An In-school Exercise Intervention to Enhance Bone and Reduce Fat in Girls: The CAPO Kids Trial

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ABSTRACT

The CAPO Kids trial was a 9-mo, controlled, school-based intervention to examine the effects of a novel, brief, high intensity exercise regime on indices of musculoskeletal and metabolic health in pre- and early pubertal girls. **Methods:** A total of 151 pre- and early pubertal girls (10.6 ± 0.6 years), recruited from two different schools consented to participate; 75 in the exercise group (EX) and 72 in the control group (CON). EX performed 10 min bouts of thrice-weekly jumping plus *capoeira* (a Brazilian sport that combines martial art with dance), along with usual physical education (PE) activities. CON continued usual PE alone. Maturity, weight, height, waist circumference, resting heart rate and blood pressure, maximal vertical jump, and aerobic capacity were determined using standard clinical and field measures. Calcaneal broadband ultrasound attenuation (BUA) and stiffness index (SI) were determined from quantitative ultrasonometry. A subsample of children also underwent DXA and pQCT measures. Prior physical activity participation and daily calcium consumption were determined from validated instruments. **Results:** EX girls improved BUA more than CON (+4.5% vs. +1.4%, $p=0.019$). Resting heart rate (-7.2% vs. -1.8%, $p<0.01$), maximal vertical jump (+13.4% vs. -1.2%, $p<0.001$), estimated maximal oxygen consumption (+10.6% vs. +1.0%, $p<0.001$), and waist circumference (+2.7% vs. +5.6%, $p<0.001$) also improved more for EX than CON. **Conclusion:** Ten minutes of high intensity exercise (*capoeira* and jumping) three times a week in the primary school setting enhances musculoskeletal and metabolic outcomes in pre- and early-pubertal girls without disrupting the academic schedule. The program, amenable to broad-scale school implementation, would confer meaningful public health benefits.

Key words: bone mineral density; lean mass; obesity; pediatric; physical activity.

1. INTRODUCTION

Exercise during childhood is important to optimise health in the short and longer term. It is a common assumption that children naturally lead active lifestyles. In reality, many children participate in very little physical activity [1]. As a consequence, rates of childhood obesity and metabolic disease have increased considerably, including premature development of chronic diseases that were previously the domain of the adult population [2-5]. Insufficient musculoskeletal loading during childhood may be particularly deleterious to long term health, as bone growth ceases in early adulthood, after which time the benefits of physical activity for bone are markedly reduced. It has been proposed that if adequate physical activity is not performed in youth, a window of opportunity to reduce osteoporotic fractures in later life may be missed [6-8]. Early puberty and the years immediately prior to puberty may be particularly opportune to harness the osteogenic potential of exercise in the presence of increasing levels of circulating growth factors [8].

Women are more likely to suffer from osteoporosis than men [9] and less likely to exercise, including during childhood [10, 11]. In addition, sexual dimorphism of bone development during growth results in greater bone strength in boys than girls following puberty [12-14]. In-school exercise interventions are an effective strategy to broadly increase the physical activity levels of both sexes [15]. Exercise programs that incorporate brief but high rate of loading activities such as jumping (for weight bearing bones) or tennis (upper extremity bones) induce the greatest gains in bone [6, 16], while longer duration aerobic activities have historically been utilised to minimise the accumulation of fat and optimise cardiovascular health [17, 18]. A recent meta-analysis of the outcomes of exercise interventions designed to enhance pediatric bone revealed that osteogenic exercise may also reduce fat [19]. It was not known if an exercise program designed to specifically *target* both tissues would be effective [20].

While dual-energy x-ray absorptiometry (DXA) remains the gold standard of bone mass estimation, its utility for large scale pediatric studies is limited by cost, scan duration, radiation exposure, and lack of portability [21-23]. Such limitations compound the considerable challenges of data collection and exercise intervention in pediatric trials. By contrast, quantitative ultrasound (QUS) is an inexpensive, portable and rapid method of estimating bone quality that does not expose growing children to ionising radiation. Furthermore, calcaneal measures exhibit strong positive relationships with BMD and fracture risk [24, 25]. For these reasons previous investigators have relied on QUS measures to track outcomes of pediatric exercise interventions [26-28].

