

**Bone health, activity and sedentariness at age 11-12 years:
Cross-sectional Australian population-derived study**

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ABSTRACT

Aim To examine cross-sectional associations of children's bone health (size, density, strength) with moderate-vigorous physical activity (MVPA) and sedentary behaviour by considering: (1) duration of activity, (2) fragmentation, and (3) duration/fragmentation combined.

Methods *Design:* Population-based cross-sectional study. *Participants:* 11-12 year-olds in the Longitudinal Study of Australian Children's Child Health CheckPoint. *Exposures:* MVPA and sedentary behaviour (7-day accelerometry), yielding (1) daily average durations (min/day) and (2) fragmentations (the parameter alpha, representing the relationship between activity bout frequency and bout length). *Outcomes:* Tibial peripheral quantitative computed tomography (bone density, geometry, strength). *Analysis:* Multivariable regression models including activity durations and fragmentations separately and combined.

Results Of 1,357 children attending the CheckPoint, 864 (64%) provided both bone and accelerometry data (mean age 11.4 years (standard deviation (SD) 0.5); 49% male). Mean daily MVPA and sedentary behaviour durations were 34.4 min/day (SD 28.3) and 667.9 min/day (SD 71.9) respectively for boys and girls combined. Each additional daily hour of MVPA was associated with small bone health benefits comprising greater periosteal and endosteal circumference (standardised effect sizes 0.25, 95% CI 0.10 to 0.40 and 0.21, 95% CI 0.03 to 0.39, respectively) and bone strength (0.26, 95% CI 0.14 to 0.38). Sedentary duration and fragmentation of either MVPA or sedentary behaviour showed little association with bone health.

Conclusions In early adolescence, moderate to vigorous physical activity showed associations with better bone health that, while modest, could be of population-level importance. MVPA fragmentation and sedentary behaviour duration and fragmentation seemed less important.

Keywords

Bone health, physical activity, sedentary behaviour, pQCT, accelerometry, adolescents

Abbreviations

DXA – Dual X-ray Absorptiometry; BMI – Body mass index; CheckPoint – Child Health CheckPoint; LSAC – Longitudinal Study of Australian Children; MVPA – moderate-vigorous physical activity; pQCT – peripheral Quantitative Computed Tomography; SEIFA – Socio-economic Indexes for Areas; SSI = strength-strain index; vBMD – volumetric Bone Mineral Density; VPA – vigorous physical activity

1. INTRODUCTION

Peak bone mass is reached in the early twenties and accounts for approximately 60% of osteoporosis risk.^{1,2} Individuals with good bone health have bones that are generally bigger, denser and hence stronger. Genetics are considered responsible for 60-80% of bone mass variation leaving only a small but important modifiable component.³ Pre- and peri-pubertal years are considered a critical window when modifiable factors, such as physical activity, can influence bone mass accrual.^{2,4,5}

Structured exercise programs demonstrate small improvements in bone measures (e.g. 5% greater bone mass),⁶ but may not reflect effects of habitual activity. Studies investigating habitual activity consistently show small associations between larger durations (minutes/day) of moderate-vigorous (MVPA) or vigorous (VPA) physical activity and better bone health,⁷⁻¹¹ while larger durations of sedentary behaviour show small associations with poorer bone health.¹²⁻¹⁵ However, most studies have evaluated bone with dual x-ray absorptiometry (DXA) that measures areal bone density, which does not necessarily reflect true volumetric bone density due to variations in growth and bone size.¹⁶

Peripheral Quantitative Computed Tomography (pQCT) evaluates volumetric density, as well as differential measures of bony compartments (cortical vs trabecular), geometry and strength.¹⁷ One study has examined activity associations with bone health using pQCT in a large paediatric population sample, reporting small associations between larger durations of VPA and greater bone content.¹⁰ However, trabecular bone was not considered, and at age 15 participants had passed peak bone mass accrual (around 12.5 years for girls and 14 years for boys),⁴ reducing the potential for exercise interventions on bone.^{18,19} Recent findings from a moderate-sized cohort study, using high-resolution (HR) pQCT across a wide age range (9-20 years) also suggested MVPA was associated with improved bone strength and size, especially peripubertally, while sedentary behaviour was negatively associated with bone size and cortical density.¹⁵ However, only VPA frequency, rather than duration, seemed important for bone strength.¹¹

Activity fragmentation should also be investigated, as the same total duration of activity can be accumulated in different ways. For example, cardiometabolic health guidelines recommend small bouts of sedentary behaviour broken up by frequent activity.^{20,21} Animal studies suggest a long period of rest between short bursts of exercise may optimise bone formation.²² In the only study to investigate activity fragmentation and bone health in humans, Chastin et al¹² reported longer bouts of activity interspersed with extended periods of sedentariness were associated with better bone health in 1,348 8-22 year-olds – suggesting optimal fragmentation of activity could differ between cardiometabolic and bone outcomes. However, the broad age range limits generalizability to the ages of greatest interest to bone health.

This study utilised a unique opportunity to examine associations between activity accumulation and bone health using pQCT in Australian children prior to peak mass accrual. In a population-derived study of 11-12 year-olds, we **aimed** to examine whether bone health was cross-sectionally associated with a) physical activity and b) sedentary behaviour, considering:

1. Duration (minutes/day),
2. Fragmentation, and
3. Duration and fragmentation combined.

2. MATERIALS AND METHODS

2.1 Study design and participants

The Child Health CheckPoint (CheckPoint) was a cross-sectional wave within the national population-based Longitudinal Study of Australian Children (LSAC).²³ CheckPoint data collection took place February 2015–March 2016 between LSAC’s 6th and 7th waves, when children were aged 11-12 years. The project was approved by the Royal Children’s Hospital Ethics Committee (HREC33225) and the Australian Institute of Family Studies Ethics Committee (AIFS14-26). A parent/guardian provided written informed consent.

