***A cross-sectional study of the relationship between recreational sporting activity and calcaneal bone density in adolescents and young adults.***

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Abstract

*Objective*: Childhood and adolescence are critical periods of bone development. Sporting activity is thought to impact peak bone mass acquisition, but most studies have used dual energy X-ray absorptiometry (DXA) to assess bone health and reported associations between bone mass and elite sporting activity. The objective of this study was instead to assess the relationship between recreational sporting activity (RSA) and another bone assessment, calcaneal quantitative ultrasound (cQUS), in adolescents and young adults.

*Methods*: We related recreational sporting activity, assessed through a lifestyle questionnaire, to heel ultrasound bone parameters in a cohort of New Zealand students aged 16 - 35 years. Complete datasets with data on all relevant confounders (body mass index (BMI), pubertal timing, smoking status and alcohol consumption) were available for 452 participants. cQUS was performed using a Lunar Achilles EX II machine to obtain bone parameters, broadband ultrasound attenuation (BUA) and speed of sound (SOS); stiffness index (SI) was derived from these measures. All descriptive statistics and statistical analyses were carried out using SPSS Statistics for Macintosh, Version 23.0 (IBM Corp., Armonk, NY, USA). Results are presented as *p*-values and 95% CI.

*Results*: Reported lifetime sport participation declined after an individual’s mid-teens. Bone cQUS parameters (SI and BUA and T-score) were all positively associated with BMI, and current physical activity (SI, SOS, BUA, T-score and Z-score) with SI and SOS measures most strongly associated with current high impact and past recreational sporting activity (all p<0.05).

*Conclusion*: Calcaneal heel ultrasound bone parameters were associated with physical activity, with SI and SOS rather than BUA more strongly related to current and past recreational sporting activity in young New Zealand adults.

*Keywords*: Adolescent, Bone, Calcaneal quantitative ultrasound (cQUS), Cross-sectional, Recreational sporting activity (RSA)

**Introduction**

Osteoporosis is a major public health problem through its association with fragility fracture [1]. Osteoporosis is often described as a disease which occurs when one becomes older, is more commonly described in females and preventative methods often focus on older people [2]. However, optimisation of peak bone mass (PBM) is critical during the periods of childhood and adolescent bone development [3]. Modifiable lifestyle behaviours such as physical activity (PA) have a major impact on the development of bones throughout life, with impact sports appearing more beneficial to bone mineral accrual than non-impact sport [4]. PBM is hence a major determinant of later fracture risk [5,6]. The importance of engagement in sporting activity in childhood and adolescence was recently demonstrated by Mantovani and colleagues, who found that men who reported they were inactive in childhood and adolescence had bone mineral content around 11% lower than active men in childhood or adolescence, whereas for women, this difference was larger at 14% [7].

Despite these observations, only a limited number of studies have examined the impact of recreational sporting activity (RSA), that is sport undertaken at a non-elite level, on the bone health of young people in adolescence and young adulthood. To date, most studies that have been undertaken have focused on the effect of elite levels of sporting activity on bone health as assessed by dual energy X-ray absorptiometry (DXA) [2,8], with a recent systematic review by our group highlighting the need to consider the relationship between RSA and other bone outcomes [9].

Although DXA is considered the “*gold standard*” of diagnostic tests for osteoporosis, an advantage of other techniques such as calcaneal quantitative ultrasound (cQUS) over DXA is that cQUS may reflect subtle changes to the bone such as the microarchitecture, giving insight to the elasticity and thickness mediated by lifestyle factors such as PA in adolescence [10-12]. There has been an increasing interest in the use of cQUS as an alternative assessment of bone health that also provides structural information on bones, and which has been shown to be responsive to exercise in all age groups [13]. Furthermore, ultrasound technology is a non-invasive, widely available, inexpensive and portable tool that provides an assessment of bone density and quality at a readily accessible weight-bearing site with a high trabecular bone content. It has been shown to be able to discern risk of fragility fracture in older adults [14,15], with the cQUS bone outcomes stiffness index (SI) appearing more predictive of fracture than speed of sound (SOS) or broadband ultrasound attenuation (BUA) [16]. The technique provides estimates of SOS and BUA by the transmission of a signal through the calcaneus bone of the heel. SI is derived from these variables, as is an estimate of bone mineral density (BMD), reported as a T-score or Z-score. Previous studies have found cQUS to be a practical method of assessing the skeletal health of normal healthy children [17].

The aim of this study was therefore to assess the relationship between RSA and bone health, as assessed by cQUS in a group of New Zealand adolescents and young adults, recruited from schools, a local university and local gyms.

**Methods**

Study design

Study recruitment was undertaken from March 2017 to October 2018 in the Wellington region, New Zealand. Ethical approval was obtained from the New Zealand Health and Disability Ethics Committee (reference # HDEC 18/CEN/18). A student population was recruited, together with other adolescents and young adults associated with the university campus or local gyms, with school children recruited from local colleges and all participants aged 16 to 35 years; no other restrictions were in place. Recruitment took place through appropriate email mailings, interactions with teachers at the college and advertising on the university campus and at local gyms in the Wellington area. Participants were invited to pass study flyers to others to encourage participation. We obtained written informed consent forms signed by participants.

The questionnaire incorporated validated sporting activity questions adapted from studies by Weeks et al (2008) and Kowalski et al (2004), modified for the New Zealand population, to determine the modified past recreational sporting activity history score, modified weekly PA score and weekly sporting activity [18-23]. The modified Kowalski questionnaire items are scored between 1 (low) and 5 (high PA), with previous studies in similar samples typically reporting scores of approximately 2.6 for school aged children [24,25]. PA recorded included the duration and types of sport undertaken regularly. Respondents self-reported up to eight habitual recreational sporting activities engaged in over their lifetime in order to calculate a past habitual recreational sporting activity history score from age 0 to the present day. This score was based on the bone physical activity questionnaire score (BPAQ) which uses an algorithm that includes the effective load stimulus (ELS) derived from ground reaction force test, years of sport participation and an age-weighting factor [18,26]. Age-weightings were incorporated into the algorithm by Weeks et al (2008) to recognise the greater osteogenic effects of exercise during growth as opposed to exercise after skeletal maturity [18]. As such, weighting did not specifically differentiate between those younger participants aged 16 - 17 years compared to those in their thirties except through their linear age. Respondents also detailed current PA and current sporting activity [19]. Current weekly sporting PA was further categorised as either: high impact, weight-bearing; weight-bearing; or non-weight-bearing sporting activities based on supporting literature [18,27].