The aim of the CAPO Kids intervention trial then was to determine the effect of a brief, simple, enjoyable, musculoskeletal- and fat-targeted exercise program on QUS-derived bone quality, fat and metabolic health in pre- and early pubertal girls over the course of a school year. We hypothesized that girls in the exercise group would experience greater improvements in parameters of bone, muscle and fat than age- and sex-matched controls.

2. MATERIALS AND METHODS

2.1 Ethical approval

Approval to perform the study was obtained from the Griffith University Human Research Ethics Committee (PES/25/11/HREC). Two Gold Coast primary schools adopted study activities (i.e. testing only for the control school[CON], and testing plus exercise for the exercise school[EX]) as part of their physical education and/or health curricula for all year 5 and 6 students. Test results of children whose parents declined to consent to their involvement in the study were not included in study analyses.

2.2 Study design

We conducted a 9-mo, controlled, school-based exercise intervention. The 10-min exercise sessions were incorporated into the daily schedule by the EX school on three consecutive days each week (Tues - Thurs), every week of the school year, with the exception of holidays. In both EX and CON schools, baseline testing occurred at the commencement of the school year (T0), before the intervention was initiated, and follow up testing was undertaken in the final weeks of the school year after 9 months of the intervention (T9). School-based testing occurred over two school days. Lab-based testing occurred an average of 2.5 weeks of either T0 or T9 time points.

2.3 Participants

Two local independent primary schools (Gold Coast, Australia) of essentially identical size and demographic (ethnicity and socioeconomic profile); having comparable school fees, school hours, curricula and time devoted to physical education and other physical activities, were randomly allocated to control (CON) or exercise (EX). Of the 152 girls enrolled in fifth and sixth grades at those schools, 151 consented to inclusion of their test measures in the CAPO Kids data analyses. Students were eligible for inclusion in the study if they were of sound general health, fully ambulatory, and gave their consent to participate. Students were excluded if they were taking medications known to affect bone, muscle or metabolism, were recovering from a limb fracture or other immobilizing injury in the past six months, were affected by any condition not compatible with physical activity, or their parents declined to consent.

2.4 Intervention activities

The EX group participated in instructor-led (RN) exercise bouts comprising 10 minutes of continuous high intensity movements intended to improve musculoskeletal and metabolic health. The approach was based on previous evidence that short duration, high intensity impact loading has been observed to enhance, not only bone in children [16, 29], but also, somewhat unexpectedly, metabolic parameters of adolescents [30]. The goal of the CAPO Kids trial was to determine if a similarly brief exercise intervention for bone with a targeted aerobic component, could also improve metabolic outcomes of pre and peri pubertal children.

The program was largely based on *capoeira*, a Brazilian sport that combines dance with martial arts, and a broad range of continuous movements of medium to high impact, applied at varying speeds and directions in order to increase heart rate, and to load a variety of muscle groups and skeletal regions in the upper and lower body. Activities varied from session to session; however, they comprised at least some of the following on each occasion: *capoeira*-specific movements (*Ginga*, kicks, and defence movements); jumps; hops; tuck jumps; jump-squats; star jumps; cartwheels; and handstands (Table 1). The number of repetitions was increased gradually such that, by the end of the year, a single session would include approximately 150 jumps and at least 50 kicks, in addition to 30 to 40 movements in an inverted position (with upper extremity weight bearing). The majority of jumps were performed with maximal effort, while other movements had an emphasis on speed. Occasional small prizes (e.g. balls and game vouchers) were provided to reward participation and improvement.

The intention was for children to expend maximal energy during the session, but be ready to return to classroom activities directly afterwards. Average total time away from the classroom was 15 minutes. In all other respects EX school continued to undertake usual school activities.

