POOR BONE QUALITY IS ASSOCIATED WITH GREATER ARTERIAL STIFFNESS: INSIGHTS FROM THE UK BIOBANK

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ABSTRACT

Osteoporosis and ischaemic heart disease represent important public health problems. Existing research suggests an association between the two conditions beyond that attributable to shared risk factors, with a potentially causal relationship. In this study, we tested the association of speed of sound (SOS) from quantitative heel ultrasound with 1) measures of arterial compliance from cardiovascular magnetic resonance (aortic distensibility, AD); 2) finger photoplethysmography (arterial stiffness index, ASI); and 3) incidence myocardial infarction and ischaemic heart disease mortality in the UK Biobank cohort. We considered the potential mediating effect of a range of blood biomarkers and cardiometabolic morbidities and evaluated differential relationships by sex, menopause status, smoking, diabetes, and obesity. Furthermore, we considered whether associations with arterial compliance explained association of SOS with ischaemic cardiovascular outcomes. Higher SOS was associated with lower arterial compliance by both ASI and AD for both men and women. The relationship was most consistent with ASI, likely relating to larger sample size available for this variable (n=159,542 vs n=18,229). There was no clear evidence of differential relationship by menopause, smoking, diabetes, or body mass index. Blood biomarkers appeared important in mediating the association for both men and women, but with different directions of effect and did not fully explain the observed effects. In fully adjusted models, higher SOS was associated with significantly lower ischaemic heart disease mortality in men, but not in women. The association of SOS with ASI did not explain this association. In conclusion, our findings support a positive association between bone and vascular health with consistent patterns of association in men and women. The underlying mechanisms are complex and appear to vary by sex.

KEYWORDS: Epidemiology, Osteoporosis, Arterial stiffness, Ischaemic heart disease, Cardiovascular disease

INTRODUCTION

Osteoporosis is a significant public health problem, particularly in aging populations. In the UK, approximately one in three women and one in five men will sustain an osteoporotic fracture in their lifetime[1]. Ischaemic heart disease is the most common cause of morbidity and mortality in the world[2].

Osteoporosis and atherosclerosis share a number of risk factors, such as older age, smoking, and sedentary lifestyle. Interestingly, several studies demonstrate an association between the two conditions beyond these shared risk factors[3–6]. Additionally, biological and genetic studies have proposed common mechanisms driving bone mineralisation and atherogenesis[7–9]. Overall, there is evidence for common causal pathways linking the two disease processes. However, existing literature is limited by small sample sizes, lack of objective measures of bone and heart health, and inability to adequately consider potential mediators and confounders. Further, whilst sex differential disease patterns and the modifying effect of menopause on bone and cardiovascular health are well-recognised, such distinctions have not been clearly elucidated with regard to relationships between these two disease areas.

We studied, in the UK Biobank (UKB), the association of speed of sound (SOS) assessed by quantitative heel ultrasound with measures of arterial compliance on cardiovascular magnetic resonance (CMR) imaging and finger photoplethysmography. We considered the potential mediating effect of a range of blood biomarkers and cardiometabolic morbidities and evaluated differential relationships by sex, menopause status, smoking, diabetes, and obesity. Furthermore, we considered the importance of this relationship in explaining association of SOS with ischaemic cardiovascular outcomes.

METHODS

Setting and recruitment

UKB is a population study incorporating over half a million participants recruited between 2006-2010 from across the UK[10]. Individuals aged 40-69 years old were identified through National Health Service (NHS) registers and invited to participate. The baseline assessment included detailed review of demographics, lifestyle, medical history, a series of physical measures, and blood sampling. The protocol is publicly available[11]. Individuals who were unable to consent or complete baseline assessment due to illness or discomfort were not recruited. Linkages with Hospital Episode Statistics (HES) and death registers enable longitudinal tracking of health outcomes for all participants. Additionally, UKB has produced algorithmically defined outcomes for incidence of key illnesses through checks across multiple data sources[12]. The UKB Imaging Study, which includes cardiovascular magnetic resonance (CMR) imaging, aims to image a subset of 100,000 participants; since its launch in 2015, over 48,000 (July 2020) participants have been scanned[13].

Ethics

This study was covered by the ethical approval for UK Biobank studies from the NHS National Research Ethics Service on 17th June 2011 (Ref 11/NW/0382) and extended 10th May 2016 (Ref 16/NW/0274).

Calcaneal quantitative ultrasound

Calcaneal quantitative ultrasound (QUS) is a non-invasive and radiation-free method of assessing bone quality. QUS parameters are good predictors of fragility fractures and correlate reliably with bone mineral density (BMD) measured by DXA (dual-energy x-ray absorptiometry)[14,15].

Calcaneal QUS was performed for the whole UKB cohort at baseline using the Sahara Clinical Bone Sonometer (Hologic, USA) according to a predefined standard operating procedure (SOP)[16]. Daily quality control checks of the sonometer were performed using a phantom. Measurement was not taken for individuals with open wounds around the heel or metal implants in the heel.

