Title Page

Title: A small amount of precisely measured high intensity habitual physical activity predicts bone health in pre- and post-menopausal women in UK Biobank

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Abstract (250 words)

**Background:** Physical inactivity is a highly modifiable risk factor for the development of osteoporosis but due to a lack of research that has precisely and objectively measured physical activity (PA) relevant to bone, the specific contribution that PA can make to bone health is poorly understood. This study examined whether a more precise measure of PA relevant to bone was associated with measures of bone health in pre- and post-menopausal women in UK Biobank. **Methods:** Time spent at intensities specific to bone health ($\geq 750$ milli-gravitational units [mg] and $\geq 1000$ mg) were analysed from raw tri-axial acceleration data averaged over 1-second epochs from seven-day monitoring of habitual PA using accelerometry-based activity monitors (100 Hz; AX3, Axivity, UK) of 1218 pre- and 1316 post-menopausal healthy women. In a cross-sectional analysis, associations between categories of time (<1, 1-2 and $\geq$2 minutes) spent above the intensity thresholds and calcaneal quantitative ultrasound measures of bone health (bone mineral density T-score, BMDT-score; speed of sound, SOS and broadband ultrasound attenuation, BUA) were examined. **Results:** Compared with <1 minute, spending 1-2 or $\geq$2 minutes/day at intensities $\geq 1000$ mg in pre-menopausal and $\geq 750$ mg in post-menopausal women was positively associated with BMDT-score, SOS and BUA. **Conclusion:** Brief bursts of high intensity PA relevant to bone health can be captured by applying bone-specific thresholds of intensity to raw tri-axial accelerations averaged over 1-second epochs. Accumulating 1-2 minutes/day of high-intensity PA, equivalent to running in pre-menopausal women and slow jogging in post-menopausal women, is associated with better bone health.

**KEYWORDS (3-10)**

Osteoporosis, Accelerometer, Raw Acceleration, Quantitative Ultrasound

**Medical Subject Headings (MeSH):** Osteoporosis, bone, exercise therapy
Key Messages (3-5 succinct bullet points)

Brief bursts of high intensity habitual physical activity beneficial to bone health can be quantified from accelerations measured at the wrist with accelerometry-based activity monitors.

This method provides a step-change in the ability to precisely and objectively measure physical activity relevant to bone from commercially available tri-axial wrist-worn monitors typically employed in large population studies.

Accumulating 1-2 minutes or ≥2 minutes per day of high-intensity physical activity, equivalent to running in pre-menopausal women and slow jogging in post-menopausal women, is associated with better bone health.

Future research should further exploit high-resolution accelerometry-based activity monitor data to determine the optimal temporal characteristics of physical activity for bone health to inform the development of manageable and effective physical activity interventions.
**Introduction**

Osteoporosis is a brittle bone disease that affects women (1 in 3) more than men (1 in 5) especially over the age of 50 [1,2]. It causes over 300,000 people a year in the UK [3] and over 2 million in the US [4] to suffer a fragility fracture resulting in significant pain, disability, loss of independence and increased risk of morbidity especially in the first 6 months after fracture [2,3]. In women the incidence of osteoporosis increases dramatically post-menopause [1,2,3] therefore identification of strategies that may optimise bone health in both pre and post-menopausal women is a priority.

Physical inactivity is a highly modifiable risk factor for the development of osteoporosis [5,6,7] but the specific contribution that physical activity (PA) can make to accruing, maintaining or minimising the loss of bone mass is poorly understood compared to other modifiable lifestyle risk factors such as diet, smoking and alcohol [2,7,8,9]. While PA guidelines recommending the accumulation of at least 150 minutes/week of moderate PA, in bouts of 10 minutes or more, exist for cardiovascular and metabolic health [10,11], there are no specific PA recommendations for reducing the risk of poor bone health which likely benefits from a different dose of activity characterised by short, dynamic, sporadic bursts [12,13]. The development of bone-specific PA guidelines is limited by a lack of research that has precisely and objectively assessed the influence of exercise interventions [2,14] or habitual PA on bone health outcomes. Consequently there is a lack of evidence for positive associations between bone mineral density (BMD) and moderate or vigorous intensities of PA in women [15,16].