In 2004, LSAC recruited a nationally representative sample of 5,107 infants (age 0 to 1 years) to its Birth (‘B’) cohort, using a two-stage cluster randomized design detailed elsewhere.²³ LSAC follows children and their families biennially, with 6 waves of data collection completed up to 2015. CheckPoint was a one-off physical health and biomarkers module offered to all families enrolled in LSAC’s B-cohort who participated in Wave 6 (retention rate 74%). Families ‘opted-in’ to be contacted about the CheckPoint module by providing written consent to the Wave 6 interviewer to pass contact details to CheckPoint.

2.2 Procedures

From December 2014, the CheckPoint team sent an invitation pack and telephoned each family to ascertain interest and book an appointment between February 2015 and March 2016. Most families attended a 3.5-hour appointment comprising multiple stations at a main assessment centre; those attending mini-centers in smaller regional cities (2.5-hour appointments) and home visits (1.5 hours) did not have bone health assessment. All participants were fitted with a wrist-worn accelerometer and given an ‘Activity Card’ at the end of the visit to record activity over 8 full days and note wake/sleep and non-wear times.

2.3 Measures

Table 1 provides technical details for all measures.

Table 1. Measures used in this study

Measure	Materials	Additional information
‘Exposure’		
Physical activity	GeneActiv Original Accelerometer	A researcher fitted each participant with a tri-axial accelerometer watch (GENEActiv Original) on their non-dominant wrist, and provided an activity card and a reply-paid envelope. Participants wore the watch 24 hours a day for 8 consecutive days and were instructed to only remove the watch for prolonged immersion in water. The accelerometer recorded participants’ activity in the form of counts of acceleration. Researchers crosschecked accelerometry data using the activity cards. On the activity cards participants recorded date, wake time, bed-time, a brief description of their day (e.g. school) and any periods of non-wear including reason for removal (e.g. sports or water related activities).
‘Outcomes’		
Bone health	Stratec XCT 2000 peripheral quantitative computed tomography scanner (pQCT)	One of three licensed researchers conducted each pQCT scan. Researchers measured quality control daily using a standard phantom and every 30 days using a cone phantom (XCT2000L®). Seven minutes were allowed for each scan of the non-dominant lower leg, classified as the leg used to kick a ball. Parents with a history of fracture had the dominant tibia scanned; no child had had previous ankle fractures. A researcher measured tibial length with a tape measure by palpating and marking the distal edge of the medial malleolus and medial edge of the tibial plateau. ²⁹ Participants positioned their non-dominant leg (shoeless) through the gantry of the pQCT scanner secured to a footrest. Participants whose calf diameters were too large for the gantry ($\geq 140\text{mm}$) proceeded only with a scan at the 4% site. A scout scan identified the distal epiphyseal plate and allowed placement of a reference line at the proximal edge. Researchers identified the 4 % and 66% sites of the tibia using this reference line in relation to the limb length. Scans had a speed of 20mm/s, slice thickness of 2.4mm and voxel size of 0.4mm. ²⁹
Potentially Confounding Variables		
Height	Portable rigid stadiometer, model I0955 (Invicta, Leicester,UK)	Height measured twice without shoes or socks and average used; if values differed by >0.5 cm, a third measurement taken and average of two closest values used
Weight	InBody 230 Scales (InBody Co. Ltd., Ca, U.S.A)	Weight measured once wearing light clothing without shoes or socks, using 4-limb bioelectrical impedance scales with arms and legs abducted to limit skin-skin interaction in the axilla and inguinal regions.
Body mass index and z-score	As per height and weight above	BMI was calculated as weight (kg)/height (m) ² . BMI z-score was calculated according to US Centre for Disease Control reference values using STATA “zanthro” function ²⁴
Index of relative socio-economic disadvantage	Socio-Economic Indexes for Areas (SEIFA)	Index based on ranking at the home postcode level by relative socio-economic disadvantage, according to the 2011 Census of Population and Housing administered by the Australian Bureau of Statistics ²⁵ . Factors assessed include household education levels, income levels, employment status and disability. Scores are standardised to a national mean of 1000 and standard deviation of 100.
Pubertal status	Pubertal developmental scale (PDS)	Self-reported and self-administered method for rating secondary sexual characteristic growth; comprised of a series of questions about growth spurt, body hair growth and skin change for both genders; breast development and the age of onset of menarche for females; facial hair growth and voice change for males. ²⁶

‘Exposures’: Episodes of physical activity and sedentary behaviour were measured continuously at 50 Hz for 8 days using triaxial accelerometers. The wrist-worn GeneActiv accelerometer was chosen for its increased compliance while still having high correlation with hip-worn accelerometers,²⁷ which are less prone to overestimation and are more specific to the site of bone imaging. From raw acceleration data, the Euclidean Norm Minus One (ENMO) was calculated, then averaged over 60-second epochs using custom Matlab code (R2015b, the Mathworks, Inc). Non-wear time was classified as continuous periods of 60 minutes or more with ENMO = 0. Self-reported removals were classified as non-wear, independent of acceleration. Sleep was identified using the activity cards, and visually adjusted if necessary (e.g. missing self-report logs). Every 60-second epoch of waking wear time was classified as sedentary, light, moderate or vigorous physical activity, using the Phillips cutpoints.²⁸ Phillips cutpoints were chosen as they are validated specifically for GeneActiv in this age group and take the absolute acceleration value for negative values. We grouped moderate and vigorous physical activity together (MVPA) in the analysis due to very low recorded durations of vigorous physical activity (median 1.3 minute per day). Non-wear periods less than 4 hours with reason for removal listed as ‘sport’ were replaced with MVPA. A valid day comprised at least 10 hours wear (including sleep), and at most 6 hours non-wear during wake time. All non-valid days were discarded; inclusion required at least 4 valid days. These participants were further classified as having an ‘optimal’ combination of valid days (at least one weekend day and three schooldays) or not.