The device automatically generates two parameters: SOS and broadband ultrasound attenuation (BUA). SOS measures the speed at which ultrasound travels through bone, it is calculated by dividing the ultrasound transit time by the length of body part studied. BUA is the slope between the attenuation of sound signal and its frequency as it travels through the bone and soft tissue. Higher SOS and BUA values indicate better bone health (Figure 1, Panel A). Within UKB, if BUA data were missing, it was estimated from the SOS measure. We therefore used SOS in this analysis as it was always directly measured. In cases where bilateral measurements were available, we used the mean.

Arterial stiffness

Arterial stiffness is a measure of vascular compliance; increased stiffness indicates adverse remodelling of the medial layer and impairment of arterial bio-elastic function. Greater arterial stiffness indicates higher risk of atherosclerotic disease and has been validated in a variety of settings[17,18].

Arterial stiffness index

Arterial stiffness index (ASI) is an indirect estimate of large artery stiffness derived from the contour of a pulse waveform as it propagates and is reflected within the arterial tree[17]. Higher ASI represents greater stiffness in the large arteries and is associated with adverse ischaemic cardiovascular outcomes[18]. Lower ASI indicate greater arterial compliance and better vascular health (Figure 1, Panel C).

ASI was measured at the baseline visit using finger photoplethysmography with the PulseTrace PCA2 (CareFusion, USA) device in accordance with a predefined SOP[19]. The participant was seated, and restrictive clothing removed from the upper arm. The PulseTrace infrared sensor was clipped onto a finger and measurement taken over 10-15s. The device provides a pulse waveform, which demonstrates a systolic and diastolic peak, and the time delay between the two peaks (Figure 3). The peak-to-peak time (PPT) represents the transit time for the pulse wave from the root of the subclavian artery to the point of reflection and back. The stiffer the large arteries, the quicker the transit time

(shorter PPT). The path length for the pulse is proportional to the height of the individual, therefore an ASI may be calculated by dividing height by PPT (Figure 3). ASI (m/s) was measured for 169,791 participants at baseline.

Aortic distensibility

Aortic distensibility (AD) is a direct measure of local arterial stiffness determined by the change in aortic cross-sectional area in systole-diastole (i.e. aortic strain) divided by central pulse pressure (CPP in mmHg) [(20)]. It is calculated as in Equation 1. Higher AD indicates a more compliant aorta and better vascular health (Figure 1, Panel B).

Equation 1. Formula for calculation of aortic distensibility

$$AD = \frac{A_{MAX} - A_{MIN}}{A_{MIN} \times CPP}$$

where A_{max} is the maximal and A_{min} the minimal aortic lumen area (mm^2)

AD was measured on cine CMR images showing transverse cross-sections of the ascending and descending aorta throughout the cardiac cycle (Figure 2). A fully automated image analysis workflow has been developed and validated on a large subset of UKB studies (n=5,065)[(21)]. The analysis pipeline has been propagated to cover the first 20,000 UKB CMR scans.

Cardiovascular outcomes

We considered outcomes occurring from point of recruitment (2006–2010) to the latest UKB censor dates (mortality outcomes: 31/01/2018, incident AMI: 31/03/2017) giving follow-up duration of 7-12 years. IHD mortality was defined as primarily cause of death attributed to IHD on death registration documents. Incident AMI was derived from algorithmically defined outcomes, which includes HES and death register data[(22)]; AMIs occurring after the baseline visit were considered.

Definition of covariates

Age, sex, and ethnicity were taken as recorded at baseline. Smoking and alcohol intake were defined according to self-report at baseline. Material deprivation is recorded in the UKB as the Townsend index. We calculated a continuous measure for level of physical activity in metabolic equivalent (MET) minutes/week by weighting different types of activity (walking, moderate or vigorous) by its energy requirements as per the International Physical Activity Questionnaire (IPAQ) study[23]. Hypertension, diabetes, hypercholesterolaemia, and menopause were defined based on self-report at baseline. Body mass index was calculated from height and weight recorded at baseline. The following serum biochemistry measures (from bloods collected at the baseline visit) were considered as potential mediators: C Reactive Protein (CRP), Creatinine, Vitamin D, Calcium, Alkaline Phosphatase (ALP), Insulin-like Growth Factor 1 (IGF1), Sex Hormone Binding Globulin (SHBG), Testosterone, Testosterone/SHBG, Oestradiol, Phosphate, Cystatin C.

Statistical analysis

Statistical analysis was performed using R studio version 3.6.0 [https://www.R-project.org/] and Stata version 14 [StataCorp. 2015. *Stata Statistical Software: Release 14*. College Station, TX: StataCorp LP]. Continuous variables are summarised as mean (standard deviation) for normally distributed parameters and median [interquartile range (IQR)] for skewed distributions. We used a 1.5× IQR rule to remove outliers from the SOS, ASI, and AD variables.