Until recently, the outcome for objectively measured PA in large cross-sectional bone health studies has been time accumulated in sedentary, light, moderate or vigorous intensity categories determined from proprietary counts (device specific) from hip-worn monitors summed over user-defined 15- or 60-second epochs [15,16]. The classification of the intensities corresponds to energy expenditure during steady state exercise, making them most relevant to cardiovascular and
metabolic health [17,18,19]. Chastin and colleagues [16] suggest that their counterintuitive finding
for the absence of an association between BMD and PA at moderate and vigorous intensities may be
due to averaging proprietary counts from hip-worn accelerometry-based activity monitors over 60-
second epochs. For short dynamic episodes of activity, averaging has the effect of over-smoothing,
misclassifying and underestimating time spent in moderate or vigorous intensities, thus failing to
capture the very activities that are likely to benefit bone [16]. Classification of activity into intensity
categories calibrated with energy expenditure from steady state activity relevant to cardiovascular
and metabolic health outcomes may also contribute to the failure to detect an association between
more dynamic intensities of PA and bone health [20,21,22].

The commercial availability of high-resolution tri-axial accelerometry-based activity monitors that
collect and store raw acceleration data at up to 100 Hz for seven days provides the opportunity to
more precisely measure intensities of PA beneficial to bone. We calibrated raw peak acceleration
from these monitors worn on the hip and wrist with external ground reaction force in adults [21]
and determined the magnitude of acceleration associated with ground reaction forces that are
beneficial to bone in pre-menopausal women [23]. Providing a valid measure of activity relevant to
bone from wrist worn monitors is particularly important because, compared to hip-worn monitors,
they result in higher levels of participant compliance, greater wear-time and therefore more
accurate measures of habitual PA [24]. The use of wrist-worn monitors to objectively measure PA is
becoming more common in large population surveys and national health databases including UK
Biobank.

UK Biobank is a new open access large-scale prospective epidemiological resource that holds
baseline measures on 500,000 adults including quantitative ultrasound scanning (QUS) of the heel
and, in a sub-sample of approximately 100,000 participants, objective measurement of habitual
(free-living) PA from seven-day monitoring using a commercial wrist-worn tri-axial accelerometer
that sampled and stored raw accelerations at 100 Hz. These high-resolution files present a unique opportunity to derive a more precise measure of PA relevant to bone from raw acceleration data in a large cross-sectional study. Brief bursts of high intensity activity can be quantified using intensity thresholds specific to bone health. We hypothesise that precise bone-specific measures of PA will predict measures of bone health in both pre- and post-menopausal women independent of PA accrued at all other intensities and other factors thought to influence bone.

Methods

Questionnaire and baseline physical measures including QUS of the heel were collected from 500,000 adults aged 40-69 years attending one of 21 assessment centres across Britain between 2006 and 2010. Objective measurements of PA were collected in a sub-sample (approximately 100,000) of the same cohort between 2013 and 2015. Details of recruitment and measurements used to obtain data for this resource can be found on the UK Biobank website: https://www.ukbiobank.ac.uk.

Study Sample

To reduce the influence of conditions or treatments affecting either bone health or PA, only ‘healthy’ individuals, in the order outlined in Figure 1, were selected. For comparison, where complete sets of data were available, general health and activity characteristics for excluded pre- and post-menopausal samples are presented (Figure 1). Pre-menopausal (n=1218) and post-menopausal (n=1316) women forming the included sample were analysed separately due to the potential for different PA intensity thresholds to predict bone health in each group.