Table 1: Descriptions of Exercise Activities

<i>Activity</i>	<i>Description</i>	<i>Repetitions per class</i>
Jumps	Two-leg take off, followed by a two-leg landing	≈ 20
Hops	Single-leg take off, followed by a single-leg landing on the same side	≈ 20
Tuck-jumps	Double-leg jump, with hips and knees flexed during flight, bringing the knees into close proximity to the chest, and arms momentarily holding the knees when they reach the chest	≈ 20
Jump-squats	Double-leg jump, where the start and final position is with hips and knees flexed to approximately 90 degrees	≈ 20
Star jumps	With feet together, jumping and positioning legs apart, at the same time the arms go up touching the hands above the head, and returning to the initial position, repetitively	≈ 60-70
Jump-Lunges	Taking a large step forward so that the knee is flexed to 90 degrees as a start position, jumping while swapping legs, returning to start position with the opposite leg	≈ 10 each leg
<i>Ginga</i>	Feet positioned shoulder-width apart, and then one foot is placed behind on the ball of the foot (<i>ginga</i> position). The back foot returns to the initial position, and the other is placed back, imagining that a triangle is being drawn on the floor with the feet	≈ 50-60
Handstands	From <i>ginga</i> position, the hands are placed on the ground shoulder-width apart and the legs kick up, together, open or with one leg forwards	≈ 15
Cartwheels	Traditional movement, but performed slowly and with arms and legs slightly flexed	≈ 15
<i>Bênção</i> with jumps	Starts from the <i>ginga</i> position. A straight forward push kick is performed, with the ankle dorsi-flexed before returning to the base position. The movement was executed combined with a jump	≈ 8 each leg

2.5 Control activities

Control school participants were aware of neither the intervention activity nor the overall purpose of the study. Testing was undertaken as an educational activity focussed on measures of physical health. In all other respects the control school continued to undertake usual school activities over the course of the intervention year.

2.6 Data collection

Testing was designed to evaluate metabolic and musculoskeletal parameters. The majority of testing was conducted by the study investigators during physical education (PE) class time, while additional bone, muscle and fat measures took place at the Griffith University Bone Densitometry Research Laboratory.

2.7 Anthropometrics

Participants' height and sitting height were measured to the nearest millimeter using the stretch stature method with a portable stadiometer (HART Sport and Leisure, Brisbane, Australia) and a 50 cm flat stool. Weight was measured to the nearest 0.1 kg using digital scales (Soehnle, Hamburg, Switzerland), with output blinded from the children. Waist circumference was measured using an anthropometric tape measure (Lufkin Executive Thinline, Apex, USA) at the mid-point between the margin of the last pair of ribs and the iliac crest, taken at the end of gentle expiration [31]. Neither weight nor waist circumference measures were divulged to participants at the time of testing.

2.8 Assessment of maturity

Maturity was estimated by calculating years to age of peak height velocity (YAPHV) based on the single measurement of several anthropometric parameters and an algorithm formulated by Mirwald and colleagues [32]. The algorithm utilises sex, date of birth, height, sitting height and weight to predict the age of peak height velocity, which is then subtracted from chronological age to derive

YAPHV. We opted to use this non-intrusive method over Tanner staging in order to maintain participant privacy and to avoid recognised issues of under- and over-estimation of maturity during self-assessment [6].

2.9 Performance measures

Muscle power was determined by a maximal vertical jump test using a Yardstick (Swift Performance Equipment, Brisbane, Australia). Participants stood with their feet shoulder-width apart, shoulders level, and their preferred arm raised with an extended elbow to determine the height of a standing reach. A jump for maximal height was performed in countermovement fashion without arm swing to touch the highest possible peg on the Yardstick. Maximal vertical jump height was determined as the difference between the height of a standing reach and total jump height. The best of five attempts following a practice attempt was recorded.

Aerobic capacity was determined by a 20-meter shuttle-run test (a.k.a. the beep test). Participants ran between two points marked 20 meters apart with lap pace determined by a pre-recorded audible tone sounding at progressively shorter levels. When a participant was unable to meet the required pace on successive laps, the number of successfully completed levels was recorded. Using the algorithm developed by Leger et al. maximal oxygen consumption ($\text{VO}_2 \text{ max}$) was estimated according to the velocity associated with the level reached by the participant ($\text{VO}_2 \text{ max} = 31.025 + (3.238 \times \text{velocity}) - (3.248 \times \text{age}) + (0.1536 \times \text{age} \times \text{velocity})$) [33].

2.10 Cardiovascular measures

Resting heart rate (beats/min) was measured by a single investigator (RN) from the radial pulse after the participant had been resting supine for 15 minutes. Resting blood pressure (mmHg) was then measured manually using a stethoscope, sphygmomanometer and cuff. Participants were seated with their left arm extended and supported by the investigator. The cuff was placed around

the arm and inflated, before steadily deflating. The first and fifth Korotkoff sounds were used to designate systolic and diastolic blood pressures per usual practice.