We tested the association of SOS with the ischaemic cardiovascular outcomes separately for men and women using competing risk regression models[24]. We report subdistribution hazard ratios (SHR) per one standard deviation increase in SOS with the corresponding 95% confidence intervals (CIs) and p-values. We next considered the association of SOS with measures of arterial compliance (ASI, AD) using multivariate linear regression models adjusting for age, exercise, smoking, material deprivation, alcohol intake, hypercholesterolaemia, diabetes, and hypertension. Results are presented as standard deviation change in vascular measure per one standard deviation increase in SOS, tested separately by sex. We checked for non-linearity of this relationship using restricted cubic splines. In addition, we

performed subgroup analyses by menopause (women only), smoking status, diabetes, and obesity. We tested whether associations of SOS with vascular compliance explained relationships with ischaemic cardiovascular outcomes.

Finally, we evaluate the mediating effect of a range of blood biomarkers (CRP, Creatinine, Vitamin D, Calcium, ALP, IGF1, SHBG, Testosterone, Testosterone/SHBG, Oestradiol, Phosphate, Cystatin C) and cardiometabolic morbidities (hypertension, diabetes, hypercholesterolaemia) selected based on evidence outlined in existing cardiovascular disease literature. The mediating effect of each mediator was first tested individually, if a significant effect was detected ($p<0.003$, corrected for 15 mediators), the mediator was taken forward for multiple mediation analysis. Independent indirect effects were calculated for each mediator as described by Van Der Weele and Vansteelandt[25]. Confidence intervals were constructed using bootstrap re-sampling. We thus calculate the direct and indirect effect of each mediator and present the proportion of effect mediated as a percentage of the total effect.

RESULTS

Baseline characteristics

Complete data for SOS and ASI were available for 71,949 men and 87,593 women (Table 1). Average age was 58 [50-63] years. Rates of smoking, hypertension, diabetes, and high cholesterol were 28.0%, 5.6%, and 19.7% respectively. Men had poorer cardiometabolic profile compared to women. The majority of women (73.0%) were post-menopause. There were 18,229 participants with SOS and AD data; their baseline characteristics are summarised in Supplementary Table 1.

Association of SOS with measures of arterial stiffness

In fully adjusted linear regression models, higher SOS was associated with lower ASI; the relationship appeared significant and of similar magnitude for both men and women (Table 2). Higher SOS was associated with greater AD at the ascending aorta in fully adjusted models for women, but not for men. Higher SOS was associated with greater AD at the descending aorta in fully adjusted models for men, but not for women. There was no evidence of non-linearity for these relationships (Supplementary Table 2). There were no significant differences in the relationships between men and women.

In stratified analyses, we did not find a differential pattern of association by menopause (Table 3). Higher SOS was associated with lower ASI in pre- and post-menopausal women. There was loss of statistical significance in the AD associations, likely due to small sample size, again with no evidence of differential relationship by menopause.

Association of SOS with measures of arterial stiffness by smoking status, diabetes, and BMI

We found no significant difference in pattern of associations in subgroup analysis by smoking status (Supplementary Table 3). Subgroup analysis by diabetes appeared to show a differential relationship with greater effect in non-diabetics; the interaction term was significant for the relationship between SOS and ASI in men ($p=0.012$) (Supplementary Table 4). With regards BMI (Supplementary Table 5), there appeared to be differential effect of SOS on ASI in men with BMI in the normal or

overweight categories vs those in the obese category with significant interaction term (p=0.0008). These findings should be interpreted with caution, given the different sizes and composition of the sub-cohorts.

Mediation analysis

We considered the role of mediators in the relationship between SOS and ASI as this appeared the most consistent relationship in previous analyses. We considered, separately for men and women, potential mediating effects of the following variables: CRP, Creatinine, Vitamin D, Calcium, ALP, IGF1, SHBG, Testosterone, Testosterone/SHBG, Oestradiol, Phosphate, Cystatin C, hypertension, diabetes, and hypercholesterolaemia. We first checked the mediating effect of each variable individually (Supplementary Tables 6 and 7); variables with significant mediated effects were taken forward for multiple mediation analysis. In the final models, we included variables that had statistically significant effects in the multiple mediator model (Supplementary Tables 8 and 9).

In multiple mediation analysis, biomarkers relating to bone mineralisation appeared important for both men and women. For men, ALP, phosphate, and vitamin D accounted for 7.5%, 4.6%, and 3.2% of the observed effect. In women, ALP and phosphate accounted for 9.6% and 13.2% of the observed effect. CRP accounted for 6.1% of the mediated effect in men and -8.6% in women. SHBG had an important suppressing effect for both men and women as adjustment for this variable increased the effect by 17.14% and 19.55% respectively.

In men the overall effect was mediation i.e. the magnitude of the main exposure-outcome relationship effect was reduced by adjustment for the mediators. In the women the effect was one of suppression rather than mediation as the magnitude of the exposure-outcome relationship increased when we added the potential mediators. The association between ASI and SOS remained significant with all the mediators in the model.

