Bone mineral density and incidence of stroke: EPIC-Norfolk population-based study, systematic review and meta-analysis

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Summary

Background and Purpose: The prospective link between osteoporosis and future risk of stroke requires evidence from large scale population-based long term studies.

Methods: Calcaneum broadband ultrasound attenuation (BUA) was measured in the Norfolk cohort of the European Prospective Investigation into Cancer (EPIC-Norfolk) between 1997 and 2000. Incident strokes were ascertained by hospital record linkage and death certificates in March 2009 and December 2011 respectively. A search of MEDLINE and EMBASE was conducted to evaluate the relationship between bone mineral density and incident stroke. After data extraction of relevant studies, pooled risk of stroke was estimated using meta-analysis.

Results: In 14290 participants (mean follow-up of 9.3 years; total person years132574), there were 599incident strokes. Participants in the lowest 10% of the calcaneum BUA distribution had an increased stroke risk (HR 1.41 (95% CI 1.02-1.94)) compared to those in the top 30% of the distribution after adjustments. A decrease of about 1 SD in BUA (20 db/MHz) was associated with a 17% increase in relative risk of stroke (95%CI:5%-30%).

Meta-analysis of 4 studies (25760 participants, 1237 cases of stroke) found that for every decrease in 1 SD in bone mineral density there was an increased risk of incident stroke among women (pooled RR 1.22 95% CI 1.09-1.37, I^2=0%, 3 studies) but not in men (pooled RR 1.05 95% CI 0.94-1.17, I^2=0%, 2 studies).

Conclusion: Bone mineral density predicts total stroke risk. The evidence is stronger in women with regard to the continuous relationship.

Keywords

- Bone mineral density (BMD)
- Osteoporosis
- Stroke
- Epidemiology
- Risk factors

Introduction

Osteoporosis and increased fracture risk are recognized complications following a stroke [1]. However, the relationship between bone health and stroke is not straight forward although there appears to be a complex mutual connection between stroke and bone health [2]. The current evidence suggests that these two conditions are risk factors for each other albeit more clear support for the effect of stroke on bone health. To date, very few population level evidence exists with regard to the association of low bone mineral density (BMD) with subsequent stroke risk.

Bone mineral density (BMD) can be assessed using different methods. Dual-energy X-ray absorptiometry (DEXA) is regarded as a standard non-invasive method to assess BMD but it is costly and therefore not always available to use in daily clinical practice. Quantitative ultrasound at the peripheral sites is relatively cheaper, easy to perform and hence has potential to be useful in daily practice. The validation studies against DEXA suggest the usefulness of quantitative ultrasound at heel (calcaneus) in diagnosing osteoporosis and future fracture risk [3].

We have previously reported that quantitative ultrasound of the calcaneus predicted total and hip fracture risk in men and women in the European Prospective Investigation into Cancer (EPIC)-Norfolk prospective population study [4]. In this paper we examine the relationship between BMD assessed using broadband ultrasound attenuation (BUA) and velocity of sound (VOS) and subsequent stroke risk and conducted a systematic review and meta-analysis to quantify the existing evidence of predictive value of bone mineral density on subsequent stroke risk.

Methods

The Norfolk cohort of the European Prospective Investigation into Cancer (EPIC-Norfolk) comprises 25,000 men and women aged 40–79 years at baseline who were resident in Norfolk, UK, at the time of recruitment. Data collection and follow-up in EPIC-Norfolk was expanded to enable assessment of chronic disease determinants; recruitment and study methods have been detailed elsewhere [5] [6]. Briefly, between 1993 and 1997, participants completed health questionnaires, and attended a first clinic visit, at which detailed health and lifestyle measurements were taken.

Between 1997 and 2000, surviving participants were invited to attend a second clinic visit. About 15 000 responded, a response rate of 58% of those mailed, after excluding those who had moved from the area or died. At this visit, ultrasound measurements of the calcaneum were obtained [4]. Trained nurses examined participants. Height and weight were measured in light clothing without shoes. Broadband ultrasound attenuation (BUA; db/MHz) and speed of sound (VOS; m/s) were measured at least twice on each calcaneum with a CUBA sonometer (McCue Ultrasonics, Winchester, UK). We used the mean of left and right ultrasound measures for analysis. The coefficient of variation was 3·5%. We recorded ambient temperature. The five CUBA machines used were calibrated daily with a physical phantom. Machines were also compared on one calcaneus.

Weight was measured with participants wearing light clothing without shoes. Height was measured up to the nearest 0.1 cm using a stadiometer with shoes removed. Body mass index

(BMI) was calculated as weight (kg) divided by height squared (m^2). Blood pressure (BP) was measured with an Accutorr monitor (Datascope, Huntingdon, UK) after the participant had been seated for 5 min. We used the mean of two BP measurements for analysis. From non-fasting venous blood samples we measured serum total cholesterol with the RA 1000 (Bayer Diagnostics, Basingstoke, UK).

At the baseline survey (1993-1997) participants completed a detailed health and lifestyle questionnaire. Participant's educational status, occupational social class, and physical activity were obtained from the baseline health and lifestyle questionnaire. Educational status was recorded as no qualification, O- level, A-level, degree or higher qualification. Social class was classified according to the Registrar General's occupation-based classification scheme [7] [8]. A four-level physical activity index was derived from the validated EPIC short physical activity questionnaire designed to assess combined work and leisure activity [9].

We ascertained medical history with a question on the health questionnaire repeated in second health survey (1997-2000)—"Has a doctor ever told you that you have any of the following?"—followed by a list of conditions, including cancer, stroke, myocardial infarction, and diabetes mellitus. Smoking history was obtained from the questions "Have you ever smoked as much as one cigarette a day for as long as a year?" and "Do you smoke cigarettes now?" Participants were also asked to report the medications they were taking (name, dose frequency etc.).

For comparisons of categorical variables with 1SD decrease in BUA and VOS, we grouped these into dichotomous variables: physical activity as physically inactive (physical activity categories

1 & 2) and physically active (categories 3 & 4), occupational social class as lower (class III-manual, IV and V) and higher (class III-non manual, II and I), educational attainment as lower (no or less than A level qualification) and higher educational attainment levels (at least A level).