Durations were expressed as average minutes per day of MVPA and sedentary time. Fragmentations were calculated using the parameter alpha for MVPA and sedentary behaviour separately. Alpha quantifies the slope of a least-squares regression line relating the log of bout frequency to the log of bout duration, where bout frequency is expressed as a proportion of total bouts.²⁹ A bout was a continuous period of at least 1 minute with at least 80% MPVA or 10 minutes with 100% sedentary behaviour. A higher alpha indicates a tendency to accumulate MVPA/sedentary behaviour in short fragmented bouts, while a lower alpha implies more long continuous bouts.

‘Outcomes’: pQCT (Stratec XCT2000®) scanner and software (version 6.20C) provided tomographic image slices of the distal 4% and 66% tibial sites.

One of two operators (WO, NI) set regions of interest around the bone and total image, and used the MACRO analysis function to generate measures with almost perfect inter-rater agreement (ICC>0.999). The following analysis parameters were determined in collaboration with a Senior Scientist (JB, see Acknowledgements):

- 4% site trabecular density (area:45%, threshold:169.0 mg/cm³, contour and peel modes:1);³⁰
- 66% site cortical density, endosteal circumference and periosteal circumference (threshold:710.0 mg/cm³, contour mode:1, peel and cort modes:2);^{31, 32}

- polar strength-strain index, calculated as a measure of torsional strength from geometry and density measures at the 66% site (threshold:480 mg/cm³, contour and peel modes:1, filter:F01);³³
- muscle area, calculated by subtracting bone cross-sectional area from combined muscle and bone cross-sectional area (threshold:50-540 mg/cm³, contour mode:3, peel mode:1, filter:F03F05).^{31, 33}

The operators graded scans for motion artefact by visual inspection from 1 (no artefact) to 5 (severe artefact) against reference images, similar to the method used by Blew et al.³⁴ To retain the maximum number of scans, we excluded only those images graded 4 and 5 with motion artefact affecting the regions of interest (inner 45% of trabecular bone in 4% scans or cortical bone in 66% scans).

A priori potential confounders were age,³⁵ sex,³⁵ height,³⁶ neighbourhood disadvantage,²⁵ weight³⁸ and muscle cross-sectional area (Table 1), all known to be associated with both bone health and activity.

2.4 Statistical analysis

Inclusion in the analytic sample required at least 4 valid days of accelerometry data and at least one pQCT measure. Data were analysed using Stata version 14.0.

Multivariable linear regression models were used for all aims. For ease of interpretation, durations were converted from minutes to hours, and both fragmentation and bone health variables were internally standardised to have a mean of zero and a standard deviation of one. Regression coefficients represent the standard deviation change in bone health for a one-hour change in duration or a one standard deviation unit change in alpha, interpreting effect sizes of ≥ 0.20 , ≥ 0.50 and ≥ 0.80 standard deviations (SD) as small, medium and large respectively.³⁹

For Aims 1 and 2, durations and fragmentation were considered separately in two models. In Model 1 effect sizes for bone health according to duration and fragmentation of activity were minimally adjusted for age, sex, height, weight and whether or not the valid days occurred in an ‘optimal’ combination. Model 2 was further adjusted for the remaining potential confounders. In Model 3 duration and fragmentation of activity were included simultaneously to estimate independent associations with each activity characteristic. Thus, Model 3 was equivalent to Model 2 + duration *and* fragmentation of activity.

Partial coefficients of determination (partial R²) summarised the variance in bone measures explained by each activity measure, and variance inflation factor analyses checked for collinearity between duration and fragmentation in Model 3.⁴⁰ We conducted interaction tests for sex to investigate whether there was evidence of an interaction between activity and sedentariness with sex in explaining bone health, as bone

health may differ at this age largely due to pubertal development. We found no evidence of an interaction and therefore present models including boys and girls rather than stratifying by sex. Sensitivity analyses of ‘optimal’ vs ‘other’ accelerometry days examined effects of type of days on outcomes. We ran post-hoc sensitivity analyses including only images with the best quality (grades 1 and 2) to assess the influence of images with greater motion artifact. We considered puberty as a confounder but data were incomplete for 66 children. Therefore we also conducted a post hoc sensitivity analysis including puberty to determine if it made a difference.

3. RESULTS

3.1 Sample characteristics

Figure 1 shows the participant flow, and Table 2 the sample characteristics.

Figure 1. Recruitment and retention of participants in the Child Health CheckPoint.