The questionnaire was used to obtain participants’ age, height, weight, ethnicity, personal and family history of fracture, alcohol intake, calcium intake, physical and sporting activity and past medical history. Pubertal timing was self-assessed with reference to the age of menarche for females and start of growth of facial hair for males. Use of the oral contraceptive pill and menstrual function was recorded. Smoking status was recorded as either never smoked or smoker. The questionnaire was implemented through the Qualtrics software programme (Qualtrics, Provo, UT).

A heel ultrasound test was performed (using an GE Achilles heel ultrasound machine) to obtain measures of BUA, SOS and SI. Estimated BMD was calculated by the machine from an algorithm utilising these parameters, and T- and Z-scores were recorded for females (aged 20 and above), representing a comparison to a young adult mean (T-score) and an age matched mean (Z-score) [28]. Quality Assurance (QA) was performed each time the machine was used. Following this the participant’s heel was sprayed with alcohol to ensure correct membrane contact, with the foot placed on the foot plate and their calf securely placed against the calf rest. The scan took approximately 10-12 seconds. Measurements were made of both heels.

In cQUS measurement, ultrasound pulses are generated as sound waves to produce two bone measures: velocity (SOS in m/sec) and frequency attenuation (BUA in dB/MHz). Sound wave energy is absorbed as it travels through heel bone and soft tissue. As such, the measures generated are dependent on the structure, chemical composition and mineral content in bone (hydroxyapatite) and soft tissues. Given this, these measures are qualitatively different to DXA, that uses ionising radiation [29]. The Achilles machine expresses ultrasound results as SOS and BUA; the SI measurement is a composite measure that is formed from the algorithm of the SOS and BUA measures [16]. There is good correlation (r>0.8) between measurements obtained by ultrasound and heel bone density *in vitro* [30].

The same ultrasound machine was used throughout the study. We evaluated the short-term *in vivo* precision of cQUS from triplicate scans in 15 participants. The overall coefficient of variation (%CV) varied from -0.5% to 0.67% for bone mass measurements for all bone measures: (T-score from -0.5% to -0.33%; Z-score from -0.30% to 0.67%; SI from 0.0% to 0.06%; BUA from 0% to 0.09%; and SOS from 0% to 0.01%) [16].

Statistical methods

Four hundred and fifty two participants had a heel ultrasound and completed the full online questionnaire. All descriptive statistics and statistical analyses were carried out using SPSS Statistics for Macintosh, Version 23.0 (IBM Corp., Armonk, NY, USA). Results are presented as p values and 95%CI. We first assessed univariate associations between predictors and cQUS bone measures.

Predictors included: gender; ethnicity; age; BMI; height; weight; pubertal timing; smoking; alcohol consumption; fracture (age of first broken bone, fracture history, family fracture history); daily calcium consumption; weekly PA Kowalski score; weekly sporting PA; past recreational sporting activity history; and oral contraceptive use. Sporting PA was further categorised into: 1) high impact, weight-bearing (HIWB) activity; 2) a combination of HIWB and weight-bearing (WB) activity; 3) a combination of HIWB, WB + non-weight-bearing (NWB) activity; and 4) NWB activity (see Table 1). Ethnicity was self-reported and then categorised into European, Māori and Pacific people, Asian, Indian, Middle Eastern, Latin American and African groups. Detailed sub-group analysis was prohibited by the sample numbers of the different ethnicities recruited in the study. In the preliminary analyses the participants’ ethnicity was divided into three categories (“European”, “Māori and Pacific people” and “Others” who were neither European, Māori nor Pacific people). In the final analysis participants were divided into two groups (“European” and “Others” that included non-Europeans, Māori and Pacific people).

Those variables that were associated on univariate analysis at a level of *p* ≤ 0.1 were then included in multiple linear regression analyses. Variables included in the regression analysis were: BMI; age; gender; ethnicity; smoking status; alcohol consumption; pubertal timing; daily calcium consumption; PA; weekly sporting categories; and past recreational sporting activity. For each analysis, approximate normality was confirmed with histograms, Q-Q plots of residuals, residuals versus fitted plots indicated homoscedasticity of residuals, and Cook’s distance did not identify any influential cases.

An additional analysis for the top ten popular sports was included, using multiple linear regression analysis to predict bone health from the sports played, age, gender, BMI and ethnicity. For descriptive statistics, the results were reported as mean ± standard deviation (SD) or count and percent in each category. All models that considered cQUS bone parameters as an outcome had >90% power to detect R2 > 0%.

**Results**

We recruited a total of 920 participants who all underwent heel ultrasound. However, in this analysis 452 participants undertook the cQUS assessment and provided complete questionnaire data regarding sporting activity. Summary statistics for the study sub-population were considered. There were no significant differences between age, sex, BMI, scores for Kowalski's PA, pBPAQ, and heel ultrasound parameters in the subsample compared with the number recruited for heel ultrasound. Summary statistics for the participants included in this analysis are displayed in Table 1 and include smoking, alcohol consumption and dietary calcium intake.

*Physical activity*

Current weekly PA was similar in both sexes, the time spent in these activities and the mean past recreational sporting activity history score are displayed in Table 1.

*Physical activity and cQUS*

Supplementary Table 1 demonstrates relationships between lifestyle and demographic factors and cQUS bone measures. Associations were found with BMI, later puberty, PA, high impact weight-bearing sporting activity, non-weight-bearing sporting activity, past recreational sporting activity score, ethnicity and alcohol intake above the median of 3.5 units per week. Prior fracture was associated with a lower SOS in women, but not in men. Current PA was positively associated with all bone parameters in both sexes but interestingly, despite a smaller sample size, many relationships were stronger in men. For example, past recreational sporting activity score was positively associated with SOS and SI for men, but not for women. Weekly number of minutes spent performing weight-bearing high impact sporting activity was positively associated with SI and SOS in both sexes, but was additionally associated with BUA in men.