Case ascertainment

All participants were flagged for death by cause at the Office of National Statistics. Participants were also linked to NHS hospital information system so that admission anywhere in the UK was notified to EPIC-Norfolk. They were also linked to ENCORE (East Norfolk COmmission Record) for admission episodes. Incident strokes were identified from the death certificates (Office of National Statistics) or hospital discharge code ICD10 –I60-I69. The follow up time started at baseline for this study (date of second health check) and ended at the censor date defined as date of the event (date of death with stroke as cause of death in the death certificate or incident stroke) or last follow up (December 2011). ENCORE data linkage was available up to end of March 2009 and the ONS linkage was available up to end of December 2011. These ascertainment methods of EPIC-Norfolk for stroke incidence have been previously validated [10].

No absolute diagnostic cutpoints exist for osteoporosis with quantitative ultrasound [11][12][13]. Criteria in studies include a specified number of SDs below either the sex-specific and age-specific mean, or the sex-specific mean for a young adult. WHO recommended a cutpoint of more than 2·5 SDs below the mean for a young adult on the basis of bone densitometry. The paucity of data from men makes definition of cut-points even more difficult.

For these analyses, we used an arbitrary lowest 10% of the sex-combined distribution for the ultrasound measures BUA and VOS to define a high-risk group. This absolute value was about 2 SDs below the mean at age 45 years for men and 1·5 SDs below the mean at age 45 years for women. We used sex-combined percentiles to enable direct comparison of men and women with the same absolute values. We categorized the remainder of the cohort by 40th and 70th percentile cutpoints to obtain four groups: the lower 10%, and then three equal groups of 30% of the population. We assessed stroke rates and relative risks by these categories. We used the Cox multivariate regression model to examine the independent predictive value of calcaneum measures for stroke incidence.

Statistical analyses were carried out using STATA version 11.2/SE (Texas, USA). Participants with missing data were excluded from analyses. After excluding participants with prevalent cancer and stroke at second health visit, multivariate adjustments were made to examine how far the association between BMD at the calcaneus and the risk of stroke might be explained by other known demographic, lifestyle, socioeconomic and cardiovascular risk factors. Calcaneum ultrasound measures were used as continuous variables. Stepwise adjustments were constructed. We adjusted for age and sex in model A, age, sex, systolic BP and cholesterol in model B, and we additionally adjusted lifestyle factors- smoking, physical activity and BMI in model C. Model D additionally adjusted for occupational social class and educational level, and model E was constructed as of model D but additionally adjusted for prevalent myocardial infarction (MI) and diabetes. The final model (model F) adjusted for variables as in model E along with lipid lowering medication and anti-hypertensive medication use.

We also assessed the relative predictive value of 1 SD decrease in BUA and VOS using Cox multivariate model compared with other risk factors: every 1 year increase in age, being male sex, every 5 mmHg increase in systolic BP, every 1 mmol/L increase in total cholesterol, being a current smoker, being physically active, having BMI >30kg/m2, having no or lower than A-level educational attainment, being in the lower occupational social class, and having prevalent diabetes or MI. We further examined the association of calcaneum measures with stroke stratified by sex (male vs. female), age group (<65 vs. >=65 years), and cigarette smoking status.

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The EPIC-Norfolk study was approved by the Norwich Research Ethics Committee.

Systematic review and meta-analysis

Studies which reported the association between bone mineral density and incident stroke were included in the systematic review. We searched PubMed and EMBASE from inception until July 2013 using the terms described in Supplementary Data 1, with no language limitations, and we checked bibliographies of included articles. One reviewer (CSK) screened abstracts and titles and then two reviewers (CSK and YKL) independently reviewed all potentially relevant studies to confirm eligibility. Data extraction of included studies was performed by CSK and JKY, and checked by YKL. Study validity was evaluated based on methods used in ascertainment of BMD and incident stroke events as well as steps taken to reduce confounding in the primary studies. We pooled data using the inverse variance method and random effects model in RevMan 5.2

software (Nordic Cochrane Center, Copenhagen, Denmark). For these comparisons, we used the multivariable adjusted measures of association (hazard ratios, relative risks or odds ratios) for a one standard deviation reduction in BMD and incident stroke. We performed analysis considering sex- and race- specific results. Heterogeneity was estimated using I^2, and we considered a value greater than 50% to demonstrate substantial heterogeneity[14]. We planned to evaluate publication bias through asymmetry testing if there were >10 studies in the dataset, and no evidence of significant heterogeneity [15].

Results

Of 15786 who attended second health check in 1997-2000 with available heel bone ultrasound measures, 382 were excluded from the analysis as they had a previous stroke and 1136 were excluded due to a previous diagnosis of cancer. A total of 14 290 men and women aged 42–82 years were included. During mean follow-up of 9.3 years (SD 1.7; total person year 132574), there were 599incident strokes. Figure 1 demonstrates the reasons for exclusions and numbers of participants included in the different models. A total of 12795 participants were included in the final models (models D, E and F). There were no significant differences of participants' characteristics between the model A and final models with regards to age and sex.

Mean values & ranges of the percentile categories are 48.75 (14.95-56.12); 66.28 (56.13-74.59); 81.96 (74.61-89.84) and 103.10 (89.85-145.54) for BUA and 1563.75 (1456.25-1581.50); 1605 (1581.75-1623.25); 1638.92 (1623.33-1655.25) and 1682.75 (1655.50-1802.50) for VOS, respectively. Table 1 shows the baseline sample characteristics of participants included in the EPIC-Norfolk study who had calcaneum ultrasound performed during their second health check by BUA categories. People in the lowest 10% of BUA distribution were older, and were more likely to be women. They had higher systolic blood pressure and cholesterol levels. They were more likely to be current-smoker, less likely to be physically active, more likely to have BMI <30 kg/m^2. Despite overall statistical significant difference between the groups with regard to social class, the differences were small except lower proportion of professionals (5%) in the bottom 10% compared to three other categories (8%). The proportions of people with prevalent diabetes were lower in this group. Whilst there appeared to be no difference in percentage of

people on lipid lowering treatment, a higher proportion of people in the bottom group were on anti-hypertensive medication compared to other categories.

Table 2 shows consistent significant trends with significant higher risk of stroke in those in the lowest 10% of BUA distribution compared to the highest 30%. Additional adjustments attenuated the estimates but remained statistically significant. Notably, adjusting for lifestyle behaviors attenuated the association. Further adjustments did not attenuate the associations further. Similar but statistically non-significant trends were observed for VOS analysis.