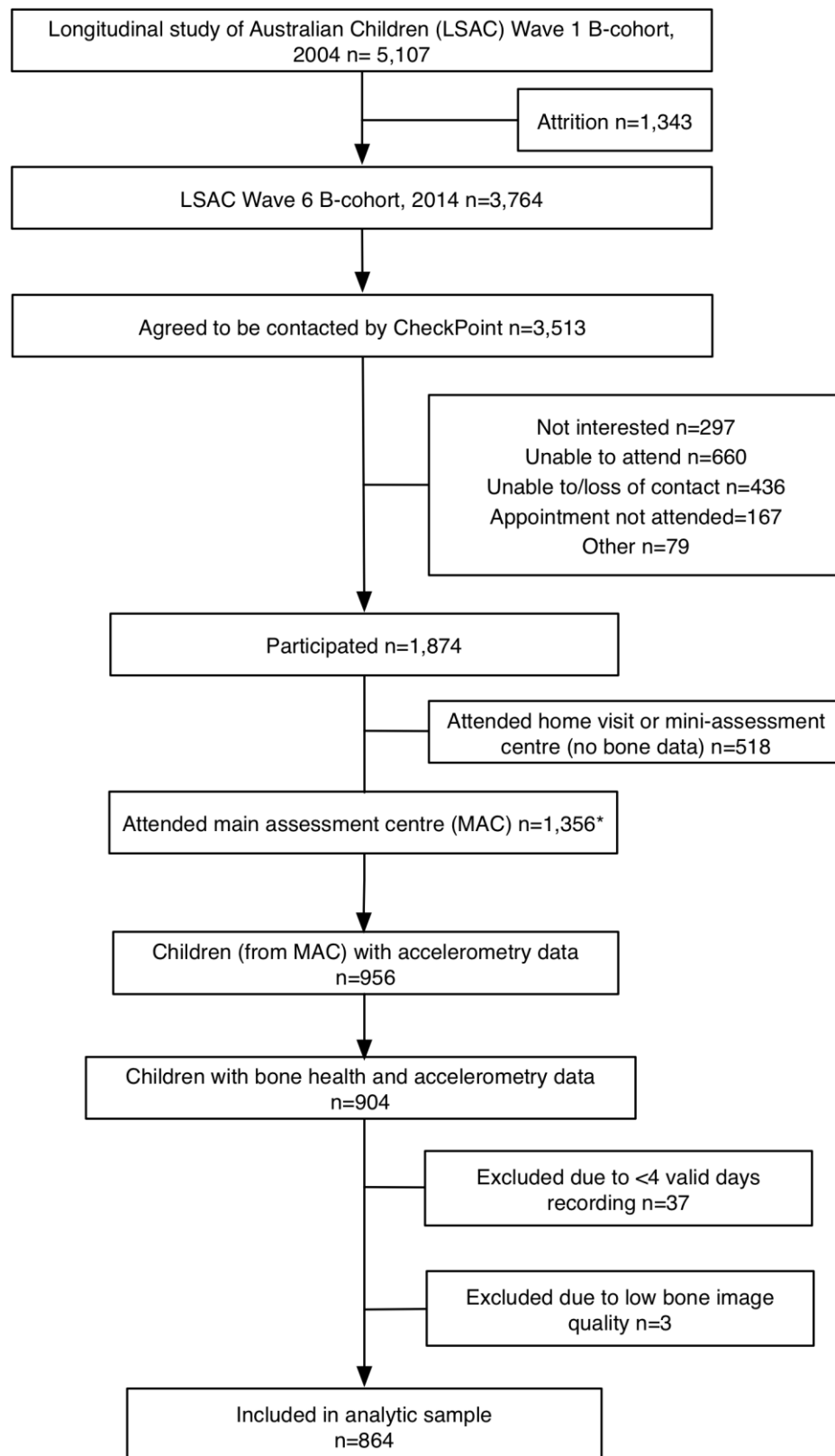


Table 2. Descriptive characteristics of participants

Variable	Mean (SD)	Mean (SD) by sex	
	Total (n = 864)	Males (n = 424)	Females (n = 440)
Potential confounders			
Age (years)	11.4 (0.5)	11.4 (0.5)	11.4 (0.5)
Puberty, %			
Pre-pubertal	10.9	16.3	5.5
Early/mid pubertal	76.3	79.9	72.6
Late/post-pubertal	12.8	3.8	21.9
Disadvantage (SEIFA)	1029 (59)	1031 (59)	1028 (60)
Height	152.9 (7.9)	152.3 (8.0)	153.5 (7.8)
Body fat (%)	21.8 (8.3)	20.8 (8.7)	22.7 (7.7)
Weight (kg)	44.7 (10.3)	44.2 (10.6)	45.3 (9.9)
Body mass index category, %			
Not overweight	77.9	77.4	78.5
Overweight/obese	22.1	22.6	21.5
Physical activity			
MVPA (min/day)	34.4 (28.3)	42.3 (31.0)	26.7 (23.1)
Sedentary behaviour (min/day)	667.9 (71.9)	662.8 (75.2)	672.8 (68.3)
Alpha, MVPA	1.99 (0.18)	1.95 (0.18)	2.03 (0.17)
Alpha, sedentary behaviour	2.13 (0.18)	2.12 (0.17)	2.14 (0.18)
Bone and muscle			
<i>Volumetric bone mineral density (vBMD)</i>			
Trabecular (mg/cm ³)	198.0 (25.5)	195.8 (23.2)	200.2 (27.4)
Cortical (mg/cm ³)	1019.2 (37.1)	1011.5 (36.2)	1026.7 (36.5)
<i>Bone geometry</i>			
Periosteal circumference (mm)	81.0 (7.2)	81.7 (7.5)	80.3 (6.9)
Endosteal circumference (mm)	61.8 (8.3)	62.9 (8.4)	60.8 (8.0)
<i>Bone strength</i>			
Polar strength-strain index (mm ³)	1692 (388)	1702 (396)	1684 (380)
<i>Muscle</i>			
Cross-sectional area (mm ²)	4355 (695)	4314 (728)	4394 (660)

Body mass index category based on CDC cut points;⁴¹ n = 852-864, except pubertal status n = 798; SEIFA = Socio-Economic Indexes for Areas (national average 1000 (SD: 100), with higher scores indicating less disadvantage); MVPA = moderate-to-vigorous physical activity; Alpha = measure of activity bout frequency and length; a higher alpha indicates a tendency to accumulate MVPA/sedentary behaviour in short fragmented bouts, while a lower alpha implies more long continuous bouts.

