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Reduced bone loss is associated with reduced mortality risk in subjects exposed to nitrogen bisphosphonates: a mediation analysis

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Abstract

Bisphosphonates**,** potent anti-resorptive agents, have been found to be associated with mortality reduction. Accelerated bone loss is, in itself, an independent predictor of mortality risk but the relationship between bisphosphonates, bone loss and mortality is unknown.
This study aimed to determine whether the association between bisphosphonates and mortality is mediated by a reduction in the rate of bone loss.
Participants from the population-based Canadian Multicentre Osteoporosis Study were followed prospectively between1996 and 2011. Co-morbidities, and life-style factors were collected at baseline and BMD at baseline, years 3 (for those aged 40-60), 5 and 10. Rate of bone loss was calculated using linear regression. Information on medication use was obtained yearly. Bisphosphonate users grouped into nBP (alendronate or risedronate) and etidronate and non-users (NoRx) were matched by propensity score including all baseline factors as well as time of treatment.
Cox's proportional hazards models, unadjusted and adjusted for annual rate of bone loss, were used to determine the association between nBP and etidronate versus NoRx. For the treatment groups with significant mortality risk reduction, the percent of mortality reduction mediated by a reduction in the rate of bone loss was estimated using a causal mediation analysis.
There were 271 pairs of nBP and matched NoRx and 327 pairs of etidronate and matched NoRx. nBP, but not etidronate use was associated with significant mortality risk reduction [HRs, 0.61 (0.39-0.96) and 1.35 (95%CI, 0.86-2.11) for nBP and etidronate, respectively]. Rapid bone loss was associated with over 2-fold increased mortality risk compared to no loss. Mediation analysis indicated that 39% (95% CI, 7%-84%) of the nBP association with mortality was related to a reduction in the rate of bone loss.
This finding provides an insight into the mechanism of the relationship between nBP and survival benefit in osteoporotic patients.

Introduction

Osteoporotic (fragility) fractures are very common, affecting 3 in 5 women and 1 in 3 men over the age of 50(1). Furthermore, their prevalence is expected to rise as a consequence of increasing life expectancy and thus the number of individuals at risk (2). The burden of osteoporotic fracture resides not only in their increased risk of subsequent fracture (3-5) but also in increased mortality risk (6-9).

Bisphosphonates are currently considered first-line treatment for osteoporosis world-wide (10, 11) with abundant evidence of their ability to reduce both vertebral and non-vertebral fracture risk (12-14). More recently, bisphosphonates have been also linked to improved survival (15, 16). The first evidence of a positive association between bisphosphonate use and survival came from an RCT of zoledronic acid post hip fracture (15). In this trial, zoledronic acid was associated with a 28% mortality risk reduction compared to placebo (15). Subsequently, a meta-analysis of anti-osteoporosis medication from 8 RCTs (including risedronate, zoledronic acid, denosumab and strontium ranelate) (16) found a pooled benefit (~11 %) of these agents on mortality risk (16). Interestingly in this meta-analysis, the mortality risk reduction was predominantly observed in the trials with the highest background mortality risk. However, a more recent RCT of zoledronic acid in women with osteopenia, has also shown a mortality risk reduction of ~ 35% over a longer follow-up time of 6 years although it did not quite reach statistical significance (17). A positive association between bisphosphonates and mortality risk has also been reported in several observational osteoporosis cohorts (18-20), a fracture liaison service setting (21) and registry-based studies (22, 23).

The mechanism by which bisphosphonates impact survival is not fully understood. Several hypotheses have been proposed to explain this effect. The most obvious explanation would be through a reduction in the rate of subsequent fractures. However, in the zoledronic acid RCT, only 8 % of total deaths prevented were estimated to be mediated through this mechanism (24). It is also possible that a reduction in the rate of bone loss may explain the mortality risk reduction observed in bisphosphonate treated groups. Excessive bone loss is associated with increased mortality risk in both the general population as well as post-fracture (25, 26). However, there is no information as to whether bone loss may play a role in mortality risk reduction associated with bisphosphonate use. Thus, this study aimed to determine whether

the association between bisphosphonates and survival is mediated by a reduction in the rate of bone loss.

Methods

Subjects and setting

The study population consisted of women and men aged 50+ participating in the Canadian Multicentre Osteoporosis Study (CaMos), an ongoing prospective population-based study that was started in 1995. Participants were recruited randomly using residential telephone lists from the region surrounding nine urban centres in Canada. A detailed description of the study design and population sampling has been published previously(27).

The present study included participants who had at least 2 bone mineral density measurements, and who either used one of bisphosphonates available (alendronate, risedronate or etidronate) or did not use any bone related medication. Alendronate and etidronate were available at baseline, while risedronate became available later and participants started using it on average 9 years (± 3 years) later, resulting in a smaller number of risedronate users. Medication was classified, based on mechanism of action, as nitrogen-bisphosphonates (alendronate or risedronate) and non-nitrogen (etidronate) bisphosphonate. In order to avoid misclassification, all the participants who switched between bisphosphonate types were excluded. Furthermore, this study used an 'intention-to-treat' approach. Thus, participants were analysed in the treatment group they were initially assigned regardless of whether they adhere or not to treatment.

A number of bisphosphonates and other treatments were excluded due to small numbers of users; viz. clodronate, pamidronate, zoledronic acid, calcitonin, denosumab, raloxifene, tamoxifen, testosterone or other sex hormone therapy.

Of the 9,423 participants recruited, 7,689 aged 50+ were screened for medication uptake and had had at least 2 bone mineral density measurements. After the first screening, it was found that very few men met the study criteria (n=68 on nitrogen-bisphosphonate and n=87 on etidronate), and therefore analysis was restricted to women only (Figure 1).