Figure 1. Here: Study inclusion flow chart
Bone Health Outcome Measures

Participants had calcaneal QUS measurements of their left and right calcaneus performed using the Sahara Clinical Bone Sonometer, (Hologic, Bedford, MA). BMDT-scores (number of standard deviations above or below peak BMD from a young sex-matched average) were derived from estimated BMD, calculated using the following formula:

\[
\text{Heel BMD} = 0.002592 \times (\text{BUA} + \text{SOS}) - 3.687 \text{ g/cm}^2
\]

where \(\text{SOS}\) is the speed of sound (m/s) and \(\text{BUA}\) is the broadband ultrasound attenuation (dH/MHz) [25]. The QUS measurements were averaged between the left and right calcaneus (one measurement from each) for each participant. In accordance with good practice, daily quality control and cleaning procedures were conducted in line with the manufacturer’s recommendations across all assessment centres. Further details of the QUS testing protocol are available on the UK Biobank website.

Physical Activity Monitoring

Raw acceleration files (.cwa) containing seven-day, 100 Hz data from tri-axial AX3 (Axivity, Newcastle, UK) accelerometers worn on the dominant wrist were downloaded from UK Biobank and auto-calibrated, resampled (100 Hz) and converted to .wav format using open-source software (Omgui Version 1.0.0.28; Axivity). An open access package (GGIR Version 1.3-2) in R (http://cran.r-project.org/) was used to convert raw accelerations (x,y and z axes) in .wav files to magnitudes of dynamic acceleration (resultant vector magnitude, corrected for gravity, expressed as Euclidean Norm Minus One, ENMO in milli-gravitational units, mg; [26,27]) averaged over 1-second epochs from which time accumulated at different intensities from 6 valid days (16 hours/day), including one weekend day, of wear was used to calculate an average day of activity. Month of PA measurement was extracted to allow for any adjustments in PA due to seasonal variation to be made.
Using wrist-worn monitors that produce acceleration magnitudes equivalent to the AX3 [28], Hildebrand and colleagues [19] found thresholds of approximately 100 \( mg \) and 400 \( mg \) represented moderate and vigorous intensities of activity based on energy expenditure for 3 and 6 METs, respectively, in adults (aged 34 ±10 years). The moderate intensity approximated brisk walking, with the vigorous threshold just over half the 750 \( mg \) output elicited during running (8 km/h), an activity that has been found to exceed impact magnitudes and loading rates beneficial to bone [23,29,30]. When calibrating acceleration magnitudes with ground reaction force beneficial to bone [23], the thresholds we identified corresponded to the acceleration magnitudes found during running at 8 km/h (slow jogging) and 10 km/h, equivalent to 750 \( mg \) and 1000 \( mg \) when averaging over 1-second epochs [19,31]. Time spent at intensities \( \geq 750 \) \( mg \) (PA \( \geq 750 \) \( mg \)) and \( \geq 1000 \) \( mg \) (PA \( \geq 1000 \) \( mg \)) were therefore used in the present study to examine thresholds of activity specific to bone.

Covariates

Variables collected by UK Biobank that were believed to be, or have previously been shown to be, associated with bone health and/or PA were treated as potential covariates. Baseline measures for age, height, fat-mass and fat-free mass (bioelectrical impedance; Tanita BC418MA), self-reported alcohol, nutritional intake and current medications were extracted from UK Biobank. While estimated calcium intake (mg) could intuitively be an important determinant of bone health, it was not included as a covariate in this report due to only half the sample providing data for it and the absence of any correlation \( (r=0.001) \) between calcium intake and BMDT-score in the half that did provide a measure. Estimated alcohol consumption (units/week) was calculated from self-reported volumes of intake multiplied by units for each alcohol type [32]. Continuous variables for age at menarche, the number of years taking contraceptive and years since the menopause (where applicable) were extracted or calculated from female-specific factors from the touchscreen.
questionnaire. The number of years between baseline and PA measures was also calculated to allow any influence in time between measures of bone health and PA to be examined. Covariates for PA (50-99 mg and 100-749 or 100-999 mg) were created to allow associations between time spent at higher intensities and measures of bone health to be analysed independent of time spent being in activities at all other intensities. To reduce the amount of dilution that light intensity activity (which may also be beneficial to bone; [16]) has on measures of moderate activity and above, time spent in 50-99 mg was used as a separate PA covariate to time spent between 100-749 mg and 100-999 mg for respective analyses.