Of 3764 eligible children, 1874 (50% of Wave 6) participated in CheckPoint; 864 (23%) had both useable accelerometry and pQCT data. This analytic sample was similar in terms of age and sex to CheckPoint participants without accelerometry and pQCT data, but came from less disadvantaged neighbourhoods (mean disadvantage index 1030 (SD 59) vs 1017 (SD 60); national mean 1000 (SD 100)). As expected, girls were further through puberty and spent less time in MVPA than boys. pQCT values were similar to reference ranges for age.⁴²

Approximately one third of participants (36%) with at least four valid days of accelerometry did not fit the ‘optimal’ criteria of at least three schooldays and one weekend day, mainly because so many parents chose to attend the Assessment Centre during school holidays. Mean (SD) for MVPA and sedentary behaviour durations for children with optimal vs non-optimal combinations were 37.7 (28.9) vs 28.4 (26.3) and 661.5 (69.7) vs 679.1 (74.3) minutes/day, respectively.

3.2 Associations between physical activity/sedentariness and bone health (see Table 3)

3.2.1 Activity/sedentariness duration (Aim 1)

3.2.1.1 Activity duration

In the minimally-adjusted Model 1, larger durations of MVPA were associated with higher (better) trabecular density, bone size (periosteal and endosteal circumference) and strength (polar SSI). Less MVPA was counterintuitively associated with slightly greater cortical density; this appeared to reflect a tendency towards lower density in the geometrically larger bones seen in the more active children ($r = -0.32$ between cortical density and periosteal circumference). Associations with MVPA and bone indices showed little change on further adjusting for, neighbourhood disadvantage, and muscle cross-sectional area (Model 2: partial R^2 ranging from 0.9 to 4.9%).

3.2.1.2 Sedentariness duration

In contrast to MVPA, more sedentary behaviour was associated with slightly greater cortical density but lower (poor) trabecular density, bone size and strength in the minimally adjusted model. As for MVPA, with further adjustment in model 2 associations changed only slightly. All bone indices, except cortical density worsened with increasing sedentariness (partial R^2 0.7 to 1.4%), although these associations were very small.

3.2.2 Activity/sedentariness fragmentation (Aim 2)

3.2.2.1 Activity fragmentation

Associations of fragmentation of MVPA with bone health were small and again similar in Model 1 and 2. For MVPA, more long continuous bouts (smaller alpha) were associated with better bone health, i.e. greater trabecular density, periosteal circumference and strength (all significant; partial R^2 0.6 to 2.4%). For example, 1 SD lower standardised MVPA alpha was associated with slightly larger periosteal circumference (effect size (ES) -0.08, 95% CI -0.13 to -0.03; $p < 0.001$; partial R^2 1.6%).

3.2.2.2 Sedentariness fragmentation

Similarly, associations of sedentariness fragmentation with bone health were also very small in model 1 and 2. A larger number of short, fragmented bouts (larger alpha) were associated with slightly greater periosteal circumference and polar strength-strain index. Conversely, longer, continuous bouts of sedentary behaviour were associated with greater cortical bone density.

Table 3. Multivariable associations of physical activity and sedentary behaviour with standardised bone measures

Standardised Outcome*	Moderate-to-vigorous activity				Sedentary behaviour			
	Duration (hours/day)		Fragmentation (alpha*)		Duration (hours/day)		Fragmentation (alpha*)	
	β (95% CI)	<i>P</i>	β (95% CI)	<i>P</i>	β (95% CI)	<i>p</i>	β (95% CI)	<i>p</i>
Model 1: separate duration and fragmentation models minimally adjusted for age, sex, height, weight, ‘optimal’ 4 days accelerometry (yes/no)								
Cortical vBMD	-0.16 (-0.31, -0.01)	0.03	0.07 (-0.00, 0.12)	0.055	0.10 (0.04, 0.15)	<0.001	-0.09 (-0.16, -0.03)	0.007
Trabecular vBMD	0.28 (0.14, 0.43)	<0.001	-0.11 (-0.18, -0.05)	<0.001	-0.08 (-0.14, -0.03)	0.004	0.04 (-0.03, 0.10)	0.29
Periosteal circumference	0.27 (0.16, 0.38)	<0.001	-0.09 (-0.14, -0.03)	0.001	-0.08 (-0.12, -0.04)	<0.001	0.06 (0.00, 0.11)	0.03
Endosteal circumference	0.18 (0.04, 0.31)	0.011	-0.05 (-0.11, 0.02)	0.14	-0.06 (-0.11, 0.01)	0.016	0.04 (-0.02, 0.10)	0.24
Polar SSI	0.32 (0.23, 0.41)	<0.001	-0.10 (-0.15, 0.06)	<0.001	-0.08 (-0.12, 0.04)	<0.001	0.06 (0.02, 0.11)	0.004
Model 2: separate duration and fragmentation (Model 1), further adjusted for disadvantage, muscle cross-sectional area								
Cortical vBMD	-0.16 (-0.31, -0.01)	0.03	0.07 (-0.00, 0.13)	0.06	0.10 (0.04, 0.16)	<0.001	-0.09 (-0.16, -0.03)	0.007
Trabecular vBMD	0.22 (0.07, 0.36)	0.003	-0.11 (-0.17, -0.04)	0.001	-0.06 (-0.12, -0.01)	0.02	0.02 (-0.05, 0.08)	0.58
Periosteal circumference	0.26 (0.15, 0.37)	<0.001	-0.08 (-0.13, -0.03)	0.002	-0.08 (-0.12, -0.03)	<0.001	0.05 (0.00, 0.11)	0.04
Endosteal circumference	0.18 (0.04, 0.32)	0.01	-0.04 (-0.11, 0.02)	0.16	-0.07 (-0.12, 0.01)	0.01	0.04 (-0.02, 0.10)	0.20
Polar SSI	0.29 (0.20, 0.38)	<0.001	-0.10 (-0.14, -0.06)	<0.001	-0.07 (-0.11, -0.04)	<0.001	0.06 (0.01, 0.10)	0.01
Model 3: Model 2 + jointly considering MVPA duration and fragmentation					Model 3: Model 2 + jointly considering SB duration and fragmentation			
Cortical vBMD	-0.10 (-0.30, 0.10)	0.33	0.04 (-0.05, 0.13)	0.43	0.08 (-0.01, 0.15)	0.02	-0.03 (-0.12, 0.04)	0.46
Trabecular vBMD	0.10 (-0.09, 0.29)	0.29	-0.08 (-0.16, 0.01)	0.08	-0.09 (-0.15, -0.02)	0.01	-0.04 (-0.12, 0.04)	0.28
Periosteal circumference	0.25 (0.10, 0.40)	0.001	-0.01 (-0.08, 0.06)	0.86	-0.08 (-0.13, -0.02)	0.004	0.00 (-0.07, 0.06)	0.95
Endosteal circumference	0.21 (0.03, 0.31)	0.02	0.03 (-0.06, 0.10)	0.88	-0.07 (-0.14, -0.01)	0.03	0.01 (-0.09, 0.07)	0.78
Polar SSI	0.26 (0.14, 0.38)	<0.001	-0.02 (-0.08, 0.04)	0.49	-0.07 (-0.11, -0.03)	0.002	0.03 (-0.05, 0.06)	0.84