2.11 Lifestyle characteristics

Validated questionnaires were used to estimate relevant physical activity participation and nutrient intake. The bone-specific physical activity questionnaire (BPAQ) was used to quantify historical bone-relevant physical activity participation [29]. Participants were asked to record all regular physical activities that they have participated in during their life and the years when participation took place. Participants recorded the specific frequency of regular physical activities performed in the last 12 months. Total, current and past BPAQ scores were then calculated from questionnaire responses using the on-line BPAQ calculator (<http://www.fithdysign.com/BPAQ/>). The intraclass correlation coefficients (ICC) for intra- and inter-operator reliability have previously been reported as 0.93, 0.97 and 0.97, and 0.86, 0.93 and 0.92, respectively for current, previous and total BPAQ-derived physical activity scores [34].

The Australian Child and Adolescent Eating Survey food frequency questionnaire (ACAES) was used to record food intake in order to estimate average nutritional intake over a three-day period [35]. Participants answered 120 items about their diet, and 15 supplementary questions about age, use of vitamin supplements and sedentary behaviour. The questionnaire has been validated for self-administration for children from nine to 16 years old and was completed independently during class time. The ACAES was computer analysed remotely per the questionnaire guidelines, and calcium consumption derived.

2.12 Bone measures

2.12.1 Calcaneal ultrasound

Broadband ultrasound attenuation (BUA) (dB/MHz) and stiffness index (SI) (%) of the non-dominant calcaneus were obtained from quantitative ultrasonometry (Lunar AchillesTM Insight, GE). Coefficients of variation for BUA and SI from a subsample (n=10) of repeated QUS measures with repositioning were 1.8% and 1.0%, respectively.

2.12.2 DXA and pQCT measures – a subsample data collection

Participants were invited to attend the Bone Densitometry Research Laboratory to undergo a full suite of dual-energy x-ray absorptiometry (DXA) (XR800, Norland Medical Systems, USA) and peripheral Quantitative Computed Tomography (pQCT) (XCT-3000, Stratec Medizintechnik GmbH, Pforzheim, Germany) measures, however only a small subsample (13%) were willing to do so. Bone mineral content (BMC) and density (BMD) of the whole body (WB), lumbar spine (LS) and non-dominant hip (femoral neck, FN; and trochanter, TR) were examined using DXA. Total lean and fat tissue mass as well as trunk lean and fat mass were derived from whole body DXA scans. Bone structural strength (IBS), material stiffness (E) and cross-sectional moment of inertia (CSMI) were calculated from hip and spine BMD scans as previously described.[36] Non-dominant tibial (4% and 38% proximal to the distal endplate) and radial (4% and 66% proximal to the distal endplate) bone strength measures were undertaken with pQCT. PQCT bone parameters reported were: total content, total density, total area, trabecular content, trabecular density, trabecular area, total and trabecular bone strength index (BSI) at 4% sites, and cortical content, cortical density, cortical area, cortical thickness, cross-sectional area (CSA), periosteal and endosteal circumferences at the proximal (38% and 66% sites). In addition, muscle cross-sectional area and muscle density from the 66% tibial site were measured from pQCT.

2.13 Statistical analyses

Both per protocol and intention to treat (ITT, mean values imputed) analyses were conducted. One-way ANOVA was used to examine baseline differences between EX and CON, while two-way

analysis of covariance (ANCOVA) was used to determine exercise effects. Covariates of weight, maturity, baseline values, physical activity and calcium consumption were employed to examine between-group differences.

A priori sample size estimation was calculated on the bone variable that typically exhibits the lowest measurement precision, that is, BUA. Based on a previously observed between-group effect, to observe a mean difference of 4 dB/MHz with a standard deviation of 12 dB/MHz [6] using two-way ANOVA, for 80% power with an alpha level of 0.05, we required a total of 142 participants (i.e. group n of 71). All statistical analyses were performed with SPSS version 21.0 for Windows (IBM, Chicago, USA).

3. RESULTS

3.1 Participant characteristics at baseline

Of the 151 girls who consented to participate in the study, 147 were available to undertake baseline testing (T0), and 138 for follow up testing (T9). There were 76 girls in EX and 75 in CON, 97% being Caucasian, and 3% Asian or Black. Three participants from CON did not attend baseline testing, while another five did not attend follow up, for a total of 67 participants in CON (11% attrition). One participant from EX did not attend baseline testing and four others did not participate in follow up testing, for a total of 71 participants in the EX (9% attrition) (Fig. 1). There were no differences in baseline physical or lifestyle characteristics between those who did not undergo follow up testing and those who did. As results of the intention to treat and per protocol analyses were essentially the same, we elected to report per protocol findings unless otherwise indicated. In total, data from 138 children were included in the per protocol analysis (i.e. 91% of the original consenting cohort).