Association of SOS with ischaemic cardiovascular outcomes

SOS was available for 477,683 participants at baseline. We considered association with IHD mortality and incident AMI for this cohort (Table 4); baseline characteristics are summarised in Supplementary Table 10. Follow-up time for mortality was 2,342,445 person years for women and 1,888,767 for men. There were 388 IHD deaths in women (rate=0.17 per 1000 person years) and 1,722 (rate=0.91 per 1000 person years) in men. Follow-up time for incident AMI was 2,123,170 person years for women and 1,659,850 person years for men. During this time, there were 2,415 AMI events in women (rate=1.14 per 1000 person years) and 5,616 events (rate=3.38 per 1000 person years) in men.

In crude models including age only, higher SOS was associated with significantly reduced hazard of both incident AMI and IHD mortality in men, but not in women. There was loss of significance for the relationship with incident AMI with addition of exercise, material deprivation, and alcohol to the model. The negative association with IHD mortality remained significant in men in this model and in a further model additionally including hypertension, hypercholesterolaemia, and diabetes. In this fully adjusted model, for men, one standard deviation increase of SOS was associated with 14% lower hazard of IHD mortality [SHR 0.86 (0.75-1.00), p-value 4.0×10^{-7}].

We tested whether the relationship of SOS with IHD mortality may be explained by observed associations of the former with ASI. Addition of ASI as covariate to competing risk models did not alter the association of greater SOS with lower IHD mortality in men, and no association in women. Therefore, it appeared that the association with IHD mortality is likely to occur through other mechanisms.

DISCUSSION

Summary of study findings

Our findings support association of higher SOS with lower arterial compliance (higher AD, lower ASI), that is, that better bone health is associated with better heart health. The relationship appeared consistent in men and women and by menopause status. There was no clear differential relationship by smoking status, diabetes, or BMI. A range of blood biomarkers were considered as potential mediators of the association between SOS and ASI; the mediation pattern appeared different for men and women and these markers did not adequately explain the observed associations. Higher SOS was associated with lower IHD mortality in men, but not in women. This relationship was not attenuated with addition of ASI to models, suggesting mediation through independent mechanisms. In summary, higher SOS was associated with better vascular health by ASI and AD and with lower IHD mortality in men; underlying mechanisms are complex and likely vary by sex.

Strengths and limitations

The large, broad population sample in UKB permitted investigation of sex-specific, and in women menopause status specific, relationships using validated measures of bone and cardiac health, incorporating a wide range of covariates and mediators. The age range in UKB was limited to 40-69 years at recruitment, as such, our results may not be applicable to younger or older ages. There is limited information in terms of the performance characteristics of the heel ultrasound device used in UK Biobank. Several instruments of the same type and same software version were used across the centres, but coefficients of variation and long-term stability data not available currently. If anything, inability to accommodate these factors is likely to add noise and thus bias towards the null rather than generate any spurious relationships. Finally, we cannot exclude the possibility of residual confounding due to the observational design of the study, and it should be recognised that although mediation analysis partitions variance in the associations, we cannot conclude causal relationships directly from this analysis.

Comparison with existing literature

Several smaller studies have investigated the relationship between bone quality and arterial stiffness made by pulse waveform analysis, but none using AD. In general, there is under-representation of men and pre-menopausal women and there are scant data through which to infer sex or menopausal status specific effects. A number of studies have been conducted in small highly selective cohorts with specific risk profiles, which limits the generalisability of their findings and has the inherent potential to introduce bias.

Consistent with our findings, in a study of 7,865 Japanese men and women, Hirose et al.[26] report a significant association between better bone quality on calcaneal QUS and lower arterial stiffness by pulse wave velocity (PWV), with the relationship appearing stronger for post-menopausal women. Avramovski et al.[27] and Zhang et al[28] also report significant negative associations between BMD and arterial stiffness. Distinctions between men and women or menopause status were not considered, perhaps due to sample size limitations. These findings are consistent with results from small cohorts of Korean[29] and Turkish women[30]. In a study of 633 individuals, Giallauria et al.[31] report a significant association between higher bone quality assessed by computed tomography and lower arterial stiffness by PWV for women, but not for men.

Several studies investigated the relationship between bone quality and vascular health in populations with specific risk profiles. For instance, Masugata et al.[32] demonstrated a negative association between BMD and arterial stiffness in 52 hypertensive men and women. Interestingly, Li et al.[33] documented a negative association between BMD and arterial stiffness by PWV in hypertensive men (n=355), but not in a comparator group without hypertension. Similarly, Li et al.[34] reported a significant negative association between lumbar spine BMD and arterial stiffness by PWV in 334 men with silent brain infarction, but not in 368 matched controls. Van Dijk et al.[35] identified no association between arterial stiffness measures and BMD or QUS parameters in 519 older men and women with hyperhomocystinaemia. The findings to date from these generally small studies are therefore somewhat variable, but in general support the notion of positive associations between bone and cardiovascular health.

Our findings, in the largest sample studied to date, confirm the association of better bone quality (higher SOS) with lower arterial compliance (lower ASI, higher AD) with consistent relationships by sex and menopause status.