*All bone outcomes and alpha values were internally standardised (mean of zero, standard deviation of one). Regression coefficients represent SD unit change in the outcome for a 1-unit increase in the exposure. Higher scores on all bone metrics indicate better bone health, with the exception of endosteal circumference, which may reflect larger size or reduced cortical thickness. *p* = p-value; vBMD = volumetric bone mineral density; SSI = strength-strain index; CI = confidence interval

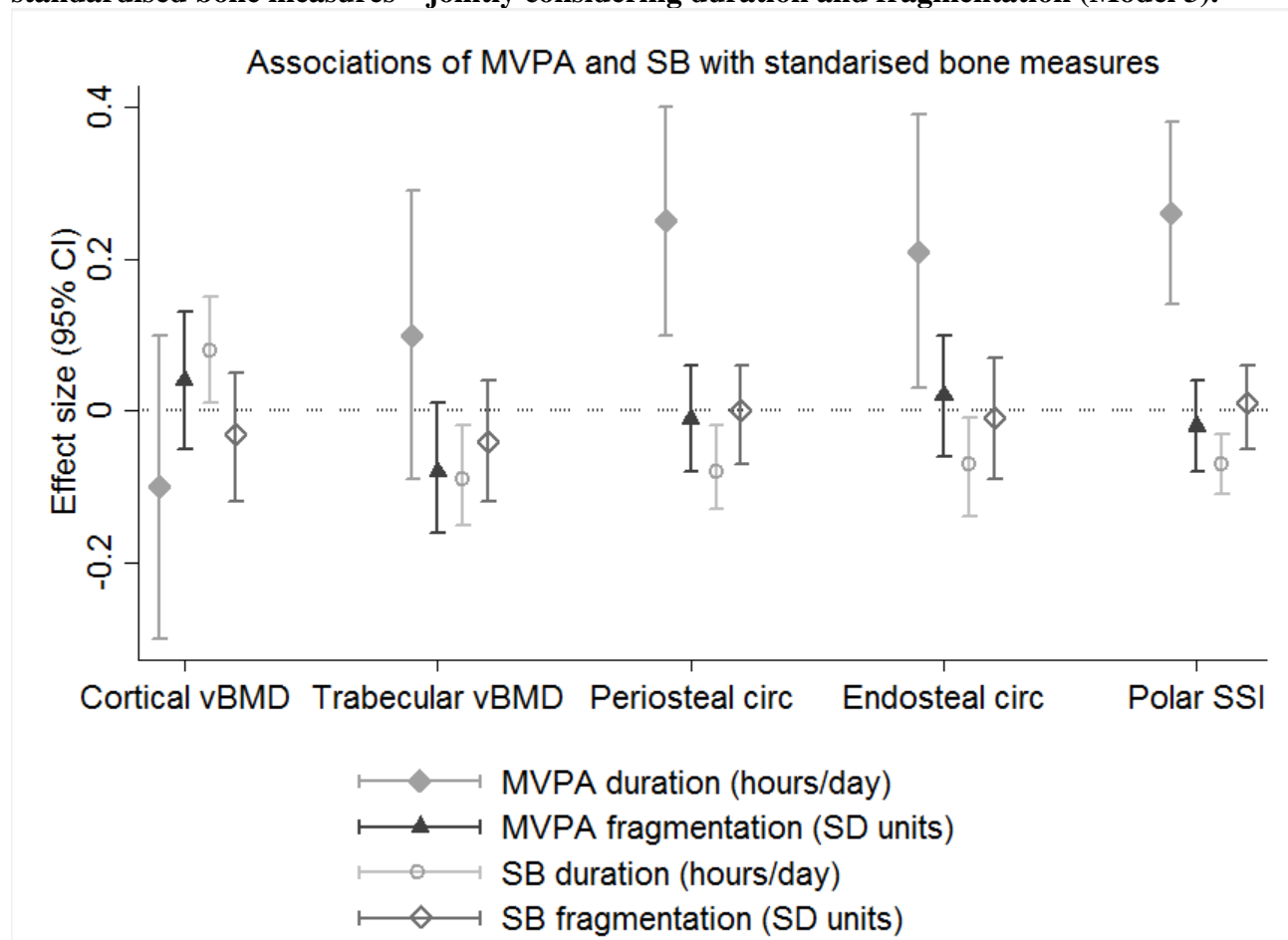
Number of children in analyses: model 1 n=849-855; model 2 n=838-849; model 3 n=838-846

There were no evident interactions between sex and MVPA or sedentary behaviour in explaining bone health.