Statistical Data Analysis

For the first stage of the model building process all of the covariates were entered simultaneously into the regression model (Model 1) without removal (e.g. all entered covariates remained in the model irrespective of their p-value). Plotting the residuals of this covariate model against PA ≥750 mg or PA ≥1000 mg indicated that the relationship was curvilinear requiring a second order polynomial to model it. For ease of interpretation we decided to address the curvilinear relationship by converting the continuous PA ≥750 mg/PA ≥1000 mg variables into categorical variables (<1, 1-2, ≥2 mins/day). The parameters of these categories were chosen after examining the distribution of time spent at intensities ≥1000 mg and ≥750 mg for pre- and post-menopausal women respectively and consideration of the lowest accumulated dose of physical activity that would lend itself to a plausible public health message. Consequently for the second stage of the model building process (Model 2), we entered the categorical variables for PA ≥750 mg/PA ≥1000 mg (<1 min/day being the reference category) into the model that contained all of the covariates with BMDT-score as the outcome measure. The models were repeated with BUA and SOS as the outcome measure. A sample size of n~1200 and n~1300 in each group provides ~90% power (at p=0.05) to detect very small
(~1%, partial R² change = 0.011) increases in the explained variance of bone health by adding PA≥750mg/PA≥1000mg to a covariate model that already explains ~10% of the variance. All analyses were carried out in IBM SPSS Version 23 (IBM, Chicago, IL).

**Results**

Descriptive statistics for measures of bone health, covariates and PA-by-intensity variables were reported in Table 1 for pre-menopausal and post-menopausal women separately. Means and standard deviations are reported for normally distributed variables and medians and interquartile ranges for variables that are positively skewed. There was no need to adjust PA data for the potential effects of seasonality as there was no evidence in this sample that PA differed by season (e.g. summer vs autumn, vs winter and vs spring were all p≥0.20 for PA≥1000mg in the pre-menopausal group and PA≥750mg in the post-menopausal group). Tables 2 and 3 report the beta-coefficients (with 95% CIs and p-values) for all the PA-by-intensity variables obtained from the full model (Model 2) that best predicted bone health measures for pre-menopausal and post-menopausal women. In addition, the R² increase for the PA≥750mg or PA≥1000mg variable was reported.

Table 1 here: Summary characteristics of pre-menopausal and post-menopausal women.

**Pre-menopausal Women**

While there was some evidence that the time spent in PA≥750mg was positively associated with BMDT-score (p=0.04), the evidence for PA≥1000mg was much stronger (p=0.001). Additional analysis implied that time spent in PA at 750-999 mg (p=0.16) did not contribute at all to the association of PA≥750mg, it was due almost completely to time spent at PA≥1000mg. For this reason we are not reporting the PA≥750mg variable for pre-menopausal women as this would lead
to inappropriate recommendations, we are only reporting the results of the model that examined PA\(\geq 1000\)mg. In this final model, PA\(\geq 1000\)mg was the only PA-by-intensity variable that was associated with BMD (e.g. BMD was 0.20 \([\text{p}=0.024]\) and 0.29 \([\text{p}<0.001]\) T-scores higher in pre-menopausal women who spent 1-2 mins/day and \(\geq 2\) mins/day respectively in PA\(\geq 1000\)mg than in pre-menopausal women who spent <1 min/day at that intensity, \(R^2\) increased by 1.2\% \([\text{p}=0.001]\) from the 1.4\% covariate model). There was no evidence that time spent in PA at 50-99 mg or PA at 100-999 mg were related to BMD with or without PA\(\geq 1000\)mg in the model (with: \(p=0.943\) and \(p=0.987\) respectively; without: \(p=0.674\) and \(p=0.211\) respectively). The pattern of results was very similar when SOS and BUA were used as the markers of bone health.

Table 2 here: Relationship between physical activity (by intensity) and measures of bone health in pre-menopausal women (n=1218).