A number of studies have examined the relationship between serum markers of bone metabolism and arterial stiffness. In a study of the relationship between plasma regulators of bone metabolism in 1,003 individuals with type 2 diabetes, Sharif et al.[36] identify significant association between higher levels of plasma osteopontin and greater arterial stiffness. In a study of 144 post-menopausal women, Albu et al.[37] identify significant association between higher plasma osteoprotegerin levels and greater arterial stiffness on PWV, but not with osteopontin (as per Sharif et al.[36]), suggesting possible differences in pathophysiology in men and women. However, given that osteoprotegin and osteopontin have been implicated directly in vascular pathology as well as bone metabolism, such findings do not necessarily demonstrate direct bone-heart mechanisms[38]. Indeed, such observations clearly demonstrate the complexity in these relationships and the difficulty in elucidating specific mechanisms in bone versus those in the vascular endothelium at the whole organ level. Our analysis of the effect of a range of blood biomarkers in mediating the relationship between SOS and ASI also suggested differences in mechanistic pathways in men and women.

Our finding of association of higher SOS with lower IHD mortality in men was consistent with multiple previous reports of association of lower BMD with cardiovascular mortality outcomes, which, similar to our findings, appeared stronger in men[39–41]. Whilst several small studies in select populations of older women, appear to show an inverse association between QUS measures of bone health and cardiovascular mortality (without specificity for IHD mortality) [42,43], these findings are not replicated in larger studies[44]. Consistent with our findings, in a study of 5,816 older women, Bauer et al.[44] do not report a significant association between BMD and cardiovascular mortality over five years of prospective follow up. Interestingly, in the present study, the observed inverse association between SOS with IHD mortality in men was not explained by association of higher SOS with lower arterial compliance, suggesting alternative mediating mechanisms are relevant here.

CONCLUSIONS

Our findings support a positive association between bone and vascular health measures which is consistent in men and women and with menopause. The association of higher SOS with lower IHD mortality appeared significant for men and was not explained by associations with arterial stiffness. The underlying pathophysiology of the bone heart axis is complex and multifaceted and likely varies in men and women. Further research into potential mechanistic pathways is needed.

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Authors' roles: All authors contributed to preparation and final approval of the manuscript. ZRE, NCH, EMC, JP, SEP led the initial project and drafting; ZRE and JC undertook statistical analysis; LB performed aortic distensibility analysis, image quality control and supervised the validation of automated results; NA, KF, EL, EH, JMP, MMS, RJT performed manual validation of aortic distensibility results; NCH/SEP oversaw the project and are guarantors.

Table 1. Baseline participant characteristics (Baseline sample)

	Whole cohort (*n*=159,542)	Men (*n*=71,949)	Women (*n*=87,593)
Age	58 [50-63]	59 [51-64]	58 [50-63]
Ethnicity (White Caucasian)	145,627 (91.9%)	65,804 (92.2%)	79,823 (91.6%)
Townsend deprivation score	-1.8 [-3.4 to 0.8]	-1.8 [-3.4 to 0.8]	-1.8 [-3.4 to 0.7]
Body mass index kg/m^2	26.7 [24.1 to 29.8]	27.2 [24.9 to 29.9]	26.1 [23.4 to 29.8]
Current smoking	16,085 (10.1%)	8,637 (12.0%)	7,448 (8.5%)
Regular alcohol use	67,664 (42.5%)	36,478 (50.9%)	31,186 (35.7%)
Physical activity (metabolic equivalent minutes/week)	1,891 [874 to 3,786]	1,908 [864 to 3,930]	1,866 [878 to 3,666]
Multimorbidity (number of non-cancer illnesses)	2.0 [1.0 to 3.0]	2.0 [1.0 to 3.0]	2.0 [1.0 to 3.0]
Hypertension	44,626 (28.0%)	23,676 (32.9%)	20,950 (23.9%)
Diabetes	8,981 (5.6%)	5,351 (7.4%)	3,630 (4.1%)
Hypercholesterolaemia	31,465 (19.7%)	18,571 (25.8%)	12,894 (14.7%)
Post-menopausal	–	–	53,940 (73.0%)
Arterial stiffness index (m/s)	9.0 [6.9 to 11.2]	9.8 [7.8 to 11.8]	8.3 [15.3 to15.7]
Speed of sound (10^2m/s)	15.5 (0.3)	15.6 (0.3)	15.5 (0.3)

Table 1 footnote: Data based on information collected at baseline assessment. Continuous variables presented as median [interquartile range] or mean (standard deviation). Discrete data presented as frequencies (percentages).