Table 3 shows the independent HRs for stroke risk during follow up for every 1SD decrease in BUA or VOS values in comparison with every one year increase in age, being male, every increase in systolic BP of 5 mmHg, every increase in 1 mmol/L of total cholesterol, being a current smoker, being physically active, having BMI >30kg/m2, having lower level of education and being in lower social class, having a prevent condition (diabetes and MI). For both measures every decrease in 1 SD was associated with an increase hazard of stroke HR 1.17 (1.05-1.30) and HR 1.12 (1.02-1.22) for BUA and VOS, respectively independently of the covariates included. Every 1SD decrease in BUA was equivalent in risk to 20 mmHg increase in systolic blood pressure or approximately 1.5 years increase in age after taking into consideration the other factors included in the model.

Table 4 shows that the independent relative risk of stroke associated with BUA and VOS were consistent in subgroups in stratified analyses.

Systematic Review and Meta-Analysis

We screened 1595 titles and abstracts and identified six relevant studies for the systematic review [16, 17, 18, 19, 20, 21],and of them three studies [16-18] were possible to be included in the meta-analysis (**Supplementary Figure 1**). Two studies were conducted in the United States [16, 17] and the other study was conducted in Sweden [18]. Two of the studies examined the relationship for both sexes [17,18] but the remaining study was limited to women only [16]. One of the two studies that examined both sexes reported results for men and women separately [17] while the other one reported a single sex-combined risk estimate [18]. Therefore including EPIC-Norfolk study a total of four prospective cohort studies with the maximum follow up of 18.7 years were included in the meta-analysis. , A total of 25760 participants and 1237 cases of incident stroke included in the meta-analysis. (**Table 5**).

Different methods were used for evaluating and ascertaining bone mineral density and incident stroke across the studies (**Supplementary Table 1**). While absorptiometry was used in all the studies only one of them evaluated bone density at the femoral neck [18] while the other two evaluated the bone density in the left hand [17] and at three different sites (distal radius, proximal radius and calcaneus) [18]. Two studies used ICD-9 codes in order to ascertain stroke cases [16, 17] and one study collected stroke cases from a validated register [18]. Three studies adjusted for potential confounders and the adjustments were limited in two studies making these studies liable to residual confounding [16,18]. One of these studies only adjusted for age [16] while the other adjusted for age, sex and body mass index [18]. The most highly adjusted study in the published literature accounted for age, smoking status, alcohol consumption, history of

diabetes, history of heart disease, education, body mass index, recreational physical activity and blood pressure medications [17].

Overall, a one standard deviation reduction in bone mineral density was associated with increased risk of incident stroke (pooled RR 1.12 95% CI 1.04-1.22, I^2=23%, 4 studies) (**Figure 2**). The significant difference was primarily driven by the results from studies of women (pooled RR 1.22 95% CI 1.09-1.37, I^2=0%, 3 studies). The results for men were not-significant (pooled RR 1.05 95% CI 0.94-1.17, I^2=0%2 studies).

Three studies were eligible to be included in the systematic review but they were not included in the meta-analysis. One of these studies was a population-based case-control study which evaluated 4175 participants in Taiwan [19]. They found that osteoporotic vertebral fracture was significantly increased among patients with incident stroke (adjusted HR 2.71 95% CI 1.90-3.86). Another study that was not included in meta-analysis was a prospective observational study of 744 participants [20]. This study found that the hazard ratio for bone mineral density and incident stroke was not significant but no numerical results were reported. The third study was an observational study of cases and controls which included 251 patients and 63 cases of stroke and found that the highest quartile of BMD had a significantly higher risk of incident stroke compared to the lowest quartile for women but not for men [21]. This study reported an increased odds ratio for stroke associated with a drop in one SD of BMD, but the 95% confidence intervals and statistical significance of this finding was not reported, and as such, we were unable to include it in the meta-analysis.

Publication bias was not formally assessed because there were fewer than 10 studies included in the analysis. However, we found clear evidence of selective reporting in one study [20] where the authors reported in the text that there was no significant association between BMD and stroke, but the odds ratios and 95% confidence intervals were not given. This null finding could not therefore be included in our meta-analysis, which means that the pooled dataset could be reporting over-inflated risk estimates.

Discussion

We found a prospective relationship between bone mineral density assessed by heel (calcaneum) ultrasound at the baseline and subsequent stroke risk in middle and older age apparently health general population. Men and women in the bottom 10% of BUA distribution had increased future risk of stroke compared to the top 30% of BUA (70-100%)(HR 1.41 (95% CI 1.02-1.94)). There also appears to be a linear relationship with every 1 SD decrease in BUA/VOS being associated with a higher risk of stroke (17% increase in relative risk of stroke (95%CI: 5%-30%)).

.Our study has several strengths which include large sample size conducted in apparently healthy community dwelling general population without previous known stroke or cancer, with complete follow up (100% follow up) using validated follow-up methods[10] andour ability to control for a range of potential confounders including demographics, anthropometry, lifestyle, social and medical risk factors. Overall, our results from both the observational study and systematic review yields supportive and consistent evidence that decreased bone mineral density is associated with risk of incident stroke in women.

In an observational study, confounding and reverse causality issues require attention. We addressed these issues in this study in several ways. First, we adjusted for possible confounders that potentially relate to both BMD and known stroke risk factors. Second, we excluded people with stroke and cancer at the baseline and third we specifically adjusted for people with prevalent MI and diabetes and medications for hypertension and hypercholesterolemia in later

models. The more consistent association with BUA suggests it is a more specific and sensitive measure than VOS [3].

There is strong and consistent evidence to suggest osteoporosis and subsequent fracture risk is substantial in people who sustained stroke [1]. Immobility with generalized bone loss compounded by region-specific bone loss at hemiplegic side was considered as the major contributing factors for the development of osteoporosis after stroke. Several pathophysiological mechanisms have been proposed. Sato et al showed evidence of bone resorption as early as within 7-days post stroke [22]. Remodelling imbalance at bone multicellular unit (BMU) has also been suggested evidenced by decline in serum biomarkers of bone formation [22]. Factors such as duration of hemiplegia [23], degree of functional recovery [24], reduced vitamin D status [25][26],and the use of anticoagulants [27] may influence bone loss after stroke.

On the other hand, the link between osteoporosis and stroke with the former being a risk factor for the latter is less well researched. It has been recognized that stroke patients are highly likely to have pre-existing osteoporosis; 40% of stroke patients admitted to a rehabilitation unit in Japan with a mean time from onset of ~40 days had established osteoporosis [28].Classical cardiovascular risk factors do not fully account for stroke mortality and burden at the population level especially in low income countries [29].Therefore understanding of the possible contribution of novel risk factors for stroke risk and stroke mortality is important.