Post-menopausal Women

In post-menopausal women the association was much stronger between BMD and PA\(\geq 750\)mg than between BMD and PA\(\geq 1000\)mg (unlike in pre-menopausal women). Additional analysis showed that the association with PA\(\geq 750\)mg was due almost completely to time spent in PA at 750-999 mg \((p<0.001)\), and not at all to time spent in PA\(\geq 1000\)mg \((p=0.79)\). For this reason we are not reporting the PA\(\geq 1000\)mg variable for post-menopausal women as this would lead to inappropriate recommendations, we are only reporting the results of the model that examined the PA\(\geq 750\)mg variable (which clearly includes time in PA\(\geq 1000\)mg). In this final model, PA\(\geq 750\)mg was the only PA-by-intensity variable that was associated with BMD (e.g. BMD was 0.16 \([\text{p}=0.024]\) and 0.27 \([\text{p}=0.001]\) T-scores higher in post-menopausal women who spent 1-2 mins/day and \(\geq 2\) mins/day respectively in PA\(\geq 750\)mg than in post-menopausal women who spent <1min/day at that intensity, \(R^2\) increased by 0.9\% \([\text{p}=0.002]\) from the 7.2\% covariate model). There was no evidence that time spent in PA at 50-99 mg or PA at 100-749 mg were related to BMD with or without PA\(\geq 750\)mg in the model (with:
p=0.823 and p=0.226 respectively; without: p=0.408 and p=0.808 respectively). The pattern of results was very similar when SOS and BUA were used as the markers of bone health.

Table 3 here: Relationship between physical activity (by intensity) and measures of bone health in post-menopausal women (n=1316).

**Discussion**

Using a bone-health specific, precise and objective measure of time spent in high-intensity dynamic activity we have demonstrated a step-change in the ability to measure PA relevant to bone and revealed a positive association between habitual physical activity and bone health in both pre and post-menopausal women. In contrast to previous research, which summed proprietary counts from commercially available accelerometers over 15- or 60-second epochs [15,16], the averaging of raw accelerations over 1-second epochs ensured that brief bursts of high intensity habitual PA more relevant to bone were captured enabling bone-specific intensity thresholds to be applied. With a view to developing realistic and achievable bone- and population-specific public health messages, it is promising to find that relatively small amounts (1-2 minutes) of habitual PA at ≥1000 mg in pre-menopausal and ≥750 mg in post-menopausal women are positively associated with measures of bone health. High impact activity is generally considered necessary to stimulate bone cells to benefit BMD [13] but this osteogenic effect has not always been found in post-menopausal women [29].

To explain why bone health measures are associated with a different threshold of intensity in pre- and post-menopausal women, it is possible as a result of bone strength declining with age, that a lower intensity activity in post-menopausal women produces a local bone strain equivalent to a higher intensity activity in pre-menopausal women [33]. This is further supported by higher loading rates in mature women (55 BW/s, ±9) compared to younger women (37 BW/s, ±8) when running at the same speed [34]. Therefore a lower threshold of high intensity activity (750 mg equivalent to a
slow jog) in post-menopausal women may provide the same mechanical stimulation as a higher threshold of high intensity activity (e.g. 1000 mg equivalent running at 10 km/h) in premenopausal women. By extension, it may also be interesting to consider the potential for a lower intensity of activity to create sufficient local strain to stimulate bone formation in a less healthy population with lower levels of bone health. However, the close proximity of BMDT-Scores of the excluded and included participants observed in the current study (-0.21 and -0.11 respectively for pre-menopausal women and -0.72 and -0.63 respectively for postmenopausal women) suggest that the activity intensities associated with bone health in each excluded menopausal group may not be that dissimilar to respective intensities of the included samples. Nonetheless, it would be interesting to further explore these intensities in a wider, potentially less healthy population with full consideration of a comprehensive list of covariates relevant to the sample.