Table 2. Linear regression models showing association of SOS with measures of arterial stiffness in men and women

		Model 1: Age	Model 2: Age, exercise, smoking, deprivation, alcohol	Model 3: Model 2+ hypercholesterolaemia, diabetes, hypertension
	ASI			
Men	B (95% CI)	-0.030 (-0.037, -0.023)	-0.021 (-0.028, -0.013)	-0.020 (-0.028, -0.012)
n=71,949	p-value	4.8×10^{-17}*	1.5×10^{-7}*	2.6×10^{-7}*
Women	B (95% CI)	-0.027 (-0.034, -0.021)	-0.024 (-0.031, -0.016)	-0.026 (-0.033, -0.018)
n=87,593	p-value	5.6×10^{-16}*	6.0×10^{-10}*	2.8×10^{-11}*
P value for interaction		0.605	0.541	0.307
	AD (ascending aorta)			
Men	B (95% CI)	0.018 (0.000, 0.036)	0.017 (-0.002, 0.036)	0.017 (-0.002, 0.036)
n=8,767	p-value	0.046	0.085	0.085
Women	B (95% CI)	0.025 (0.008, 0.042)	0.020 (0.000, 0.039)	0.020 (0.000, 0.039)
n=9,462	p-value	0.004*	0.045*	0.043*
P value for interaction		0.588	0.846	0.829
	AD (descending aorta)			
Men	B (95% CI)	0.040 (0.021-0.059)	0.037 (0.018, 0.057)	0.037 (0.017, 0.056)
n=8,767	p-value	2.2×10^{-5}*	0.0002*	0.0002*
Women	B (95% CI)	0.017 (-0.000, 0.035)	0.019 (-0.001, 0.039)	0.019 (-0.000, 0.039)
n=9,462	p-value	0.057	0.063	0.054
P value for interaction		0.081	0.194	0.217

Table 2 footnote: ASI: arterial stiffness index; AD: aortic distensibility; B: beta coefficient; CI: confidence interval; SOS: speed of sound. B= increase (number of SDs) in outcome for a 1 SD increase in SOS. *indicates p-value <0.05.

Table 3. Linear regression models showing association of SOS with measures of arterial stiffness in women stratified by menopause status

		Model 1: Age	Model 2: Age, exercise, smoking, deprivation, alcohol.	Model 3: Model 2+ hypercholesterolaemia, diabetes, hypertension
	ASI			
Pre-menopause	B (95% CI)	-0.026 (-0.041, -0.011)	-0.025 (-0.041, -0.008)	-0.025 (-0.042, -0.009)
n=33,653	p-value	0.0008*	0.003*	0.003*
Post-menopause	B (95% CI)	-0.019 (-0.028, -0.010)	-0.015 (-0.025, -0.005)	-0.018 (-0.028, -0.007)
n=53,940	p-value	4.9×10^{-5}*	0.005*	0.0009*
P value for interaction		0.433	0.327	0.449
	AD (ascending aorta)			
Pre-menopause	B (95% CI)	0.016 (-0.017, 0.049)	0.012 (-0.024, 0.048)	0.012 (-0.024, 0.048)
n=4,333	p-value	0.338	0.522	0.516
Post-menopause	B (95% CI)	0.013 (-0.011, 0.037)	0.011 (-0.016, 0.038)	0.012 (-0.015, 0.039)
n=5,129	p-value	0.288	0.483	0.370
P value for interaction		0.884	0.964	0.984
	AD (descending aorta)			
Pre-menopause	B (95% CI)	0.003 (-0.029, 0.036)	0.001 (-0.035. 0.036)	0.001 (-0.035, 0.036)
n=4,333	p-value	0.837	0.973	0.957
Post-menopause	B (95% CI)	0.004 (-0.020, 0.028)	0.008 (-0.019, 0.035)	0.009 (-0.018, 0.035)
n=5,129	p-value	0.741	0.563	0.513
P value for interaction		0.977	0.749	0.727

Table 3 footnote: ASI: arterial stiffness index; AD: aortic distensibility; B: beta coefficient; CI: confidence interval; SOS: speed of sound. B= increase (number of SDs) in outcome for a 1 SD increase in SOS. *indicates p-value <0.05.

Table 4. Competing risk models of the association of SOS with incident AMI and IHD mortality

n=477,683		Model 1: Age	Model 2: Age, exercise, smoking, deprivation, alcohol	Model 3: Model 2+ hypercholesterolaemia, diabetes, hypertension
	Incident AMI			
Men (n=214,410)	SHR (95% CI)	0.96 (0.93-0.99)	0.99 (0.96-1.02)	0.99 (0.96-1.02)
	p-value	0.002*	0.651	0.658
Women (n=263,273)	SHR (95% CI)	0.97 (0.93-1.01)	1.03 (0.97-1.08)	1.00 (0.95-1.05)
	p-value	0.159	0.352	0.987
	IHD mortality			
Men (n=214,410)	SHR (95% CI)	0.81 (0.77-0.85)	0.86 (0.81-0.91)	0.86 (0.81-0.91)
	p-value	7.8×10^{-15}*	9.9×10^{-7}*	4.0×10^{-7}*
Women (n=263,273)	SHR (95% CI)	0.92 (0.82-1.02)	0.91 (0.78-1.05)	0.86 (0.75-1.00)
	p-value	0.093	0.184	0.051

