³)	-0.09	-0.10	-0.24
	NN BR	0.14	-0.08	-0.32^a
	L3 BMC (g)	-0.12	-0.01	0.02

Data in **large bold font** served as the basis for maturity-specific PA regressions.Data in *italics* represent statistically significant partial correlation coefficients.^aPartial correlation, p<0.05;^bPartial correlation, p = 0.01.

Table 4a

Regression Results: Maturity-Specific Activity as a Predictor of Adult Bone Status (Strongest, Significant Associations Only)

DXA2 BONE STATUS (DEPENDENT)	ADJUSTED MODEL R ²	DXA1 GYNAGE	DXA1 BONE STATUS	DXA2 BODY SIZE INDEX (STATURE OR FA)	DXA1 to DXA2 BODY SIZE CHANGE (STATURE OR FA)	PHYSICAL ACTIVITY PREDICTOR
1/3 BMC (g)	0.64 ^c	-0.16 [0.09]	0.92 ^c [0.15]	-0.001 [0.02]	0.03 [0.03]	POST1 PA 0.017 ^b [0.005]
Sub-Head BMC (g)	0.69 ^c	-262.2 ^c [70.25]	0.30 ^a [0.12]	14.06 ^c [3.31]	-1.28 [3.9]	POST1 PA 7.94 ^a [3.87]
NN Width (cm)	0.40 ^c	0.03 [0.10]	0.49 ^a [0.21]	0.005 [0.005]	0.005 [0.005]	POST2 PA -0.012 ^a [0.006]
NN ED (cm)	0.46 ^c	0.09 [0.10]	0.63 ^b [0.19]	0.003 [0.005]	0.006 [0.005]	POST2 PA -0.013 ^a [0.006]
NN BR	0.51 ^c	0.66 [0.40]	0.48 ^c [0.09]	0.022 [0.016]	-0.01 [0.02]	POST2 PA -0.05 ^a [0.02]
Table 4b. Regression Results: Adolescent Physical Activity Predicts Young Adult Bone Outcomes (Significant TOTAL PA Associations Only)						
1/3 BMC (g)	0.65 ^c	-0.18 ^a [0.09]	0.84 ^c [0.15]	0.005 [0.02]	0.02 [0.03]	TOTAL PA 0.02 ^c [0.006]
Sub-Head BMC (g)	0.70 ^c	-273.8 ^c [69.7]	0.26 ^c [0.12]	15.5 ^c [3.4]	-2.0 [3.9]	TOTAL PA 10.9 ^a [4.8]

Betas [standard errors] and t significance of betas shown for each predictor; **significant predictors are in bold.**

The name of the PA predictor entered and the PA predictor results are displayed in the right-most column.

DXA1 and DXA2 measurement dates: DXA1= ~1 year pre-menarche; DXA2= ~5 years post-menarche.

FA= forearm length; PA= organized, non-aquatic physical activity exposure (h/wk); POST1 PA = 1 year to 3 years post-menarche; POST2 PA = 3 to ~5 years post-menarche; TOTAL PA= mean PA over DXA1 to DXA2 interval; 1/3= 1/3 distal radius; BMC= bone mineral content (g); Z= section modulus (cm³); NN= Femoral Narrow Neck; ED= Endosteal Diameter (cm); BR= Buckling Ratio

^a p<0.05;

^b p 0.01;

^c p 0.001.