Two sets of multivariate analyses were performed, one with gender as a covariate and one by gender.

Table 2 shows the questionnaire variables associated with the three cQUS measures (SI, SOS and BUA) on multiple linear regression. T- and Z-scores were only available for women (aged 20 and above), so the analysis for T-scores excluded gender as a predictor and the analysis for Z-scores excluded both gender and age. We saw differences in relationships between the questionnaire variables and the various cQUS outcomes (Supplementary Table 2). Hence while current PA was strongly associated with SI, SOS and BUA bone parameters (<=0.001), past sporting activity was more related to SI and SOS than BUA (p<0.1), and age, gender and alcohol intake was associated with BUA rather than SI or SOS. Current BMI was positively associated with SI and BUA.

*Recalled RSA*

Figure 1 is a graphical demonstration of recalled RSA over the lifespan of the study population. While most participants reported playing at least one sport over their lives, it was found that during the mid-teens to late-teens there was a decline in reported RSA, with a higher proportion of participants reporting that they did not engage in sporting activity at older ages.

*Participation in individual sports and cQUS*

There were 104 different types of RSA indicated by participants which were played over a range of one to 13.5 years by the participants. The top 20 most commonly reported activities were running, swimming, netball, soccer, walking, resistance, cycling, dance, rugby, badminton, basketball, tennis, hockey, cricket, hiking, volleyball, gymnastics, ballet, yoga and athletics. A positive correlation was seen in both men and women between bone cQUS measures and those with a history of running or playing basketball, soccer, rugby or cricket. Participants in those sports recorded significantly better bone health measurements than those who did not play those sports. Those participants who reported walking as a sporting activity actually had comparatively poorer bone health. Finally, we observed higher T-score and Z-scores in women who played rugby.

Finally, for each bone health measure, a multiple linear regression analysis was run with cQUS as the outcome with the top ten sports played as exposures, with age, sex, BMI and ethnicity included in these models. A summary of the results are shown in Table 3. Supplementary Table 3 results indicates that bone SI was significantly higher in participants that reported running (*p* = 0.022) and playing soccer (*p* = 0.047), and significantly lower in participants that reported walking (*p* = 0.009). Bone SOS was significantly higher in participants that reported running (*p* = 0.01). Bone BUA was significantly higher in participants that reported playing soccer (*p* = 0.042). Both T-score and Z-score were significantly higher in participants that reported playing rugby (*p* = 0.034 and *p* = 0.035, respectively). When the sexes were considered individually, playing soccer was associated with higher SI, SOS and BUA measures in males but not in females, while playing rugby was associated with higher bone parameters for females. In females, walking and cycling were negatively associated with SI and SOS but no such relationship was apparent in men. There were no statistically significant interactions between calcium intake and PA in these models (all *p* > .05).

**Discussion**

This study has found that levels of past recreational sporting activity, current PA and other lifestyle factors were associated with measures of bone health in an adolescent and young adult population in New Zealand. Our findings chime with a previous systematic review and meta-analysis of global participation in sport and leisure-time physical activities that highlighted high levels of activity among individuals from the Western Pacific (Australia, China, Hong Kong, Japan, New Zealand and the Philippines) [36]. In addition, a 2018 report by Sports New Zealand suggested that 72% of adults participate in weekly physical active play, exercise, recreation or sport, with over 90% participation amongst those aged between 15 to 17 years [37]. Therefore, the results of our study may be a reflection of the local New Zealand population in this age group. However, similar to other reports we found that this level of recalled activity declined during adolescence.

While this study was unable to indicate which specific sports were more beneficial to bone health, the results appeared to suggest that adolescents and young adults who participated in weight-bearing sports (such as rugby, soccer and running) enjoyed better bone health. These results are consistent with previously published data studies that have considered cQUS as an outcome and the benefits of participation in individual sports on bone health [4,36-38]; such studies suggested that football or dancing conferred better benefits to bone health than swimming or cycling [38,39], and that gymnasts enjoyed better bone health compared to controls [40]. Unexpectedly, walking was negatively associated with bone health in this group. It is possible that participants not engaged in regular sports chose to report “walking” as a form of sport rather than appear inactive, while others that engaged in organised sporting activity did not include time spent walking which in effect serves almost as a baseline activity level. Adiposity and consequent perturbation of the GH/ IGF1 axis [growth hormone (GH) and insulin-like growth factor-1 (IGF-1)] may contribute to these relationships, although our observations remained significant after adjustment for BMI [41,42].

This study has a number of strengths and limitations. The sample size was larger than other studies of cQUS and RSA, with information on relevant confounders collected by questionnaire, although a large number of participants failed to complete the whole questionnaire as so were not included in this analysis. Some information, including height and weight, were obtained by self-completed questionnaires, which may lead to recall bias. In addition, we were unable to provide in-depth separate analyses for each sport group separately, as many participants reported a number of activities. Sporting activity was self-reported and not validated by accelerometers, therefore intensity of the activity was not objectively reported. The questionnaire we used incorporated validated sporting activity questions adapted from studies by Weeks et al. (2008) and Kowalski et al. (2004), modified for the New Zealand population [18,19]. However, assessing historical RSA relies on recall which may be prone to bias, with the possibility of over or under reporting past levels of sporting activity. Over reporting activity intuitively appears more likely. We also note that there were a very large number of explored associations in the analysis performed in this study, and there were no adjustments made for multiple comparisons. The information provided by *p*-value may be limited and we acknowledge that the 95%CI gives more information than a single *p*-value about how consistent the relationship is rather whether it is significant or not. We have hence reported both the *p*-values and 95%CI given to allow better interpretation of our findings. Therefore, following this exploratory analysis, it is important that the associations we report are verified in subsequent studies with larger sample numbers to establish the benefits of specific recreational sporting activities.