Table 4 footnote: AMI: acute myocardial infarction; ASI: arterial stiffness index; CI: confidence interval; SHR: subdistribution hazard ratio; IHD: ischaemic heart disease. *indicates p-value <0.05

REFERENCES

1. Van Staa TP, Dennison EM, Leufkens HGM, Cooper C. Epidemiology of fractures in England and Wales. Bone. 2001;29(6):517–22.
2. Roth GA, Abate D, Hassen Abate K, et al. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980-2017: a systematic analysis for the Global Burden of Disease Study 2017 GBD 2017 Causes of Death Collaborators*. Lancet 2018;392:1736–88.
3. Kiel DP, Kauppila LI, Cupples LA, Hannan MT, O'Donnell CJ, Wilson PW. Bone loss and the progression of abdominal aortic calcification over a 25 year period: the Framingham Heart Study.[Erratum appears in Calcif Tissue Int. 2004 Feb;74(2):208]. Calcif Tissue Int. 2001;68(5):271–6.
4. Hak AE, Pols HAP, Van Hemert AM, Hofman A, Witteman JCM. Progression of aortic calcification is associated with metacarpal bone loss during menopause: A population-based longitudinal study. Arterioscler Thromb Vasc Biol. 2000;20(8):1926–31.
5. Barengolts EI, Herman M, Kukreja SC, Kouznetsova T, Lin C, Chomka E V. Osteoporosis and coronary atherosclerosis in asymptomatic postmenopausal women. Calcif Tissue Int. 1998;62(3):209–13.
6. Lee SN, Cho JY, Eun YM, Song SW, Moon KW. Associations between osteoporosis and coronary artery disease in postmenopausal women. Climacteric. 2016;19(5):458–62.
7. Luo G, Ducy P, McKee MD, et al. Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein. Nature. 1997;386(6620):78–81.
8. Bucay N, Sarosi I, Dunstan CR, et al. Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. Genes Dev. 1998;12(9):1260–8.
9. Papanicolaou DA, Wilder RL, Manolagas SC, Chrousos GP. The Pathophysiologic Roles of Interleukin-6 in Human Disease. Ann Intern Med. 1998;128(2):127.
10. Allen NE, Sudlow C, Peakman T, Collins R. UK biobank data: Come and get it. Sci Transl Med. 2014;6(224):224ed4.
11. UK Biobank: Protocol for a large-scale prospective epidemiological resource. 2007. Available

from: https://www.ukbiobank.ac.uk/wp-content/uploads/2011/11/UK-Biobank-Protocol.pdf (Accessed 25 July 2020).

12. Schnier C, Sudlow Biobank CU. Algorithmically-defined health outcomes (Chief Scientist), with input from members of the UK Biobank Follow-up and Outcomes Adjudication Group. 2017. Available from: https://biobank.ctsu.ox.ac.uk/crystal/crystal/docs/alg_outcome_main.pdf (Accessed 25 July 2020)
13. Petersen SE, Matthews PM, Bamberg F, et al. Imaging in population science: cardiovascular magnetic resonance in 100,000 participants of UK Biobank - rationale, challenges and approaches. J Cardiovasc Magn Reson. 2013; 15(1):46.
14. Krieg MA, Barkmann R, Gonnelli S, et al. Quantitative Ultrasound in the Management of Osteoporosis: The 2007 ISCD Official Positions. J Clin Densitom. 2008;11(1):163–87.
15. Chin KY, Ima-Nirwana S. Calcaneal quantitative ultrasound as a determinant of bone health status: What properties of bone does it reflect? Int J Med Sci. 2013;10(12):1778–83.
16. UK Biobank Ultrasound Bone Densitometry. 2011. Available from: http://biobank.ndph.ox.ac.uk/showcase/showcase/docs/Ultrasoundbonedensitometry.pdf (Accessed 25 July 2020)
17. Laurent S, Cockcroft J, Van Bortel L, et al. Expert consensus document on arterial stiffness: Methodological issues and clinical applications. Eur Heart J. 2006;27(21):2588–605.
18. Abdullah Said M, Eppinga RN, Lipsic E, Verweij N, Van der Harst P. Relationship of arterial stiffness index and pulse pressure with cardiovascular disease and mortality. J Am Heart Assoc. 2018;7(2):e007621.
19. UK Biobank Arterial Pulse-Wave Velocity. 2011. Available from: https://biobank.ndph.ox.ac.uk/showcase/showcase/docs/Pulsewave.pdf (Accessed 25 July 2020)
20. Pannier BM, Avolio AP, Hoeks A, Mancia G, Takazawa K. Methods and Devices for Measuring Arterial Compliance in Humans. Am J Hypertens 2000;15:743–53.
21. Biasiolli L, Hann E, Lukaschuk E, et al. Automated localization and quality control of the

aorta in cine CMR can significantly accelerate processing of the UK Biobank population data. PLoS One. 2019;14(2):e0212272.