To our knowledge, no other research producing dynamic measures of acceleration (ENMO) from raw accelerations (100 Hz) averaged over 1-second epochs to quantify PA relevant to bone is available for comparison. However, methods using a non-commercial uniaxial waist-worn accelerometer with an on-board processor to count the number of impact peaks in vertical acceleration during an activity intervention, found that positive changes in BMD and calcaneal BUA were evident from fewer than 100 daily impacts over 3.9 g (standard acceleration due to gravity), a threshold that is indicative of running and jumping [30]. This supports the positive associations found for time spent above magnitudes equivalent to running in the present study.

Our results are counter to reports of osteogenic benefits [35] and changes in bone structural properties [8] from walking, which yield average (1-second epochs) accelerations of 170 mg during steady state activity [19]. A high number (approximately 8500) of peak accelerations at low intensity (0.3-1 g represents walking; [30]) have been found to significantly predict changes in bone structure e.g. circumference and cortical thickness at the proximal tibia [8]. Given that low-level stimulations
normally ‘ignored’ by bone may become highly anabolic if performed at higher frequencies [36,37],
it may be that osteogenic benefits from lower intensity accelerations averaged over 1-second epochs can only be recognised if wider characteristics of PA frequency, bout length and intermittence are also described [8,38,39]. Therefore, further research should also consider the temporal characteristics of PA such as the distribution of activity bouts and rest periods over discrete periods of time [8,13,22,40,41].

The development of a primary population-based strategy to increase PA at all ages in order to prevent osteoporosis and reduce the risk of fragility fractures has been limited by a scarcity of research that has accurately determined the influence of exercise intervention type, uptake and compliance on bone health outcomes using precise, objective measurements of PA [2,14]. This study demonstrates that the method used to analyse raw accelerations from commercially available tri-axial wrist-worn monitors, typically employed in large population studies, can be used to precisely and objectively capture high intensity PA relevant to bone. This could be used to evaluate the influence of PA interventions on bone health and to inform the development of manageable PA guidelines specific to bone.

A number of limitations of the present study are acknowledged. Averaging accelerations over 1-second epochs captured high intensity activity relevant to bone more accurately than previous studies summing counts over 15- or 60-second epochs; however, it was not possible to count the magnitude of individual peaks in raw acceleration using this method. The thresholds used in this study however, were specific to the intensities of activity beneficial to bone and are meaningful in that they can be described in relation to running speed and duration. In UK Biobank, accelerometer data were collected from monitors worn on the dominant wrist, whereas our thresholds and those of Hildebrand et al, [19] were developed using the non-dominant wrist. Evidence suggests however, that differences in accelerometer output between the dominant and non-dominant wrist are
minimal at higher intensities [31]. Therefore, unless an activity that dominates on one side is taking place, e.g. racket sports, these high-intensity thresholds are likely appropriate for either wrist. It should also be acknowledged that accelerometers only measure acceleration and are not able to capture loading, e.g. from resistance type training, which can also benefit bone health.

QUS measurements were used in UK Biobank rather than the current gold standard of DXA for measuring bone as it provides a radiation-free and inexpensive method for measuring the density and micro-architectural properties of bone. The ultrasound derived modulus of elasticity, as measured by the SOS, correlates strongly with values of bone breaking strength derived from static loading, while BUA values are reported to be dependent upon trabecular orientation in vitro and to be significantly associated with bone structure independently of BMD. These results can be combined to provide a single estimate, which is an analogue of bone mineral density [42]. While QUS is not used clinically in the UK, it provides a useful research tool to measure calcaneal estimated BMD and is affected by weight-bearing activity; with the calcaneus having a trabecular content similar to that of the spine and representing more metabolically active bone, which is likely to respond to mechanical and hormonal stimuli more rapidly than cortical bone sites [42]. Finally, as this is a cross-sectional study, it may be susceptible to reverse causality whereby time spent being physically active at a high intensity could be influenced by bone health.

In conclusion, using precise, objective measures of high-intensity dynamic activity we found that 1-2 minutes per day of high-intensity dynamic physical activity, equivalent to running in pre-menopausal women and slow jogging in post-menopausal women, is associated with better bone health.

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Conflicts of Interest

None

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