Date of age at menarche and appearance of facial hair was reported to the nearest year for all events. Facial hair development has been reported as a marker of gonadal hormones [43,44]. We note that while self-reported secondary sexual characteristics is a more common way to assess contemporaneous progression through puberty [45,46], correlation with physical examination can be modest [47]. As such, we considered that the use of such correlations was not necessary for this study. We sought to identify outliers in regard to pubertal progression only, as this could have impacted bone development.

Importantly, we based this study at a university campus and a high proportion of the subjects recruited were university students, although we also included participants recruited at local gyms and colleges. Although the generalisability of this study may therefore be considered limited to the university environment, the participants came from different backgrounds and a range of locations within New Zealand and internationally, ensuring a wide ethnic mix; although some groups were not sufficiently represented to allow relationships between individual ethnicities and bone health to be reported. It should be noted that a high proportion of young people access university education in New Zealand, higher than in many other countries; in New Zealand in 2018 there were 61,297 domestic school leavers and of these, 59.7% (36,582 students) enrolled in tertiary education at all levels during 2019 [48]. It will of course be important to replicate these findings in other settings to ensure generalisability of our results.

We observed benefits of high impact weight-bearing activity in our study. It is generally accepted that mechanical load induced by exercise training enhances osteoblastic activity [49-51]. However, not all exercise modalities are equally osteogenic. Weight-bearing high impact exercise such as hopping and jumping has been shown to have the greatest benefits [52]. Bone formation is increased in regions of high strain, with the skeletal effect of mechanical loading being site-specific, with greater responses at skeletal sites where loading impacts are greater [2,6,53]. The majority of weight-bearing exercises elicit physical loading to the lower limbs. To generate the adaptive response of bone to mechanical loading, sufficient magnitude, rate and frequency of loading are necessary if adequate intensity of loading is to be achieved: relatively few loading repetitions are sufficient to generate an adaptive skeletal response [54]. Further, as osteocytes are desensitised by repetitive loading, short bouts with intervals of rest are more beneficial than the same number of loads performed continuously [55]. Sporting activity is particularly important as it has been suggested that adaptations to mechanical loading in adolescence and young adulthood may be translated into greater bone strength over a lifetime [56]. Bones may become less sensitive to mechanical loading after skeletal maturity is reached at 18 to 25 years of age [57].

In this study we chose cQUS as the bone outcome measure, rather than fracture. Fractures in adolescence and young adulthood often reflect significant trauma, and it is controversial how they relate to later osteoporotic fracture [58-60]. The heel bone is an easily accessible, weight-bearing site, composed mostly of trabecular bone, and intuitively might be expected to respond to exercise. Similar to the vertebral spine, it has a bone high turnover. A limitation of this approach is that we do not have measures of lean mass in this group; muscle bone interactions are very important in determining bone health, but might be expected to have a greater effect at the hip [61]. A systematic review and meta-analysis of twelve longitudinal studies attested to the sensitivity of cQUS to exercise induced changes in bone health [13]. Earlier studies found comparisons with DXA and cQUS, and indicated that cQUS may detect low BMD in children with fragility fractures and that BUA parameters have been shown to reflect BMD changes in bone development [12,14,62,63]. However, no study to date has focused on different predictors of outcome for the different cQUS measures made and this will be a fascinating area to explore further.

In addition to the differences in relationships seen with physical and sporting activity, we also observed some sex differences. Interestingly some relationships observed were stronger in men than women, and further work is required to determine whether these are real differences and what the biological explanation may be. Previous studies have suggested that sexual dimorphism may operate in the relationships seen [64,65]. Our finding of stronger relationships in men than women could reflect chance, differing sporting profiles in men and women leading to different relationships seen, or an underlying biological explanation for the sex difference observed. Given the relatively small sample size of men recruited to this study, this is a particularly intriguing question that future studies might address.

Finally, dietary dairy intake, vitamin D and PA together potentially enhance bone health during childhood and adolescence [66,67]. Previous reviews have suggested that PA and dietary calcium intake during childhood and adolescence play a critical, synergistic role with the potential to improve bone health with sufficient calcium intake [6,68]. The dietary intake of calcium by recall was assessed in this study, with the average daily calcium intake across participants falling well below the recommended dietary requirement of 1000mg or more for this age group [69]. The relationship between cQUS and sporting activity has been shown to indicate differential levels of benefit according to levels of milk intake [70,71]. In this present study, we did not find evidence of an interaction between dietary calcium with bone health in this group. This may reflect the dietary calcium intakes we observed in this group: a calcium supplementation intervention study of young gymnasts found that with sufficient intake of dietary calcium, additional calcium supplementation provides no additional benefit [72].

**Conclusion**

In this cross-sectional study, we reported levels of recreational sporting activity in New Zealand adolescents and young adults, as well as relationships between cQUS and current and past sporting activity, including weight-bearing high impact sports such as running, soccer and rugby. The benefits of RSA need further longitudinal investigation in other populations where validated measures can be made to determine a dose-relationship, and assessment of lower limb muscle mass made.

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**Table 1**: Summary of participant characteristics providing full questionnaire data (*n* = 452)