Most recently Qu and colleagues systematically reviewed the literature and they reported that low BMD was not associated with the risk of stroke mortality (HR 1.08, 95% CI; 0.89-1.28)[30];

however, there is dearth of data on the link between BMD and stroke incidence in the literature. One possible explanation of why the associations were null is because the studies were underpowered and this is supported by the researchers reporting 95% confidence intervals that are broad. In addition, our systematic review builds on the findings of their review as we have only considered stroke incidence and we reported results (including non-significant findings) from studies that were excluded from their meta-analysis. Our study provides further evidence by extending this link in men and women with wider age range (range 42-82 years) using more robust adjustment for various confounders which were not considered in previous studies. The point estimates for stroke risk at follow up for every 1SD decrease in men and women in our study were 1.08 and 1.25, respectively, and for those 65 years or older (including men) was 1.22 (1.08-1.38). Differences in population mix, sample sizes and follow-up duration, and risk factors adjusted for etc. may account for slight differences in estimates. We also considered publication bias or selective outcome reporting to be a problem and important limitation in our meta-analysis.

Whether osteoporosis is a marker rather than risk factor for stroke remains uncertain. Nevertheless, there are potential biological mechanisms underpinning the possible causal link.It has been proposed that excess calcium resorbed from the bone occurring in osteoporotic process may become incorporated into the vascular lining, in people with osteoporosis especially at atheromatous plaques making them more brittle and unstable as well as making blood vessels more rigid. Frost et al., showed BMD was negatively correlated with arterial stiffness over carotid and femoral arteries [31]. Fifty-nine percent of osteoporotic women (BMD T-score < -

2.5) had calcified plaque at one or more sites compared with 42% and 20% for women with osteopenia (T-score < -1) and normal BMD, respectively (P for trend = 0.04) [31].

BMD is a measure of lifetime exposure to estrogen and the observed age and sex differences in the association between BMD and stroke risk may stem from differences in levels of endogenous oestrogen that could affect cardiovascular risk. While it is tempting to speculate that high endogenous estrogen (and therefore higher BMD) might be associated with lowering of cardiovascular risk, the association between estrogen and stroke risk is still debated. A recent meta-analysis of randomized controlled trials on effect of hormone replacement therapy on cardiovascular outcomes demonstrated a significantly increased risk of stroke with exogenous estrogen [32]. Moreover, a recent observational study found that higher endogenous estradiol levels (particularly in those with central adiposity) were associated with increased stroke risk [33].

In another interesting study researchers assessed the potential role of oestrogen signalling on this association through putative target genes (osteoprotegerin [OPG] and interleukin-6); carriers of the OPG-1181C/C genotype had a significantly increased risk of intracerebral hemorrhage (P = 0.005) [34]. This implies that alterations in OPG-mediated signalling in the vasculature may be involved in the pathophysiology of hemorrhagic stroke. There have also been a number of studies on the effect of statins on bone mass. In two recent meta-analyses on the effects of statins on BMD and fracture risk, statin use was associated with modest but significant increase in total hip and femoral neck BMD[35] and significant reduction of fracture risk in case-control (odds ratio [OR] = 0.62, 0.45-0.85) and cohort (OR = 0.77, 0.59-1.00) studies, but not in post-hoc analyses of randomized trials (OR = 1.03, 0.91-1.16) [36].

Another possible link between bone health and stroke is through the action of vitamin D. Vitamin D is well known for its effect on bone; it has also been shown to be associated with cardiovascular risk factors such as hypertension [37] and diabetes mellitus [38] as well as with markers of subclinical atherosclerosis such as intima-media thickness and coronary calcification [39]. Hence, it was thought that vitamin D deficiency contributes to the development of cardiovascular events such as stroke through its association with risk factors. However, direct effects of vitamin D on the cardiovascular system may also be involved [40]. Recent findings have shown that vitamin D receptors are expressed in cardiomyocytes, vascular smooth muscle cells and endothelial cells and vitamin D affects inflammation and cell proliferation and differentiation in these tissues [36]. A recent meta-analysis on the limited available data suggests that vitamin D supplementation at moderate to high doses may reduce cardiovascular risk [41]. Further prospective placebo-controlled randomized trials however are less promising [42].

Naturally, our study had limitations. Because of the requirement of participants to provide detailed health and lifestyle information and to be able to undergo health checks, the initial response rate was modest (40%). This may have introduced a healthy responder bias. Nevertheless, the baseline characteristics of the study population were similar to those of other UK population samples, except for a slightly lower prevalence of smokers [5].Furthermore, as previously reported the sample characteristics of EPIC-Norfolk respondents who attended to second health check were not materially different with regards to age (0.5 years younger as a group) and other characteristics such as BMI (again 0.5 unit difference) [4]. Moreover, the truncation of distribution because of healthy responders may attenuate the associations, but this

should not have produced a spurious relation between quantitative calcaneum ultrasound measure (BUA) and stroke observed within the study participants; if anything, truncation of the distribution is likely to reduce the power of any associations. Subgroup analysis reduced the sample size and event rates in strata which are less prevalent in this cohort (e.g. smoking). This may contribute to non-significant association observed in current smokers. There also remains a possibility of residual confounding as well as there may be unknown potential confounders which we were not able to adjust for such as depression.

We used death certification and a hospital record linkage system using ICD coding to identify stroke cases. Although follow-up with the use of these methods is virtually complete, this approach may underestimate incident nonfatal stroke cases that are not admitted to the hospital. The use of self-reported stroke to exclude prevalent cases may have missed some prevalent strokes. We were not able to separately examine stroke subtypes. Nevertheless, the primary focus of the study was to assess the risk prediction of clinical stroke event severe enough to lead to hospitalization or death regardless of stroke subtype. In any case, the misclassification of strokes was likely to only attenuate any associations. Only single measurements of BUA and VOS and other covariates, such as cholesterol and BP, were made at baseline at the second health check. These measures as well as lifestyle behaviors, which may affect bone mineral density, may have changed over the follow-up period. Moreover, the blood sample taken was a non-fasting sample and was therefore less standardized for some of the variables (e.g., cholesterol concentration) than was a fasting blood sample. Nevertheless, random measurement error was likely only to attenuate any relations observed between calcaneum quantitative ultrasound and stroke.

The meta-analysis had both limitations and strengths. The main limitations of the meta-analysis were that only three studies in addition to the current study were found and two of these studies were subject to residual confounding because of minimal adjustments for potential confounders [16, 18]. Furthermore, there was methodological variation in the way bone mineral density was assessed across the included studies which may account for differences in results. However, the included studies found on the search were large (average of ~3800 subjects) prospective cohort studies with long duration of follow up (up to 18.7 years) and we were able to analyze the results for men and women separately.