Fig. 1. CONSORT diagram of participant flow.

At baseline, the combined sample averaged $10.6 (\pm 0.6)$ years old, weighed 38.3 ± 8.4 kg, and was 1.43 ± 0.07 m tall. Average BMI was 18.5 ± 3.1 kg/m² and waist circumference was 66.0 ± 9.9 cm. EX and CON were similar in almost all respects at baseline. EX exhibited a slightly lower estimated VO₂ max than CON (25.89 ± 4.98 vs. 32.80 ± 6.19 ml/kg/min, $p = 0.001$), which was managed by controlling for baseline 20-meter shuttle run test score in the analysis of treatment effect. Physical activity levels (current, past and total BPAQ scores) were very similar between groups (total BPAQ score: 37.3 ± 38.9 vs. 42.6 ± 41.2 , $p = 0.484$). Calcium intake was lower for EX than CON at baseline (848.6 ± 464.1 vs. 1148.3 ± 649.1 g, $p = 0.003$) and therefore was controlled in the ANCOVA for treatment effect.

3.2 Nine-month change in metabolic and performance measures

Changes in physical and performance measures for both groups are presented in Table 2. EX improved more than CON in resting heart rate (-7.2% vs. -1.8%, $p < 0.01$), maximal vertical jump (+13.4% vs. -1.2%, $p < 0.001$), estimated VO₂ max (+10.6% vs. +1.0%, $p < 0.001$), and waist circumference (+2.7% vs. +5.6%, $p < 0.001$) (Figure 2). Similar results were observed after controlling for skeletal maturity (i.e. YAPHV), baseline values and weight. While EX gained a significant amount of weight during the exercise period ($p < 0.05$), there was no between-group difference in weight change. No other significant between-group differences in change were observed for any other physical, metabolic, or performance parameter.

Fig. 2. Nine-month change (%) in waist circumference, vertical jump, resting heart rate and estimated VO₂ max for CON and EX groups following the 9-month exercise intervention.

Abbreviations: VO₂ max = maximal oxygen consumption.

Table 2: Baseline and Nine-month measures ($\pm$ SD) with percent change in physical and lifestyle characteristics (N = 138).

Parameter	Control (n = 67)			Intervention (n = 71)			p
	Baseline	Follow up	%	Baseline	Follow up	%	
Age	10.7 (0.6)	11.4 (0.6)	6.5	10.5 (0.6)	11.3 (0.6)	7.6	0.106
YAPHV (years)	-1.3 (0.6)	-0.7 (0.6)	-46.2	-1.2 (0.6)	-0.6 (0.6)	-50	0.394
Weight (kg)	37.2 (7.2)	39.3 (9.4) [†]	5.6	39.3 (9.4)	43.0 (10.2) [†]	9.4	0.391
Standing height (m)	1.425 (0.071)	1.464 (0.072)	2.7	1.442 (0.067)	1.482 (0.070)	2.8	0.230
Sitting height (m)	0.738 (0.035)	0.754 (0.036)	2.2	0.741 (0.035)	0.766 (0.039)	3.4	0.565
Waist Circumference (cm)	64.4 (8.2)	68.0 (8.6)	5.6	67.5 (11.1)	69.3 (10.6)	2.7	0.000*
Resting Heart Rate (beats/min)	67.2 (5.0)	66.0 (5.6)	-1.8	71.8 (7.2)	66.6 (6.5)	-7.2	0.012*
Blood Pressure (mmHg)	77.13 (7.23)	75.38 (7.58)	-2.3	72.52 (9.31)	72.83 (8.68)	0.4	0.408
Vertical jump (cm)	32.4 (5.4)**	32.0 (5.1)	-1.2	29.9 (5.5)**	33.9 (5.5)	13.4	0.000*
Estimated VO ₂ max	32.8 (6.19)**	33.12 (6.30) [†]	1.0	25.89 (4.98)**	28.63 (5.77) [†]	10.6	0.000*
BUA (dB/MHz)	94.4 (8.3)	95.7 (11.5)	1.4	94.2 (10.6)	98.8 (12.6)	4.9	0.046*
SI (%)	77.5 (7.9)	81.7 (11.3)	5.4	79.9 (8.8)	85.9 (12.4)	7.6	0.145

Abbreviations: YAPHV = Years to age of peak height velocity; BUA = Broadband ultrasound attenuation; SI = Stiffness Index; VO₂ max = maximal oxygen consumption. P values represent between-group comparisons of percent change.