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Participant descriptive characteristics | | **Female** | | | | **Male** | | | |
| Variable | Variable details | ***Mean ± SD*** | ***Category (n)*** | ***%*** | ***Respondents (n)*** | ***Mean ± SD*** | ***Category (n)*** | ***%*** | ***Respondents (n)*** |
| Age (years) | *Years* | 21.7 *±* 4.3 |  |  | 309 | 23.5 *±* 4.4 |  |  | 143 |
| Participant source | *University* |  | 217 | 70.2 | 309 |  | 119  24  0 | 83.2 | 143 |
| *Sports complex* | 43 | 13.9 | 309 | 16.8 | 143 |
| *School* | 49 | 15.9 | 309 | 0 | 143 |
| Ethnicity (six ethnicities) | *European* |  | 216 | 69.9 | 309 |  | 97 | 67.8 | 143 |
| *Māori and Pacific people* |  | 33 | 10.7 | 309 |  | 15 | 10.5 | 143 |
| *Asian* |  | 34 | 11 | 309 |  | 14 | 9.8 | 143 |
| *Indian* |  | 16 | 5.2 | 309 |  | 12 | 8.4 | 143 |
| *Middle Eastern Latin America* |  | 6 | 1.9 | 309 |  | 2 | 1.4 | 143 |
| *African* |  | 4 | 1.3 | 309 |  | 3 | 2.1 | 143 |
| BMI (kg/m2) | *Self-reported BMI (kg/m2)* | 23.7 *±* 4.2 |  |  | 309 | 24.2 *±* 4.3 |  |  | 143 |
| Bone measures | *SI* | 113.0 *±* 16.2 |  |  | 309 | 116.0 *±* 19.5 |  |  | 143 |
|  | *SOS* | 1605.9 *±* 38.2 |  |  | 309 | 1606.6 *±* 49.2 |  |  | 143 |
|  | *BUA* | 125.3 *±* 12.6 |  |  | 309 | 129.7 *±* 11.7 |  |  | 143 |
|  | *T-score* | 0.9 *±* 1.2 |  |  | 190 |  |  |  |  |
|  | *Z-score* | 0.9 *±* 1.2 |  |  | 190 |  |  |  |  |
| Oral contraceptive pill (OCP) use | *OCP use (Yes)* |  | 203 | 65.7 | 309 |  |  |  |  |
| Age of pubertal timing (years) | *Age of pubertal timing (menarche/facial hair)* | 12.7 *±* 1.4 |  |  | 309 | 14.6 *±* 2.3 |  |  | 143 |
| Smoking status (yes or no) | *Never smoked* |  | 234 | 75.7 | 309 |  | 87 | 60.8 | 143 |
| *Smoker* |  | 75 | 24.3 | 309 |  | 56 | 39.2 | 143 |
| Alcohol consumption | *Yes, alcohol consumed* |  | 284 | 91.8 | 309 |  | 133 | 93 | 143 |
|  | *Age of alcohol consumption (years)* | 15.4 *±* 2.3 |  |  | 281 | 15.1 *±* 3.1 |  |  | 133 |
|  | *Consumers of alcohol above the median\** |  | 129 | 41.7 | 309 |  | 77 | 53.8 | 143 |
|  | *Alcohol intake\*\* (units/week)* | 4.0 *±* 3.4 |  |  | 309 | 5.4 *±* 4.5 |  |  | 143 |
| Fracture status (yes or no) | *Self-reported fracture* |  | 115 | 37.5 | 307 |  | 60 | 42 | 143 |
| Age of first broken bone (years) | *Age of first fracture* | 9.9 *±* 4.6 |  |  | 115 | 12.6 *±* 5.0 |  |  | 58 |
| Daily calcium intake (mg/mL) | *Dietary calcium daily* | 666.6 *±* 628.4 |  |  | 309 | 752.7 *±* 593.0 |  |  | 143 |
| Weekly physical activity score | *Modified Kowalski PA mean score* | 2.0 *±* 0.5 |  |  | 309 | 2.1 *±* 0.6 |  |  | 143 |
| Sporting activity (mins/week) | HIWB | 126.8 *±* 250.3 |  |  | 309 | 107.5 *±* 146.5 |  |  | 143 |
|  | HIWB + WB | 467.2 *±* 456.9 |  |  | 309 | 413.9 *±* 445.6 |  |  | 143 |
|  | HIWB + WB + NWB | 494.9 *±* 470.8 |  |  | 309 | 468.6 *±* 505.1 |  |  | 143 |
|  | NWB | 27.7 *±* 73.8 |  |  | 309 | 54.7 *±* 150.0 |  |  | 143 |
| Past recreational sporting activity score | *Past BPAQ modified* | 33.0 *±* 31.7 |  |  | 309 | 28.0 *±* 24.6 |  |  | 143 |

^ Data are presented as mean ± SD (standard deviation)

Abbreviation: BMI = body mass index; BUA = broadband ultrasound attenuation; BPAQ = bone physical activity questionnaire; cQUS = calcaneal quantitative ultrasound; HI = high impact; NWB = non-weight-bearing; OCP = oral contraceptive pill; PA = physical activity; SI = stiffness index; SOS = speed of sound; WB = weight-bearing.

\* median of 3.5 units per week

\*\*includes non-drinkers

**Supplementary Table 2**: Lifestyle and demographic variables associated with cQUS measures in multiple regression models

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Variable | **n=452 (Females and males)** | | **n=309 (Females)** | | **n=143 (Males)** | |
| Source | Sig. | B | Sig. | B | Sig. | B |
| ***Dependent Variable: SI*** |  |  |  |  |  |  |
| Ethnicity (European or Other) | 0.086 | 3.018 | 0.988 | 0.03 | 0.011 | 9.017 |
| BMI (kg/m2) | **0.006** | 0.514 | 0.01 | 0.572 | 0.21 | 0.455 |
| Weekly non-weight-bearing sporting activity (minutes) | 0.066 | 0.014 | 0.41 | 0.01 | 0.195 | 0.014 |
| Past recreational sporting activity score | **0.064** | 0.05 | 0.193 | 0.037 | 0.558 | 0.041 |
| Physical activity score | **<.001** | 6.754 | 0.013 | 4.641 | 0.005 | 9.303 |
| Smoking status | 0.104 | 3.078 | 0.943 | -0.159 | 0.009 | 9.258 |
| Gender (female) | 0.326 | -1.914 |  | |  | |
| ***Dependent Variable: SOS*** |  |  |  |  |  |  |
| Ethnicity (European or Other) | 0.002 | 13.427 | 0.142 | 7.157 | 0.002 | 27.829 |
| Total daily calcium intake (100mg) | 0.079 | -0.548 | 0.04 | -0.718 | 0.763 | -0.198 |
| Weekly non-weight-bearing sporting activity (minutes) | 0.046 | 0.038 | 0.538 | 0.018 | 0.105 | 0.044 |
| Past recreational sporting activity score | **0.029** | 0.143 | 0.164 | 0.096 | 0.314 | 0.173 |
| Physical activity score | **<.001** | 15.042 | 0.033 | 9.596 | 0.011 | 20.855 |
| Smoking status | 0.13 | 6.996 | 0.554 | -3.188 | 0.002 | 26.975 |
| Gender (female) | 0.975 | 1.566 |  | |  | |
| ***Dependent Variable: BUA*** |  |  |  |  |  |  |
| Ethnicity (European or Other) | 0.473 | -0.906 | 0.083 | -2.708 | 0.38 | 1.96 |
| Consumers of alcohol above the median of 3.5 units per week | **0.059** | -2.341 | **0.032** | -3.239 | 0.995 | 0.015 |
| Age | **0.037** | -0.281 | 0.12 | -0.258 | 0.145 | -0.348 |
| Pubertal timing | 0.111 | 0.531 | **0.019** | 1.204 | 0.854 | 0.079 |
| BMI (kg/m2) | **<.001** | 0.613 | <.001 | 0.722 | 0.05 | 0.453 |
| Physical activity score | **0.001** | 3.793 | 0.042 | 2.919 | 0.013 | 5.209 |
| Gender (female) | **0.023** | -3.192 |  | |  | |
| ***Dependent Variable: T-score*** *(n=190, females aged 20 and above)* |  |  |  | | | |
| Ethnicity (European or Other) | 0.553 | 0.118 |
| BMI (kg/m2) | 0.045 | 0.042 |
| Physical activity score | 0.041 | 0.377 |
| ***Dependent Variable: Z-score*** *(n=190, females aged 20 and above)* | 0.125 |  |
| Ethnicity (European or Other) | 0.134 | 0.449 |
| BMI (kg/m2) | 0.057 | 0.04 |
| Physical activity score | 0.054 | 0.352 |