In summary, there appears to be a complex connection between bone health and stroke. Both may share common biological or lifestyle antecedents and the association warrants further exploration. In the interim, people with low bone mass may not only be at high risk for fractures but also increased risk for stroke and therefore candidates for targeted intervention not just for fracture prevention but also potentially cardiovascular risk assessment and control.

List of Tables and Figures

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Contributors

KTK and NJW are PIs of EPIC-Norfolk. RNL is responsible for data management and record linkage. PKM designed the study and developed the analysis plan. ABC analyzed the EPIC-Norfolk data. Meta-analysis protocol was developed by PKM, CSK and YKL. CSK screened abstracts and titles. CSK and YKL independently reviewed all potentially relevant studies to confirm eligibility. Data extraction of included studies was performed by CSK and JKY, and checked by YKL. PKM drafted the paper with critical input from CSK and YKL. All co-authors contributed in writing of the paper. PKM is the guarantor.

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Table 1: Baseline characteristics of men and women of EPIC-Norfolk 42-82 years old at the second health check (1997-2000) by BUA categories

	Percentile category				p-for trend
	<10	10 to <40	40 to <70	70 to 100	
Number	1420	4259	4259	4259	
Age (years)	67.6 (8.0)	62.5 (8.9)	60.4 (8.8)	60.3 (9.0)	**<0.001**
Male	126 (9)	1042 (24)	2006 (47)	3089 (73)	**<0.001**
Systolic blood pressure (mmHg)	139.5 (19.0)	134.6 (18.3)	134.2 (18.1)	134.6 (17.6)	**<0.001**
Cholesterol (mmol/L)	6.4 (1.2)	6.2 (1.2)	6.0 (1.1)	5.9 (1.1)	**<0.001**
Current smoker	136 (10)	355 (8)	362 (9)	323 (8)	0.03
Physically active	360 (26)	1775 (42)	2287 (55)	2565 (61)	**<0.001**
BMI > 30 kg/m^2	130 (9)	641 (15)	811 (19)	852 (20)	**<0.001**
Educational attainment					**<0.001**
None	626 (44)	1552 (36)	1345 (32)	1135 (27)	
O-level	171 (12)	529 (12)	474 (11)	392 (9)	
A-Level	498 (35)	1644 (39)	1789 (42)	2002 (47)	
Degree or higher	125 (9)	533 (13)	650 (15)	727 (17)	
Occupational social class					**<0.001**
Professional (I)	70 (5)	297 (7)	329 (8)	347 (8)	
Managerial (II)	491 (36)	1525 (36)	1630 (39)	1741 (42)	
Skilled non-manual (III non-manual)	310 (22)	761 (18)	666 (16)	553 (13)	
Skilled manual (III manual)	281 (20)	888 (21)	905 (22)	941 (22)	
Semi-skilled (IV)	175 (13)	557 (13)	543 (13)	505 (12)	
Non-skilled (V)	56 (4)	152 (4)	125 (3)	100 (2)	
Diabetes (yes)	33 (2)	121 (3)	125 (3)	149 (3)	**0.017**
Myocardial infarction (yes)	48 (3)	105 (2)	126 (3)	149 (3)	0.092
On lipids lowering drugs	66 (5)	211 (5)	176 (4)	184 (4)	0.192
On anti-hypertensive medication	398 (28)	942 (22)	921 (22)	866 (20)	**<0.001**

Data presented are mean (SD) for continuous and number (%) for categorical data.
BMI: Body mass index

Table 2: Incident stroke rates and hazards ratios (corresponding 95% Confidence Intervals) by BUA and VOS categories for individuals aged 42-82 years of EPIC-Norfolk (1997-2000 to 2009-2011).

	Percentile category				**p for trend**
	<10	**10 to <40**	**40 to <70**	**70 to 100**	
BUA (mean, range)	48.75 (14.95-56.12)	66.28 (56.13-74.59)	81.96 (74.61-89.84)	103.10 (89.85-145.54)	
Number	1420	4259	4259	4259	
Rate / 100 (n of events)	8.5 (120)	4.3 (181)	3.1 (130)	3.6 (155)	
Model A	**1.52 (1.13 - 2.04)**	1.12 (0.88 - 1.43)	0.90 (0.71 - 1.14)	1.00	**0.007**
Model B	**1.52 (1.12 - 2.07)**	1.21 (0.94 - 1.56)	0.90 (0.70 - 1.15)	1.00	**0.004**
Model C	**1.40 (1.02 - 1.92)**	1.19 (0.92 - 1.53)	0.85 (0.66 - 1.09)	1.00	**0.020**
Model D	**1.40 (1.02 - 1.92)**	1.19 (0.92 - 1.53)	0.85 (0.66 - 1.09)	1.00	**0.017**
Model E	**1.42 (1.03 - 1.96)**	1.20 (0.93 - 1.56)	0.86 (0.67 - 1.11)	1.00	**0.019**
Model F	**1.41 (1.02 - 1.94)**	1.21 (0.93 - 1.56)	0.84 (0.65 - 1.09)	1.00	**0.019**
VOS (mean, range)	1563.75 (1456.25-1581.50)	1605 (1581.75-1623.25)	1638.92 (1623.33-1655.25)	1682.75 (1655.50-1802.50)	
Number	1433	4261	4259	4244	
Rate / 100 (n of events)	7.8 (111)	4.6 (196)	3.5 (149)	3.1 (130)	
Model A	1.31 (0.99 - 1.74)	1.10 (0.87 - 1.39)	1.02 (0.81 - 1.29)	1.00	0.06
Model B	1.31 (0.99 - 1.75)	1.10 (0.87 - 1.40)	0.95 (0.74 - 1.22)	1.00	0.048

Model C	1.27 (0.94 - 1.70)	1.09 (0.85 - 1.39)	0.97 (0.76 - 1.25)	1.00	0.103
Model D	1.32 (0.98 - 1.78)	1.10 (0.86 - 1.41)	0.99 (0.77 - 1.28)	1.00	0.07
Model E	1.33 (0.98 - 1.79)	1.11 (0.87 - 1.43)	1.00 (0.78 - 1.29)	1.00	0.06
Model F	1.31 (0.97 - 1.77)	1.10 (0.86 - 1.41)	0.99 (0.77 - 1.28)	1.00	0.08

Variables adjusted are- Model A= age, sex; Model B = model A + systolic BP and cholesterol; Model C = model B + lifestyle factors- smoking, physical activity and BMI; Model D = model C + occupational social class and educational level; Model E = model D + prevalent MI and diabetes; Model F = model E + lipid lowering medications and anti-hypertensive medication use.