3.2.3 Activity durations and fragmentations simultaneously (Aim 3)

In the fully adjusted Model 3 analyses, associations between more MVPA and better bone health appeared largely driven by duration rather than fragmentation (as clearly illustrated in Figure 2), particularly in terms of bone size and the composite measure of polar stress-strain index. Sedentary behaviour duration and fragmentation appeared to be of minimal importance.

Figure 2. Associations of moderate-vigorous physical activity and sedentary behaviour with standardised bone measures – jointly considering duration and fragmentation (Model 3).



Effect size = regression coefficients β , representing SD unit change in the outcome for a 1-unit increase in the exposure; CI = confidence interval; vBMD = volumetric bone mineral density; circ = circumference. All analyses adjusted for age, sex, height, 'optimal' 4 days accelerometry (yes/no), disadvantage, weight, muscle cross-sectional area.

Each extra hour of physical activity was associated with moderately better bone strength (ES 0.26, 95% CI 0.14 to 0.38; $p < 0.001$; partial R^2 2.5%) and periosteal circumference (ES 0.25, 95% CI 0.10 to 0.40; $p = 0.001$; partial R^2 1.7%). As for models 1 and 2, more sedentary behaviour had very small associations with greater cortical density and lower trabecular density, bone size and strength (ES -0.07 (-0.11 to -0.03)). In contrast, for MVPA and sedentary fragmentation all associations attenuated to non-significance.

3.2.4 Interaction and sensitivity analyses

All interaction analyses for sex were non-significant, suggesting males and females should not be examined separately. Similarly, sensitivity analyses for 'optimal' days were comparable to the main

results and the variance inflation factors for Model 3 were all lower than 10, suggesting collinearity was not an important issue.⁴⁰

3.2.5 Post-hoc analyses

Sensitivity analyses for image quality (including only grades 1 and 2) were similar to the main results, suggesting that including the images with lower grades did not impact on findings. Sensitivity analyses including puberty reduced the sample size but did not substantively alter the findings (results available from the author). This is likely because for children of this age much of the variability in pubertal status is explained by sex, which was already included in the models. In order to maximise sample size we therefore excluded puberty from linear regression models.

4. DISCUSSION

4.1 Principal findings

This is the first study to investigate how duration and fragmentation of physical activity and sedentariness are mutually associated with bone health in a population-derived sample of young adolescents. MVPA was more strongly associated with bone health than sedentariness, with the association driven more by duration than fragmentation; nonetheless, even MVPA duration explained less than 5% of the variance in bone health parameters. Associations of MVPA duration with greater bone strength appeared to reflect larger size (periosteal circumference) rather than higher density.

4.2 Findings in relation to previous research

Our study concurs with several previous cross-sectional studies reporting superior bone health with higher durations of MVPA or VPA⁷⁻¹⁰ but is only the second to use pQCT. Using pQCT in adolescents, aged 15.5 years, Sayers et al¹⁰ reported slightly higher tibial bone mineral content (ES 0.08) with doubling VPA. Our observational study did not separate MPA and VPA because VPA durations were very small for the majority of children (median 1.3 min/day, mode 0); however, this does not preclude potential benefit from interventions targeting bursts of VPA. Differences in activity intensity categories (VPA vs MVPA) and time unit (doubling minutes vs additional hours) preclude comparisons of effect sizes between studies. Nevertheless, our apparently larger effect sizes (for example, 0.26 SD greater bone strength per additional hour of MVPA) may partly reflect our younger age group, with pre- and peri-pubertal years considered the optimal time for contributions of physical activity to bone mass.^{2, 4, 5} Recently published HR-pQCT data also supports this, with associations of increased strength and bone size with MVPA especially marked during early to mid puberty.¹⁵ Although accelerometry-based

measurements can vary significantly depending on device and processing parameters, estimated MVPA in our study was similar to previous population based studies in early adolescents.^{43, 44}

Similar to Sayers et al,¹⁰ higher durations of MVPA were associated with larger bones (periosteal circumference) but lower or no difference in cortical density. Increased areal bone density with greater physical activity reported in previous DXA studies^{7, 8, 45} could be artefactual as DXA, unlike pQCT, does not account for three-dimensional bone size. Similar to our findings, studies in athletes using pQCT have indicated lower or similar density but increased cross-sectional area of peripheral bones compared to non-dominant limbs or controls.^{46, 47} Reduced or unmodified cortical density with physical activity may result from increased remodelling of new less dense bone under mechanical stress.^{46, 48} This would support our finding of greater cortical bone strength (despite lower cortical density) from larger periosteal circumference with more MVPA. Mineralisation may be also delayed in the setting of rapid geometric adaptations during peak bone growth.⁴⁹ Longitudinal studies with serial pQCT could determine if mineralisation of newly-formed bone lags during adolescence, contributing to transient lower density.