**Table 2**: Lifestyle and demographic variables associated with cQUS measures in multiple regression models

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Variable | **n=309 (Females)** | | **95% Confidence Interval** | | **n=143 (Males)** | | **95% Confidence Interval** | |
| Source | Sig. | B | Lower Bound | Upper Bound | Sig. | B | Lower Bound | Upper Bound |
| ***Dependent Variable: SI*** | R Squared = .095 (Adjusted R Squared = .058) | | | | R Squared = .240 (Adjusted R Squared = .170) | | | |
| Ethnicity (European or Other) | 0.988 | 0.03 | -3.965 | 4.026 | 0.011 | 9.017 | 2.099 | 15.935 |
| BMI (kg/m2) | **0.01** | 0.572 | 0.141 | 1.004 | 0.21 | 0.455 | -0.259 | 1.168 |
| Weekly non-weight-bearing sporting activity (minutes) | 0.41 | 0.01 | -0.014 | 0.035 | 0.195 | 0.014 | -0.007 | 0.036 |
| Past recreational sporting activity score | 0.193 | 0.037 | -0.019 | 0.094 | 0.558 | 0.041 | -0.096 | 0.178 |
| Physical activity score | **0.013** | 4.641 | 0.97 | 8.312 | **0.005** | 9.303 | 2.854 | 15.751 |
| Smoking status | 0.943 | -0.159 | -4.575 | 4.257 | 0.009 | 9.258 | 2.343 | 16.173 |
| ***Dependent Variable: SOS*** | R Squared = .072 (Adjusted R Squared = .034) | | | | R Squared = .275 (Adjusted R Squared = .208) | | | |
| Ethnicity (European or Other) | 0.142 | 7.157 | -2.421 | 16.734 | 0.002 | 27.829 | 10.761 | 44.897 |
| Total daily calcium intake (100mg) | 0.04 | -0.718 | -1.401 | -0.034 | 0.763 | -0.198 | -1.491 | 1.095 |
| Weekly non-weight-bearing sporting activity (minutes) | 0.538 | 0.018 | -0.041 | 0.078 | 0.105 | 0.044 | -0.009 | 0.097 |
| Past recreational sporting activity score | 0.164 | 0.096 | -0.039 | 0.231 | 0.314 | 0.173 | -0.165 | 0.512 |
| Physical activity score | **0.033** | 9.596 | 0.797 | 18.395 | **0.011** | 20.855 | 4.945 | 36.765 |
| Smoking status | 0.554 | -3.188 | -13.773 | 7.396 | 0.002 | 26.975 | 9.914 | 44.035 |
| ***Dependent Variable: BUA*** | R Squared = .128 (Adjusted R Squared = .093) | | | | R Squared = .146 (Adjusted R Squared = .067) | | | |
| Ethnicity (European or Other) | 0.083 | -2.708 | -5.775 | 0.358 | 0.38 | 1.96 | -2.443 | 6.363 |
| Consumers of alcohol above the median of 3.5 units per week | 0.032 | -3.239 | -6.189 | -0.288 | 0.995 | 0.015 | -4.43 | -4.43 |
| Age | 0.12 | -0.258 | -0.583 | 0.068 | 0.145 | -0.348 | -0.819 | 0.122 |
| Pubertal timing | **0.019** | 1.204 | 0.195 | 2.213 | 0.854 | 0.079 | -0.772 | 0.93 |
| BMI (kg/m2) | **<.001** | 0.722 | 0.391 | 1.054 | **0.05** | 0.453 | -0.001 | 0.908 |
| Physical activity score | **0.042** | 2.919 | 0.102 | 5.736 | **0.013** | 5.209 | 1.105 | 9.313 |
| ***Dependent Variable: T-score*** *(n=190, females aged 20 and above)* | R Squared = .090 (Adjusted R Squared = .028) | | | |  |  |  |  |
| Ethnicity (European or Other) | 0.553 | 0.118 | -0.273 | 0.509 |  |  |  |  |
| BMI (kg/m2) | **0.045** | 0.042 | 0.001 | 0.083 |  |  |  |  |
| Physical activity score | **0.041** | 0.377 | 0.015 | 0.738 |  |  |  |  |
| ***Dependent Variable: Z-score*** *(n=190, females aged 20 and above)* | R Squared = .086 (Adjusted R Squared = .030) | | | |  |  |  |  |
| Ethnicity (European or Other) | 0.134 | 0.449 | -0.256 | 0.523 |  |  |  |  |
| BMI (kg/m2) | 0.057 | 0.04 | -0.001 | 0.08 |  |  |  |  |
| Physical activity score | **0.054** | 0.352 | -0.006 | 0.71 |  |  |  |  |