Table 3: Cox multivariate regression model of risk factors for incidence stroke according to BUA and VOS in 12795 individuals aged 42-82 years of EPIC-Norfolk (1997-2000 to 2009-2011)

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	BUO model		VOS model	
	Relative risk (95% CI)	p	Relative risk (95% CI)	P
Age per 1 year increase	1.12 (1.10 - 1.13)	**<0.001**	1.12 (1.10 - 1.13)	**<0.001**
Sex	0.63 (0.50 - 0.79)	**<0.001**	0.69 (0.56 - 0.85)	**<0.001**
Systolic BP per 5 mmHg increase	1.04 (1.02 - 1.07)	**0.001**	1.04 (1.02 - 1.07)	**0.001**
Cholesterol by 1 mmol/L increase	0.97 (0.90 - 1.05)	0.48	0.97 (0.90 - 1.05)	0.45
Current Smoking	1.79 (1.33 - 2.41)	**<0.001**	1.80 (1.34 - 2.42)	**<0.001**
Being physically active	0.72 (0.58 - 0.89)	**0.002**	0.72 (0.58 - 0.89)	**0.003**
BMI > 25 kg/m^2	1.02 (0.81 - 1.29)	0.88	0.98 (0.77 - 1.23)	0.83
No or lower than A-level Education	1.16 (0.96 - 1.39)	0.12	1.16 (0.96 - 1.39)	0.12
Lower occupational social Class	0.92 (0.76 - 1.11)	0.39	0.92 (0.76 - 1.12)	0.42
Prevalent Diabetes	1.50 (1.05 - 2.16)	**0.028**	1.53 (1.07 - 2.20)	**0.021**
Prevalent MI	1.92 (1.39 - 2.65)	**<0.001**	1.93 (1.40 - 2.67)	**<0.001**
BUA per 20 dB/MHz decrease	**1.17 (1.05 - 1.30)**	**0.005**		
VOS per 40 m/s decrease			**1.12 (1.02 - 1.22)**	**0.019**

All variables listed are included in the model.

Table 4: Hazard Ratios (95% Confidence Intervals) of incidence stroke stratified by sex, age and smoking status Cox multivariate regression in 12795 individuals aged 42-82 years (1997-2000 to 2009-2011)

	Events/ N	BUA (per 20 dB /MHz decrease)			VOS (per 40 m/s decrease)		
		Relative risk (95% CI)	p-value	p-value for strata difference	Relative risk (95% CI)	p-value	p-value for strata difference
By sex				0.078			0.38
Men	303/6312	1.08 (0.94 - 1.24)	0.29		1.06 (0.94 - 1.20)	0.33	
Women	296/7985	1.25 (1.04 - 1.50)	**0.018**		1.11 (0.96 - 1.29)	0.14	
By age-group				0.658			0.67
<65 years	113/8602	1.20 (0.93 - 1.54)	0.16		1.14 (0.93 - 1.41)	0.20	
>=65 years	486/5695	1.22 (1.08 - 1.38)	**0.002**		1.15 (1.04 - 1.28)	**0.007**	
By smoking status				0.733			0.93
Current smoker	60/1181	1.18 (0.86 - 1.64)	0.31		1.25 (0.93 - 1.68)	0.14	
Non-current smoker	528/13016	1.17 (1.04 - 1.32)	**0.007**		1.10 (1.00 - 1.21)	0.05	

*Estimates are for every 1 SD decrease in BUA or VOS adjusting age, sex, systolic BP, cholesterol, smoking status, physical activity, BMI, education level, social class, prevalent diabetes, prevalent MI, use of lipid modifying and anti-hypertensives, excluding the relevant factor stratified.

Table 5: Study design, participants, follow up and outcomes for studies evaluating bone mineral density and incident stroke

Study ID	Bone mineral density measure	Study design	No in analysis	Characteristics	Follow-up (years)	Outcome measures	Measures of association
Browner 1993 [16]	BMD measured at distal radius, proximal radius and calcaneus with single photon absoptiometry.	Prospective cohort study.	4024 (83 strokes).	Ambulatory women aged 65 years or older in the prospective Study of Osteoporotic Fractures in USA.	1.98 years.	Incident stroke.	Each SD decrease in BMD at the calcaneus was associated with HR 1.31 (1.03-1.65) increase risk of stroke.
Chen 2013 [19]	No BMD but based on ICD-9 codes for osteoporosis.	Population-based case-control study.	4175	Population based cohort in Taiwan.	Median follow up 3 years.	Incident stroke.	Stroke was more likely to occur in osteoporotic vertebral fracture: adjusted HR 2.71 (1.90-3.86).
Jorgensen 2001 [21]	BMD measured by DXA at both femoral necks.	Observational study of cases and controls.	251 (63 strokes).	63 stroke patients and 188 control subjects from the general population with mean age 75-77 years in Norway.	None.	Incident stroke.	OR for stroke increased 1.9 per SD (0.13 g/cm2) reduction in BMD (95% CI or p-value not given) Highest quartile vs. lowest quartile BMD and risk of incident stroke: women adjusted OR 6.6 (1.8-24.8), men adjusted OR 0.6 (0.1-2.3).
Mussolino 2003 [17]	Phalangeal BMD was determined from a baseline X-ray of left hand.	Prospective cohort study.	3402 (416 strokes).	White and black subjects 45 through 74 years of age in USA.	18.7 years.	Incident stroke.	BMD and risk of incident stroke: white men RR 1.01 (0.86-1.19), white women RR 1.13 (0.93-1.38), blacks RR 0.93 (0.72-1.21).
Nordstrom 2009 [18]	DXA of femoral neck.	Prospective cohort study.	4044 (139 strokes)	Men and women with mean age of 54 years living in Vasterbotten county of Sweden.	5 years 7 months.	Incident stroke.	Every SD decrease in neck BMD associated with increase HR for stroke of 1.23 (1.01-1.49) in total cohort.
Szulc 2009 [20]	Lumbar spine (L2-L4), right hip and whole body by DXA.	Prospective cohort study.	744 (43 strokes)	Men ≥50 years of age living in Montceau les Mines and adjacent villages..	7.5 years	Incident stroke.	HR for BMD and incident stroke was not significant (numerical results not reported).