Prior studies suggesting an association between sedentariness and bone health have generally elicited sedentary activities by questionnaire^{13, 14} (known to be inaccurate) and/or measured bone with DXA.^{11,38} Our findings of very small associations for accelerometry-measured sedentariness and tibial pQCT differ slightly from Gabel et al's⁵⁰ initial cross-sectional study of 9-20 year-olds using HR-pQCT, which showed no association. However, in Gabel's recent follow up of this cohort, sedentary behaviour had small but detrimental effects on bone geometry 4 years later.¹⁵ Another consideration is whether results for sedentary time and MVPA should be standardised, e.g. effects expressed in terms of standard deviation differences relative to the daily means of 667.9 min/day and 34.4 min/day respectively. However, public health interventions do generally make recommendations in actual minutes and sometime argue for a direct swap of time e.g. 1 hour sedentary behaviour for 1 hour MVPA. However, these interventions typically make only small and transient shifts in sedentary and activity durations (e.g. 30 min/day).^{21, 22}

Fragmentation, as well as duration, of activity is topical to cardiometabolic health research. Regarding bone, Chastin et al¹² described higher DXA measures with short bouts of physical activity interspaced with long continuous periods of sedentariness (low sedentary alpha). In contrast, we suggest that long continuous MVPA with short fragmented sedentary behaviour were associated with better bone health. Our study goes further than Chastin's by investigating *both* sedentary and MVPA fragmentation, and by considering fragmentation in light of duration. We conclude that, although fragmentation may have some influence, it appears fragmentation is mainly driven by duration — i.e. young adolescents with more

continuous MVPA and shorter fragmented sedentary behaviour are simply those who spend more time in MVPA and less time being sedentary.

4.3 Strengths and limitations

Strengths include the large nationally-derived population sample and high-quality objective measures of activity and bone health. Australia's large geographical size ensures diversity in sunlight hours (and thence Vitamin D) that could impact on exercise and/or bone growth. We were able to adjust for important potential confounders including weight, leg muscle size, and disadvantage. Furthermore, we considered both duration and fragmentation in the same model, allowing us to draw conclusions on how activity and sedentary behaviour is accumulated as well as total time.

Limitations include the cross-sectional design, precluding causal inference; however, several randomised controlled trials support a unidirectional relationship between physical activity and bone health.¹⁸ We were not able to consider diet, vitamin D, ethnicity, medical conditions and medications. All may affect the skeleton,¹ but it seems less likely that they will affect the internal associations between activity and bone. The retained sample had lower neighbourhood disadvantage than both the non-retained sample and the general population; children with less disadvantage may have better bone health,⁵⁵ leading to reduced sample variability and underestimation of effects. Accelerometry recordings may not truly represent normal activity levels (e.g. seasonal variations and removal for sport). Nevertheless, 4-7 day recordings have moderate to high intra-class reliability (0.66-0.87) in this age group.⁵⁶ In Australia, all devices must be removed for contact sport, and accelerometers are not guaranteed to be waterproof. Therefore, removal periods for such sports (e.g. netball or swimming of less than 4 hours) were replaced with MVPA. Although accounting for unrecorded activity may be a strength in determining children's true activity levels, replacing with MVPA may have led to underestimation of VPA and overestimation of MVPA. Furthermore accelerometers do not assess activity load (e.g. running on flat vs up hill). This would potentially underestimate the effect of high load activities, which may be particularly anabolic for bone and show stronger associations than MVPA. Other bone parameters could have been explored, but we balanced limiting our analyses to 5 validated, reliable measures against the increased likelihood of chance findings with more outcomes.

4.4 Future directions for research

Assessment of type of activity and load provides avenues for future research, as do other factors not included in this study such as bone measurements from the radius, measures of circulating biochemical parameters, and high-resolution pQCT assessment. It would also be of interest to examine relationships of activity and bone with muscle parameters such as cross-sectional area and density.

5. CONCLUSIONS

We conclude that MVPA has a stronger relationship with bone health in early adolescence than does sedentary behaviour, which showed only small negative associations. These MVPA associations were driven by duration rather than fragmentation; adolescents with longer bouts of continuous MVPA and shorter fragmented bouts of sedentary behaviour are simply those who spend more time in MVPA. This suggests that guidelines for improving paediatric bone development via activity should focus on increasing the total duration of MVPA – irrespective of fragmentation and sedentary behaviour.

Effect sizes of around 0.3 SD for a one hour per day increase in MVPA suggest that overall MVPA plays a modest role in total paediatric bone accrual. While these effects are small, activity modification might nonetheless benefit population-based outcomes (particularly osteoporosis and fractures).^{57, 58} This warrants ongoing investigation, especially in light of few apparent alternatives for optimising the bone health of young adolescents.

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COMPETING INTERESTS

The authors declare no potential conflicts of interest, including no specific financial interests relevant to the subject of this manuscript

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CONTRIBUTIONS

MW conceived the CheckPoint study with the wider CheckPoint team including PS and TO. KL was a post-doctoral researcher and student supervisor of WO. FF prepared the data from accelerometry recordings. JV was involved with initial data collection and analysis. WO and NI prepared the data from pQCT images. FM and JM advised on statistical issues and helped to conduct the analyses. PS and TO were student supervisors and provided expert advice throughout the study bone health and, physical activity and sedentariness, respectively. MW was the primary student supervisor and oversaw all aspects of the study and the manuscript preparation. DB, JBC, BE, TD, PA were all involved in the conception of

the Child Health CheckPoint and oversaw data collection. They each provided critical input on drafts, assisted with interpretation and approved the final manuscript as submitted. All authors contributed to and approved the final manuscript.

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