**Table 3**: Association of ethnicity, BMI and named popular sporting activity with bone parameters

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Variable** | **Female Top ten sports n=309** | | **95% Confidence Interval** | | **Male Top ten sports n=143** | | **95% Confidence Interval** | |
| Source | Sig. | B | Lower Bound | Upper Bound | Sig. | B | Lower Bound | Upper Bound |
| ***SI*** | R Squared = .120 (Adjusted R Squared = .081) | | | | R Squared = .187 (Adjusted R Squared = .105) | | | |
| Ethnicity (European or Other) | 0.392 | -1.726 | -5.688 | 2.236 | 0.004 | 10.294 | 3.322 | 17.265 |
| Running | **0.039** | 3.828 | 0.197 | 7.46 | 0.238 | 3.796 | -2.537 | 10.128 |
| Swimming | 0.531 | 1.139 | -2.437 | 4.716 | 0.273 | 3.558 | -2.835 | 9.95 |
| Soccer | 0.572 | -1.217 | -5.445 | 3.011 | **0.002** | 10.186 | 3.877 | 16.496 |
| Walking | **0.027** | -4.226 | -7.965 | -0.486 | 0.217 | -4.645 | -12.049 | 2.759 |
| Cycling | **0.017** | -5.574 | -10.137 | -1.011 | 0.969 | -0.142 | -7.353 | 7.069 |
| Rugby | **0.001** | 8.255 | 3.227 | 13.283 | 0.233 | -4.256 | -11.282 | 2.769 |
| BMI | **0.015** | 0.537 | 0.106 | 0.968 | 0.315 | 0.393 | -0.377 | 1.163 |
| ***SOS*** | R Squared = .109 (Adjusted R Squared = .069) | | | | R Squared = .186 (Adjusted R Squared = .104) | | | |
| Ethnicity (European or Other) | 0.472 | 3.454 | -5.983 | 12.89 | **0.001** | 30.874 | 13.255 | 48.493 |
| Running | **0.05** | 8.636 | -0.014 | 17.287 | 0.076 | 14.449 | -1.554 | 30.452 |
| Soccer | 0.48 | -3.621 | -13.691 | 6.449 | **0.015** | 19.924 | 3.979 | 35.869 |
| Walking | **0.011** | -11.542 | -20.449 | -2.634 | 0.263 | -10.63 | -29.34 | 8.081 |
| Rugby | **0.002** | 18.781 | 6.806 | 30.757 | 0.29 | -9.53 | -27.285 | 8.226 |
| Cycling | **0.008** | -14.811 | -25.68 | -3.942 | 0.705 | 3.498 | -14.724 | 21.721 |
| ***BUA*** | R Squared = .112 (Adjusted R Squared = .073) | | | | R Squared = .193 (Adjusted R Squared = .112) | | | |
| Ethnicity (European or Other) | 0.013 | -3.942 | -7.054 | -0.83 | 0.194 | 2.751 | -1.418 | 6.92 |
| Age | 0.214 | -0.209 | -0.538 | 0.121 | 0.103 | -0.364 | -0.803 | 0.075 |
| BMI | **<.001** | 0.699 | 0.36 | 1.037 | 0.074 | 0.42 | -0.041 | 0.88 |
| Soccer | 0.898 | -0.217 | 0.36 | 1.037 | **0.001** | 6.684 | 2.911 | 10.457 |
| Swimming | 0.958 | -0.075 | -2.885 | 2.734 | 0.083 | 3.371 | -0.452 | 7.193 |
| Dance | 0.373 | -1.37 | -4.395 | 1.654 | 0.094 | 7.477 | -1.281 | 16.234 |
| Rugby | **0.02** | 4.709 | 0.759 | 8.658 | 0.264 | -2.38 | -6.581 | 1.821 |
| ***T-score*** (n=190, females aged 20 and above) | R Squared = .097 (Adjusted R Squared = .030) | | | |  |  |  |  |
| Rugby | **0.034** | 0.609 | 0.047 | 1.171 |  |  |  |  |
| ***Z-score*** (n=190, females aged 20 and above) | R Squared = .097 (Adjusted R Squared = .035) | | | |  |  |  |  |
| Rugby | **0.035** | 0.601 | 0.042 | 1.16 |  |  |  |  |

**Supplementary Table 3**: Association of ethnicity, BMI and named popular sporting activity with bone parameters

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Variable | **Top ten sports n=452** | | **Female Top ten sports n=309** | | **Male Top ten sports n=143** | |
| *Sig.* | B | *Sig.* | B | *Sig.* | B |
| ***SI*** |  |  |  |  |  |  |
| Ethnicity (European or Other) | 0.147 | 2.593 | 0.392 | -1.726 | 0.004 | 10.294 |
| Gender | 0.311 | -2.154 |  | |  | |
| Running | **0.022** | 3.766 | 0.039 | 3.828 | 0.238 | 3.796 |
| Swimming | 0.157 | 2.292 | 0.531 | 1.139 | 0.273 | 3.558 |
| Soccer | **0.047** | 3.594 | 0.572 | -1.217 | **0.002** | 10.186 |
| Walking | **0.009** | -4.613 | 0.027 | -4.226 | 0.217 | -4.645 |
| Cycling | 0.124 | -3.056 | 0.017 | -5.574 | 0.969 | -0.142 |
| Rugby | 0.289 | 2.212 | 0.001 | 8.255 | 0.233 | -4.256 |
| BMI | 0.023 | 0.445 | 0.015 | 0.537 | 0.315 | 0.393 |
| ***SOS*** |  |  |  |  |  |  |
| Ethnicity (European or Other) | 0.003 | 12.81 | 0.472 | 3.454 | 0.001 | 30.874 |
| Gender | 0.787 | 1.397 |  | |  | |
| Running | **0.01** | 10.385 | 0.05 | 8.636 | 0.076 | 14.449 |
| Soccer | 0.14 | 6.502 | 0.48 | -3.621 | **0.015** | 19.924 |
| Walking | 0.04 | -12.481 | **0.011** | -11.542 | 0.263 | -10.63 |
| Rugby | 0.283 | 5.453 | 0.002 | 18.781 | 0.29 | -9.53 |
| Cycling | 0.175 | -6.559 | **0.008** | -14.811 | 0.705 | 3.498 |
| ***BUA*** |  |  |  |  |  |  |
| Ethnicity (European or Other) | 0.292 | -1.347 | 0.013 | -3.942 | 0.194 | 2.751 |
| Gender | 0.01 | -3.943 |  | |  | |
| Age | 0.057 | -0.259 | 0.214 | -0.209 | 0.103 | -0.364 |
| BMI | <.001 | 0.582 | <.001 | 0.699 | 0.074 | 0.42 |
| Soccer | **0.042** | 2.635 | 0.898 | -0.217 | **0.001** | 6.684 |
| Swimming | 0.316 | 1.159 | 0.958 | -0.075 | 0.083 | 3.371 |
| Dance | 0.679 | -0.591 | 0.373 | -1.37 | 0.094 | 7.477 |
| Rugby | 0.433 | 1.168 | 0.02 | 4.709 | 0.264 | -2.38 |
| ***T-score*** (n=190, females aged 20 and above) |  |  |  | | | |
| Rugby | **0.034** | 0.609 |
| ***Z-score*** (n=190, females aged 20 and above) |  |  |
| Rugby | **0.035** | 0.601 |