* = $p \leq 0.05$; ** = $p \leq 0.05$, Difference between groups at baseline; [†] = $p \leq 0.05$, Difference between groups at follow up.

3.3 Nine-month change in bone, fat and lean tissue parameters

EX increased calcaneal BUA more than CON (+4.9% vs. +1.4%, $p = 0.05$), with a stronger level of significance observed from the ITT analysis ($p = 0.019$) (Figure 3). No differences were found in SI (Table 2). For the sub-sample of participants who underwent DXA scans (CON = 6; EX = 12), greater improvements in LS IBS were observed for EX girls compared to CON (+24.4% vs. +12.0%, $p = 0.006$) (Table 3). Although no other comparisons of densitometric parameters reached significance, girls from EX tended to increase both lean (+15.5% vs. +9.2%, $p = 0.353$) and fat mass (+7.6% vs. +0.1%, $p = 0.598$) more than CON. There were no significant between-group differences in change in DXA-derived trunk fat and lean mass.

Radius total bone mineral content at the 4% site tended to increase more in EX than CON (+36.1% vs. +10.7%, $p = 0.065$), and there was a tendency for greater improvements in radial cortical density at the 66% site in EX than CON (+2.7% vs. +0.3%, $p = 0.072$). There were no significant between-group differences in any pQCT-derived tibial parameters, although a tendency for greater change in tibia total density at 4% was more apparent for EX than CON (+11.5% vs. -1.2%, $p = 0.234$) (Table 3).

Table 3: Baseline and Nine-month measures ($\pm$ SD) for subgroup with percent change in DXA and pQCT Tibial and radial measures (N = 18).

Parameter	Control (n = 6)			Intervention (n = 12)			p
	Baseline	Follow up	%	Baseline	Follow up	%	
Lean Mass (Kg)	23.27 (3.50)	25.40 (4.93)	9.2	23.44 (5.27)	27.08 (6.19)	15.5	0.353
Fat Mass (Kg)	13.57 (3.88)	13.59 (3.68)	0.1	15.95 (5.32)	17.16 (6.46)	7.6	0.598
WB BMC (g)	1561.67 (87.44)	1701.50 (117.21)	9.0	1523.67 (225.67)	1732.8 (314.97)	13.7	0.443
WB BMD (g/cm ²)	0.729 (0.020)	0.744 (0.045)	2.1	0.705 (0.062)	0.758 (0.085)	7.5	0.083
LS BMC (g)	21.1 (3.0)	23.2 (3.6)	10.0	20.4 (4.2)	24.8 (7.2)	21.6	0.249
LS BMD (g/cm ²)	0.686 (0.072)	0.729 (0.058)	6.3	0.653 (0.091)	0.725 (0.124)	11.0	0.318
LS IBS (g ² /cm ⁴)	0.606 (0.154)	0.679 (0.113)	12.0	0.553 (0.154)	0.688 (0.238)	24.4	0.006*
FN BMC (g)	3.051 (0.278)	3.243 (0.387)	6.3	2.770 (0.455)	3.112 (0.559)	12.3	0.309
FN BMD (g/cm ²)	0.712 (0.062)	0.757 (0.077)	6.3	0.672 (0.085)	0.739 (0.105)	10.0	0.590
Tibial total content 4% (mg)	199.38 (19.19)	197.21 (19.76)	-1.1	176.16 (28.61)	186.28 (21.67)	5.7	0.867
Tibial trabecular density 4% (mg/cm ³)	243.05 (10.15)	233.60 (10.74)	-3.9	232.64 (26.05)	230.38 (26.63)	-1.0	0.790
Tibial cortical density 38% (mg/cm ³)	1046.33 (25.57)	1049.30 (26.59)	0.3	1032.96 (34.67)	1049.43 (25.99)	1.6	.965
Radial Total content 4% (mg)	48.40 (10.91)	50.41 (5.16)	4.1	41.20 (6.65)	52.66 (11.17)	27.8	0.065
Radial Trabecular density 4% (mg/cm ³)	219.56 (19.12)	224.08 (23.39)	2.1	218.32 (29.85)	230.23 (22.75)	2.2	0.674
Radial Cortical density 38% (mg/cm ³)	1004.74 (34.50)	1008.04 (25.94)	0.3	992.41 (42.25)	1019.5 (32.61)	2.7	0.072

Abbreviations: BMC = bone mineral content; BMD = bone mineral density; FN = femoral neck; LS = lumbar spine; WB = whole body; TR = trochanter; IBS = index of bone structural strength. P values represent between-group comparison of percent change. * = $p \leq 0.05$; ** = $p \leq 0.05$, Difference between groups at baseline; † = $p \leq 0.05$, Difference between groups at follow up.