22. Schnier C, Bush K, Nolan J, Sudlow C. Definitions of Acute Myocardial Infarction and Main Myocardial Infarction Pathological Types UK Biobank Phase 1 Outcomes Adjudication Documentation on behalf of UK Biobank Outcome Adjudication Group Definitions of Acute Myocardial Infarction. 2017. Available from: http://biobank.ndph.ox.ac.uk/showcase/showcase/docs/alg_outcome_mi.pdf (Accessed 25 July 2020)

23. Craig CL, Marshall AL, Sjöström M, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003;35(8):1381–95.

24. Fine JP, Gray RJ. A Proportional Hazards Model for the Subdistribution of a Competing Risk. J Am Stat Assoc. 1999;94(446):496–509.

25. Van Der Weele T, Vansteelandt S. Mediation analysis with multiple mediators. Epidemiol Method. 2014;2(1):95–115.

26. Hirose KI, Tomiyama H, Okazaki R, et al. Increased pulse wave velocity associated with reduced calcaneal quantitative osteo-sono index: Possible relationship between atherosclerosis and osteopenia. J Clin Endocrinol Metab. 2003;88(6):2573–8.

27. Avramovski P, Avramovska M, Sikole A. Bone Strength and Arterial Stiffness Impact on Cardiovascular Mortality in a General Population. J Osteoporos. 2016; 2016:7030272.

28. Zhang M, Bai L, Kang J, Ge J, Peng W. Links between arterial stiffness and bone mineral density in middle-aged and elderly Chinese individuals: A cross-sectional study. BMJ Open. 2019;9(8):e029946.

29. Kim NL, Suh HS. Correlation of arterial stiffness and bone mineral density by measuring brachial-ankle pulse wave velocity in healthy Korean women. Korean J Fam Med. 2015;36(6):323–7.

30. Baykara M, Öztürk C, Elbüken F. The relationship between bone mineral density and arterial stiffness in women. Diagnostic Interv Radiol. 2012;18(5):441–5.

31. Giallauria F, Ling SM, Schreiber C, et al. Arterial stiffness and bone demineralization: The

Baltimore longitudinal study of aging. Am J Hypertens. 2011;24(9):970–5.

32. Masugata H, Senda S, Inukai M, et al. Association between Bone Mineral Density and Arterial Stiffness in Hypertensive Patients. Tohoku J Exp Med. 2011;223(2):85–90.

33. Li XS, He H, Zhao YL, et al. Bone Mineral Density Is Negatively Associated With Arterial Stiffness in Men With Hypertension. J Clin Hypertens. 2016;18(11):1106–11.

34. Li L lu, Li Y, Liu T, Wang R tao. Bone mineral density is negatively associated with arterial stiffness in men with silent brain infarction. Int J Stroke. 2015;10(7):E74.

35. Van Dijk SC, De Jongh RT, Enneman AW, et al. Arterial stiffness is not associated with bone parameters in an elderly hyperhomocysteinemic population. J Bone Miner Metab. 2016;34(1):99–108.

36. Sharif S, Bots ML, Schalkwijk C, Stehouwer CDA, Visseren FLJ, Westerink J. Association between bone metabolism regulators and arterial stiffness in type 2 diabetes patients. Nutr Metab Cardiovasc Dis. 2018;28(12):1245–52.

37. Albu A, Fodor D, Bondor C, Crâciun AM. Bone metabolism regulators and arterial stiffness in postmenopausal women. Maturitas. 2013;76(2):146–50.

38. Roumeliotis S, Roumeliotis A, Dounousi E, Eleftheriadis T, Liakopoulos V. Biomarkers of vascular calcification in serum. Adv Clin Chem. 2020;98:91–147.

39. Van der Klift M, Pols HAP, Geleijnse JM, Van der Kuip DAM, Hofman A, De Laet CEDH. Bone mineral density and mortality in elderly men and women: The Rotterdam study. Bone Bone; 2002;30(4):643–8.

40. Trivedi DP, Khaw KT. Bone mineral density at the hip predicts mortality in elderly men. Osteoporos Int. Osteoporos Int. 2001;12(4):259–65.

41. Mussolino ME, Madans JH, Gillum RF. Bone mineral density and mortality in women and men: The NHANES I epidemiologic follow-up study. Ann Epidemiol. 2003;13(10):692–7.

42. González-Macías J, Marín F, Vila J, et al. Relationship between bone quantitative ultrasound and mortality: A prospective study. Osteoporos Int. 2009;20(2):257–64.

43. Pinheiro MM, Castro CM, Szejnfeld VL. Low Femoral Bone Mineral Density and Quantitative Ultrasound Are Risk Factors for New Osteoporotic Fracture and Total and

Cardiovascular Mortality: A 5-Year Population-Based Study of Brazilian Elderly Women. J Gerontol. 2006;16A(2):196–203.

44. Bauer DC, Palermo L, Black D, Cauley JA. Quantitative ultrasound and mortality: A prospective study. Osteoporos Int. 2002;13(8):606–12.