**Supplementary Table 1**: Pearson correlations between demographic & lifestyle factors & calcaneal cQUS bone outcomes

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Variable | **SI** | **SOS** | **BUA** | **T-score** | **Z-score** | **SI (F)** | **SOS (F)** | **BUA (F)** | **SI (M)** | **SOS (M)** | **BUA (M)** |
|  | **(*n* = 452)** | **(*n* = 452)** | **(*n* = 452)** | **(*n* = 190)** | **(*n* = 190)** | **(*n* = 309)** | **(*n* = 309)** | **(*n* = 309)** | **(*n* = 143)** | **(*n* = 143)** | **(*n* = 143)** |
| *Age (years)* | -0.026 | -0.003 | -0.041 | -0.012 | 0.03 | -0.089 | -0.077 | -0.07 | 0.039 | 0.113 | -0.086 |
| *BMI (kg/m2)* | **.130\*\*** | 0.045 | .**199\*\*** | .144\* | 0.141 | .124\* | 0.016 | .206\*\* | 0.129 | 0.093 | 0.162 |
| *Height (cm)* | 0.047 | -0.086 | .222\*\* | 0.111 | 0.11 | 0.072 | -0.059 | .211\*\* | -0.136 | -.214\* | 0.025 |
| *Weight (kg)* | .133\*\* | -0.009 | .286\*\* | .176\* | .174\* | .157\*\* | -0.01 | .300\*\* | 0.049 | -0.019 | 0.154 |
| *Pubertal timing (years)* | 0.073 | 0.009 | **.145\*\*** | 0.093 | 0.094 | 0.1 | 0.045 | .141\* | -0.022 | -0.035 | -0.003 |
| *Daily calcium intake (100mg)* | -0.029 | -0.066 | 0.026 | -0.077 | -0.08 | -0.062 | -0.106 | 0.012 | 0.017 | 0.001 | 0.024 |
| *Physical activity score* | **.255\*\*** | **.236\*\*** | **.200\*\*** | **.179\*** | **.174\*** | .191\*\* | .167\*\* | .151\*\* | .358\*\* | .351\*\* | .281\*\* |
| *Past recreational sporting activity score* | .112\* | .138\*\* | 0.027 | 0.072 | 0.08 | 0.096 | 0.107 | 0.04 | **.179\*** | **.221\*\*** | 0.042 |
| *Weekly weight-bearing high impact sporting activity (minutes)* | **.145\*\*** | **.154\*\*** | 0.088 | 0.055 | 0.05 | **.131\*** | **.140\*** | 0.076 | **.235\*\*** | **.231\*\*** | **.185\*** |
| *Weekly weight-bearing high impact and weight-bearing sporting activity (minutes)* | 0.054 | 0.064 | 0.023 | 0.071 | 0.073 | 0.05 | 0.073 | 0.002 | 0.076 | 0.052 | 0.107 |
| *Weekly weight-bearing high impact and weight-bearing and non-weight-bearing sporting activity (minutes)* | 0.077 | 0.087 | 0.041 | 0.082 | 0.085 | 0.061 | 0.082 | 0.011 | 0.111 | 0.095 | 0.122 |
| *Weekly non-weight-bearing sporting activity (minutes)* | .120\* | .120\* | 0.088 | 0.084 | 0.088 | 0.08 | 0.069 | 0.06 | 0.146 | .167\* | 0.092 |
| *Weekly weight-bearing sporting activity (minutes)* | -0.02 | -0.013 | -0.024 | 0.053 | 0.059 | -0.027 | -0.004 | -0.05 | -0.001 | -0.025 | 0.048 |
| Smoking status (yes = 1 or no = 0) | 0.042 | 0.054 | 0.013 | 0.021 | 0.02 | 0.016 | 0.031 | -0.016 | .168\* | .185\* | 0.089 |
| Fracture status (yes = 1 or no = 0) | -0.072 | -0.091 | -0.029 | -0.082 | -0.076 | -0.094 | **-.128\*** | -0.026 | -0.082 | -0.076 | -0.042 |
| *Ethnicity (European = 1 or Other = 0)* | -0.084 | **-.149\*\*** | 0.029 | 0.006 | -0.002 | 0.012 | -0.065 | 0.101 | -.251\*\* | -.288\*\* | -0.123 |
| *Consumers of alcohol above the median of 3.5 units per week (yes = 1 or no = 0)* | 0.049 | -0.006 | **.112\*** | 0.099 | 0.09 | 0.081 | 0.014 | .135\* | -0.031 | -0.043 | 0.005 |

(F) Female

(M) Male

\*\* Correlation is significant at the 0.01 level (2-tailed).

\* Correlation is significant at the 0.05 level (2-tailed).

**Figure 1**: Recalled sports participation from age 8 to 25 years in 452 young people participating in this study