Fig. 3. Nine-month change (%) in BUA and WB, tibial and radial BMD for CON and EX groups following the 9-month exercise intervention.

Abbreviations: BUA = Broadband ultrasound attenuation.

3.4 Compliance

Overall loss to follow-up was 9% and was related mainly to student relocation or absence from school on the days of testing. Mean compliance for the exercise intervention was 90%, with missed sessions directly attributable to absence from school.

4. DISCUSSION

Our aim was to determine the effect of a novel, targeted, thrice weekly, brief, in-school exercise regime (i.e. *capoeira* combined with jumping) for a school year on parameters of musculoskeletal and metabolic health in pre- and early-pubertal girls. We found that the exercise intervention

improved calcaneal BUA, resting heart rate, waist circumference, vertical jump and VO₂ max. Improvements were also observed in lumbar spine strength and a number of parameters of bone geometry as a result of the intervention, but the subsample of DXA and pQCT measure was insufficiently powered to be conclusive. Students were highly engaged with the activity during class, and informal feedback from staff and students was very positive.

BUA, a measure of frequency dependence of ultrasound attenuation, has been shown to predict bone strength, due to the relationship with trabecular microstructure and mechanical properties [37]. There is some evidence that exercise may improve the BUA of children [6, 38, 39] and adults [40]. For example, an 8-month, 10-minute jumping-related intervention improved BUA of adolescent girls [6], and a 3-month exercise intervention improved bone strength in obese children [38]. Our intervention likewise improved BUA of EX compared to CON reinforcing the ability of brief, high intensity impact loading to stimulate positive adaptive responses in growing bones.

Numerous studies have demonstrated that school-based weight-bearing exercise interventions can improve DXA-derived bone parameters of children [6, 16, 41-49]. For instance, a 7-month jumping program (10 mins jumping, 3/week) improved FN BMC, LS BMC and BMD of prepubescent children [50]. Others reported improvement in WB and LS BMC, but not FN for pre and early pubertal girls after an 11-month trial of skipping and aerobic activities plus 30 counter movement jumps (15 mins/day, 5/week) [43]. In addition, an 8-month twice-weekly 10-minute jumping intervention improved FN BMC and LS BMAD in peri-pubertal female adolescents [6]. By contrast, combined aerobic and strength training 4 times per week with 500mg of calcium per day for 9 months improved spine BMC but not BMD at any measured site in adolescent girls [44]. A number of other exercise interventions have not evoked BMD effects in pre, early or peripubertal children [6, 44, 46]. We similarly did not observe between-group differences in DXA-derived BMD and BMC measures following the CAPO Kids intervention for the sub-sample group. While it is possible the intervention did not adequately stimulate bone, our

lack of ability to detect DXA-derived bone mass differences between groups is potentially a function of the relatively small subsample who were willing to attend the densitometry laboratory for measures. Calcium intake (CON 1150 mg/day, EX 850 mg/day) was somewhat lower than the current Recommended Dietary Allowance (RDA), which is 1300 mg per day for children from nine to thirteen years old, although CON reached the Estimated Average requirement (EAR) of 1100 mg per day [51, 52]. That our exercise intervention effected positive change in the girls in the intervention group, despite lower than recommended calcium intake, is encouraging in light of the common notion that between 850 and 1000 mg of calcium per day is required for an exercise effect in children [53, 54].

In accord with a number of other findings of pediatric exercise interventions [41, 55], we did not detect significant between-group differences in pQCT-derived parameters of bone geometry or volume. For example, nine months of twice-weekly step-aerobics did not improve tibial cortical density, CSA and BSI of pre and post menarcheal girls [41]. Likewise, a 12-week intervention composed of 25 jumps daily from a 45-cm box did not improve bone at trabecular sites (e.g. the 4% sites of tibia and radius) during a period of rapid growth [55]. Our exercise intervention was novel in that it was specifically designed to also improve the strength of upper extremity bones that are not typically loaded during weight bearing exercise, but are at considerable risk of childhood and osteoporotic fracture subsequent to a fall [56]. The tendency for the EX group to improve total content and cortical density of the radius may reflect a positive effect of the novel upper extremity loading stimulus.