BMD = Bone Mineral Density, DXA = Dual-energy X-ray Absorptiometry, SD = Standard deviation, RR = relative risk, HR = hazard ratio

Figure 1: Flow Diagram

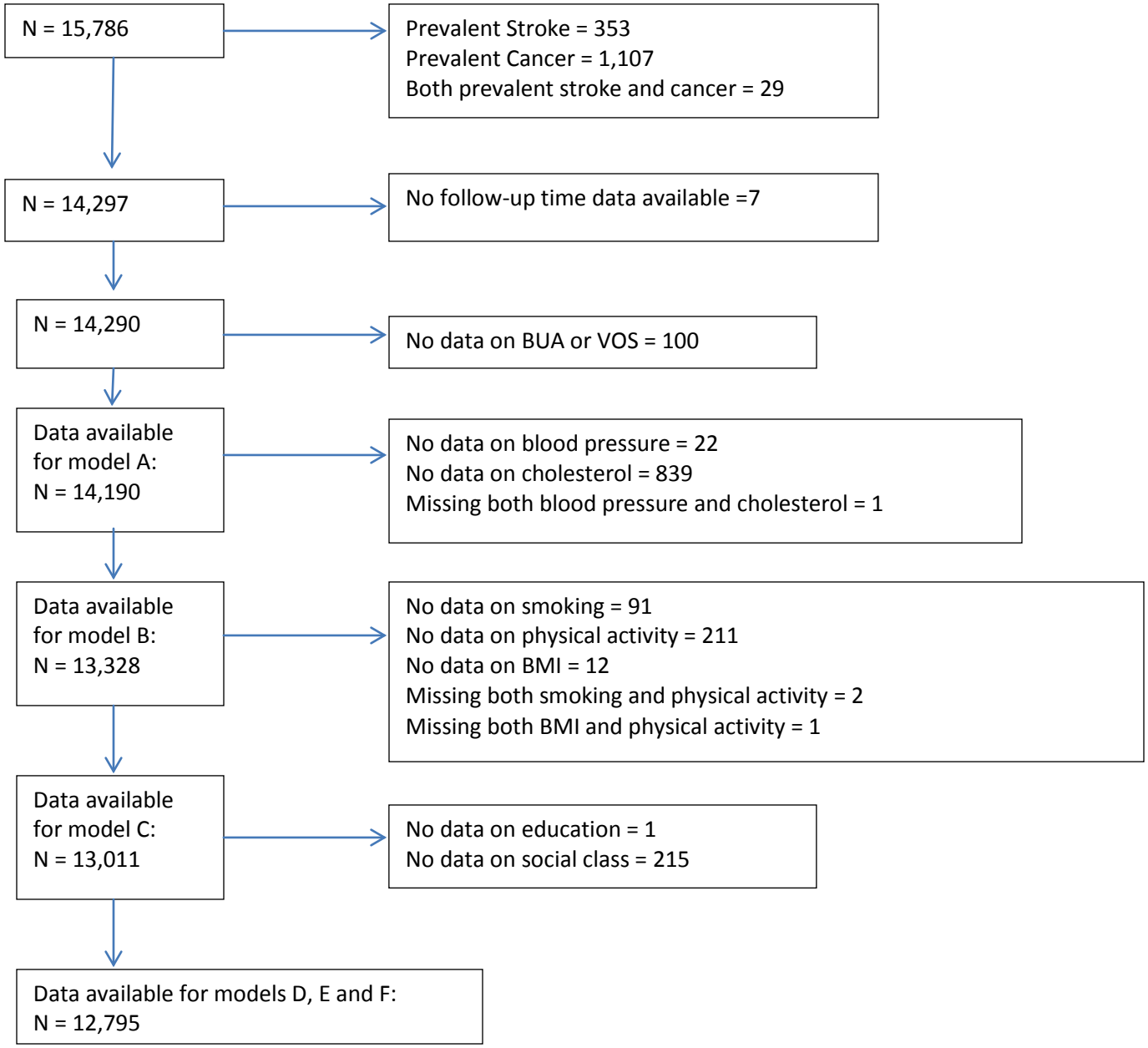
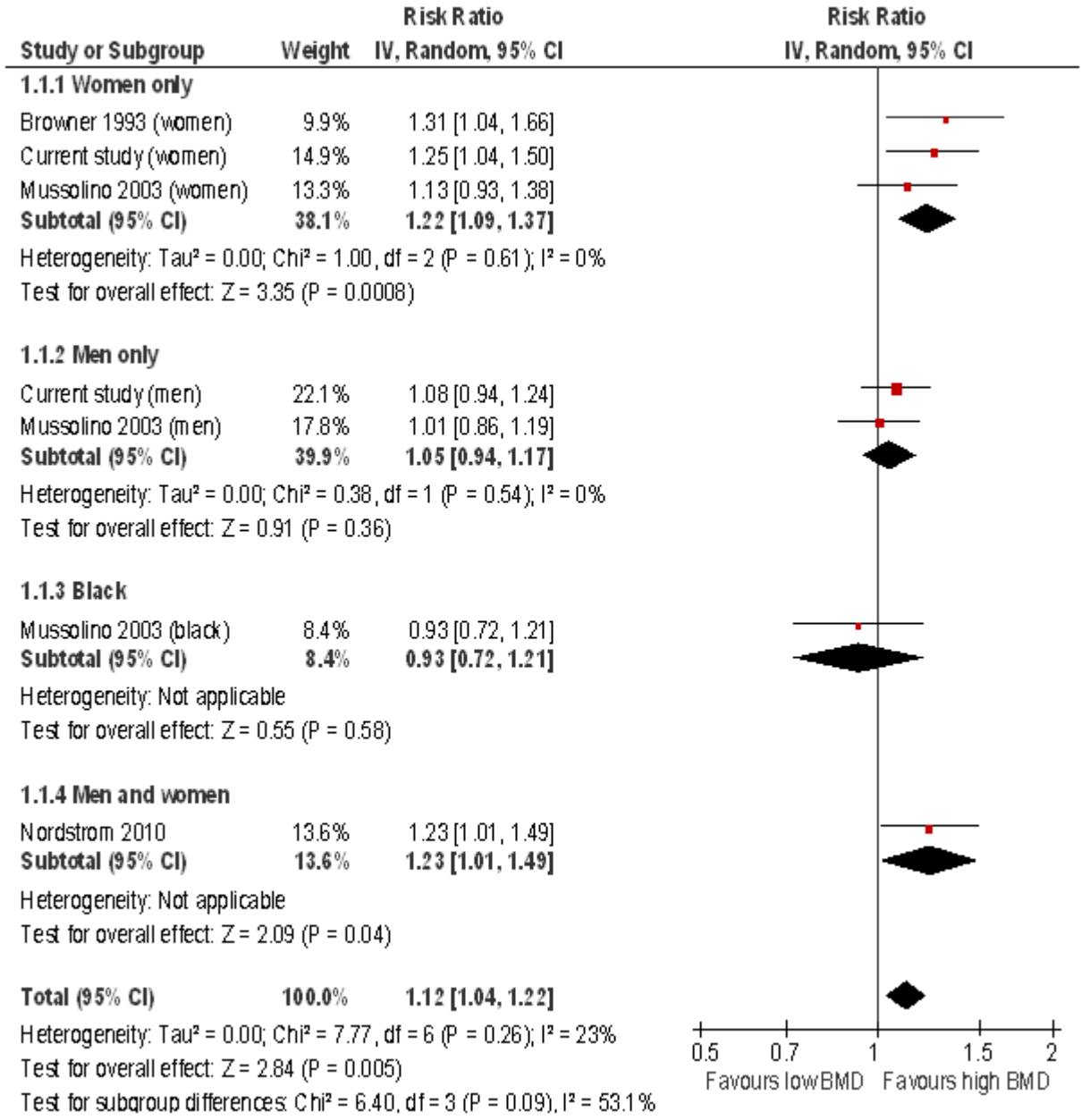