It is frequently asserted that exercise is most osteogenic if undertaken prior to puberty, and there is some evidence to support the assertion [17, 41]. It is important to note however, that much of the evidence is observational [57] or based on data sets that have only included pre-pubertal children [46]. In fact, a growing body of evidence exists to suggest it is not universally the case [6, 58]. For instance, Petit and colleagues [58] did not observe change in FN BMD in prepubertal girls

after a 7-month intervention of twice-weekly 10 to 12 minutes of high impact exercise, but significant gains were detected in early pubertal girls. We could not perform a direct comparison of response in our study in light of the narrow age range of our participants; however we note that controlling for years to age at peak height velocity did not change the outcomes of our analyses. That is, responses to our exercise program could not be attributed to maturity.

Waist circumference predicts visceral abdominal adiposity, which is closely associated with obesity and metabolic syndrome [59]. As waist circumference increases during growth, our observation of increased waist circumference in both groups was to be expected. That the increase for CON was significantly greater than EX suggests the exercise intervention prevented a degree of accumulation of abdominal adiposity over the course of the 9 months and is in accord with a previous report [30]. Our recent meta-analysis [19] revealed that a number of bone-targeted exercise programs have previously evoked positive adaptations in both bone *and* fat in childhood. For example, a 9-month, 10-minute jumping and non-impact activity intervention program improved FN BMD and BMC and reduced fat mass in prepubertal girls [48]. Studies that implemented longer duration moderate to vigorous physical activities (MVPA) plus high impact exercises especially found positive results for fat reduction after intervention [16, 45]. For instance, a 30-min MVPA combined with weight bearing training for 10 months showed improvement in not only WB, LS and FN BMD and BMC, but also total body fat [16]. Our findings also suggest that positive changes in body composition can be evoked from simple, brief, bone-specific training in youth.

In addition to improving bone and fat mass, muscle loading during high impact exercises also can increase lean mass and muscle strength [6, 16, 44-46, 48]. Our observation that the current exercise intervention tended to increase lean mass supports the findings of our meta-analysis [19] that bone-specific exercise tends to be beneficial to both bone *and* muscle mass [6, 48]. The above-mentioned weight-bearing and MVPA studies also observed improvements in lean mass

after exercise training that improved bone [16, 45]. The significant improvements we observed in estimated VO₂ max and maximum vertical jump suggest that lean mass improvements were accompanied by meaningful performance benefits.

4.1 Limitations

The primary limitation of the current study was the relatively low number of participants who were able to attend testing at the Griffith University Bone Densitometry Research Laboratory. Those low numbers likely reduced our ability to detect between-group differences in DXA- and pQCT-derived bone parameters. We attempted to address the predicted reluctance of parents to bring their children for testing on campus outside of school hours by taking our portable QUS device to the schools in order to capture heel bone data from the full cohort along with all other in-school measures. Maximal vertical jump and VO₂ max were significantly different between the intervention and control school students at baseline. Although we adjusted for baseline values in our analyses, it is possible that differences in other components of fitness that were not measured may have influenced our findings. We were unable to draw blood to examine Vitamin D status of the children, however it is likely they were producing sufficient levels of serum 25(OH)D based on the latitude and sunny environment of the study location (28° 0' 0" S / 153° 26' 0" E).

5. CONCLUSION

Our simple, novel, nine-month, in-school exercise intervention of thrice weekly, 10 minutes capoeira plus jumping (CAPO Kids) improved musculoskeletal, metabolic and fat outcomes in pre- and early-pubertal girls in comparison with controls. Furthermore, the program was found to be enjoyable and feasible for all participants and amenable to incorporation into a primary school

day without disruption to the academic schedule. Based on observations from previous data sets [60], there is reason for optimism that the beneficial effects from the intervention will be sustained. Long term follow up data collection will allow us to test the hypothesis. Findings provide optimism that similar interventions can be adopted more broadly in primary school systems to optimise the health of children at a critical stage in their physical development in order to minimise the risk of lifetime chronic diseases.

Disclosure:

All authors state that they have no conflicts of interest to disclose.

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