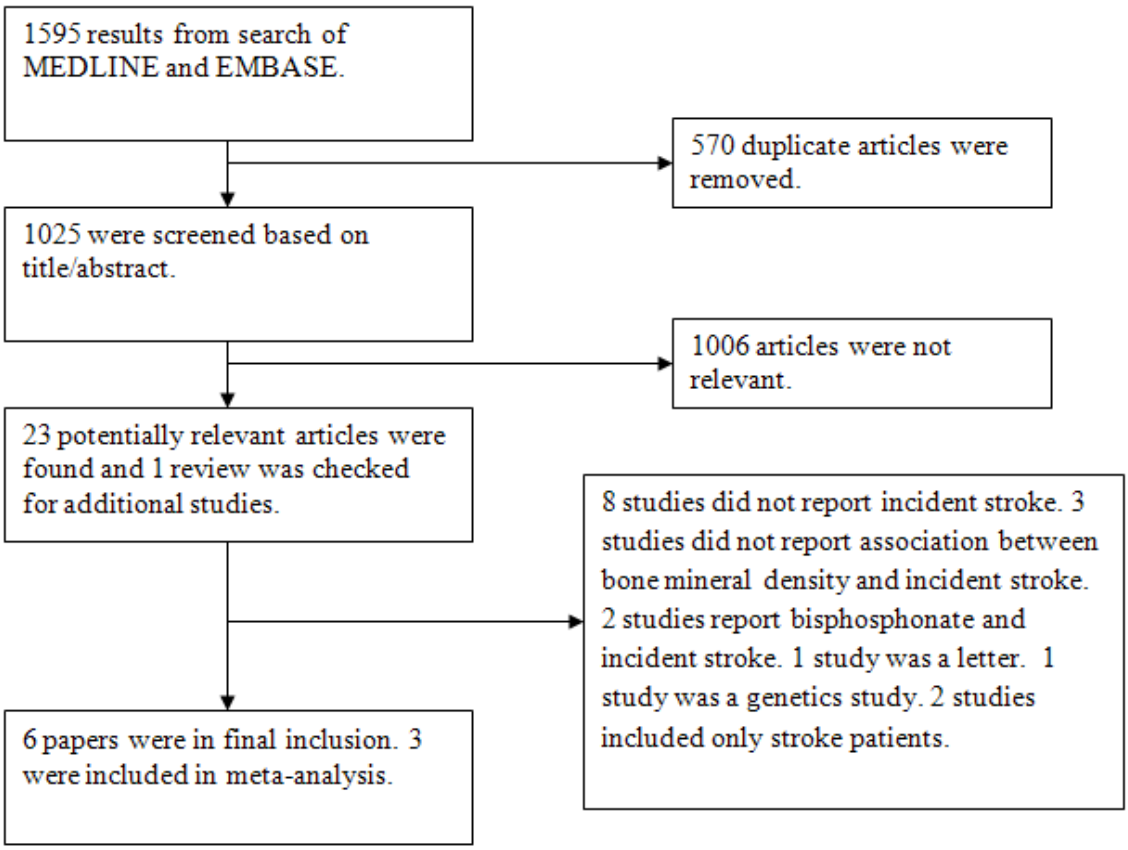
Figure 2: Meta-analysis risk of incident stroke with decreasing bone mineral density in different patient groups.



Supplementary Data I: Search Strategy

("bone density" OR "bone mineral density" OR "osteoporosis" OR "heel ultrasound" OR "broadband ultrasound") AND (stroke OR "intracranial hemorrhage" OR "intracranial haemorrhage" OR "intracranial bleed" OR "cerebrovascular disease")

Supplementary Figure 1: Search results and study selection for meta-analysis of bone mineral density and incident stroke



Supplementary Table I: Quality assessment of studies included in meta-analysis which evaluating bone mineral density and incident stroke

Study ID	Ascertaining Bone Mineral Density	Determining Incident Stroke	Adjustment for Confounders
Browner 1993 [16]	BMD was measured at three sites-the distal radius, the proximal radius and the calcaneus-using single photon absorptiometry (OsteoAnalyzer, Siemens-Osteon, Wahiawa, Hawaii).	Medical records in financial administrative database to identify hospitalizations with ICD-9-CM codes for stroke diagnoses.	Age
Mussolino 2003 [17]	Phalangeal BMD was determined from a baseline x-ray of the left hand by use of radiographic absorptiometry .	Incident stroke cases met at least 1 of the following criteria: (1) a death certificate with underlying or nonunderlying cause of death coded as stroke based on ICD-9, or (2) ≥1 hospital and/or nursing home stays during the follow-up period with any discharge diagnosis coded as stroke based on ICD-9.	Baseline age, smoking status, alcohol consumption, history of diabetes, history of heart disease, education, body mass index, recreational physical activity, and blood pressure medication. Sex is included in age- and risk-adjusted models for blacks
Nordstrom 2009 [18]	BMD were measured in the femoral neck using DPX-L.	Validated stroke register (Northern Sweden) set up by World Health Organization.	Age, sex, BMI, in the total cohort.

BMD = Bone mineral density