CaMos was approved by the Ethics Committee of McGill University and at each participating centre.

Outcomes and risk factors

A standardized interviewer administered questionnaire was obtained at baseline (1995-97). Information was obtained on lifestyle factors (i.e. smoking, physical activity), demographics, education, co-morbidities and medication use. In addition to this structured questionnaire, each participant had a clinical visit that included anthropometric (*i.e.* height, weight) and bone mineral density (femoral neck and lumbar spine) measurements.

This information was subsequently obtained in Years 5 and 10. In those aged 40 – 60 years at entry, another clinical visit was conducted at year 3. Yearly postal self-administered questionnaires for incident fractures and medications were obtained between clinical visits.

Bone mineral density assessment

Bone mineral density was measured as femoral neck and lumbar spine areal bone mineral density (BMD) at baseline and then subsequently in Years 3 (40-60 years of age), 5 and 10. BMD assessment was performed by dual x-ray absorptiometry (Discovery W, Hologic, Bedford MA, USA). Standardization between DXA scanners was assessed by scanning a single phantom, which was circulated among centres (28).

Participants and/or their primary care physician received a copy of the BMD report performed at baseline and all subsequent visits. They did not receive any formal fracture risk assessment or management suggestions from the CaMOS investigators.

The annual percent change in bone mineral density was calculated for each individual participant using a linear regression model to determine the intercept (at baseline) and the slope of BMD over time for both femoral neck and lumbar spine sites. The annual rate of bone loss was calculated as the ratio between the slope and the intercept. The annual percent bone loss was compared according to medication use. For the non medication users all BMD measurements were used in calculation, while for the medication users, the rate of bone loss

was calculated based on 1 measurement prior to first visit with medication and all the subsequent BMD measurements following that visit. For participants who started medication after Year 10 visit (n=294), this could not be calculated.

Participants with at least one BMD measurement were included in a sensitivity analysis. Their individual rate of bone loss at the site of femoral neck was estimated using mixed effects models. These models estimate a rate of bone loss in individuals with one measurement based on the general trend of the participants who have all or almost all measurements.

Fracture ascertainment

Self-reported incident clinical fractures were obtained yearly and at clinical visits. Information on the date, site, circumstance of the fracture, was obtained by interview, and an x-ray report sought. The majority of fractures (78%) were confirmed by medical report(29). This study included only incident fragility fractures. Skull, sternum, finger and toe fractures were excluded.

Mortality ascertainment

Deaths occurring during the study follow-up were ascertained by contact with the next of kin or proxy if the yearly questionnaire was not returned and in some centres verified by obituaries.

Statistical Analysis

nBP (alendronate and risedronate) and non-nBP (etidronate), due to the higher potency of the former and different mechanism of action, were analysed separately.

Baseline characteristics were examined for the 2 classes of bisphosphonates (nBP and non-nBP) in comparison to non-treated participants (NoRx) (T-tests for continuous and chi-squared tests for categorical variables). Due to significant differences in baseline characteristics between treatment groups, participants who received medication during the follow-up were matched 1:1 to NoRx based on propensity score. Propensity score was calculated using a multivariate logistic regression model with treatment as outcome and

adjustment for all baseline characteristics. The treated and not-treated participants were then matched using the SAS macro “gmatch” based on a propensity score difference of less than half SD and the condition that not treated participant should be alive when the treated participant started treatment.

The relationship between medication use, annual rate of bone loss and mortality risk was assessed in 2 nested sets of nBP and non-nBP users (etidronate) matched 1:1 to NoRx participants using a single causal mediation analysis(30). Briefly, mediation analysis seeks to explain the underlying mechanism between an independent and dependent variable by inclusion of a third variable which represents the hypothesised mediator. The mediation model thus tests the hypothesis that the independent variable influences the mediator variable which in turn influences the dependent variable. According to this model a variable is likely to be a mediator upon meeting three conditions: the independent variable is significantly associated with the mediator; the mediator is significantly associated with the dependent variable; and the association between independent and dependent variable decreases and become not significant in the presence of mediator. This study, utilises this methodology to test whether the relationship between bisphosphonates (independent variable) and survival (dependent variable) is mediated by a reduction in the annual rate of bone loss (mediator variable)(31) . Four models were thus constructed: 1) a linear regression model of treatment with annual percent change of bone loss as the outcome (path a); 2) a Cox’s proportional hazards model of annual percent bone loss with survival as the outcome (path b); 3) a Cox’s proportional hazards model of treatment and survival without adjustment for annual percent bone loss (path c); 4) a multivariate Cox’s proportional hazards model of treatment with adjustment for annual percent bone loss with survival as outcome (path c’) (Figure 2). Given that the treated participants were matched by propensity score with non-treated participants, there were only few unbalanced variables. Double adjustment has been recommended for all variables with SMD $\geq$ 0.10 after matching (32). In order to be more conservative, we have adjusted for all variables with a SMD $\geq$ 0.03 after matching. All four mediation models described above were adjusted for the variables with SMD $\geq$ 0.03 (paths a, b, c, c’) (33). Follow-up was calculated from the time of medication start for both treated and non-treated. For non-treated this starting point was obtained by the addition to baseline date his/her “pair’s” time of medication commencement. In each survival model, the strength of the association between treatment, annual percent bone change and survival was assessed by the hazard ratio and the 95% CI.

If the associations between treatment and both survival and bone loss reduction were significant, a causal mediation analysis was performed using a SAS macro(34). This analysis estimates the point and interval estimates of the percent of the treatment effect mediated by the mediator (i.e. annual percent change in bone mineral density) using Cox regression survival analysis.

All statistical analyses were performed using SAS version 9.4 for windows.

Results

This study included 1735 women aged 50+ who had at least 2 BMD measurements, followed for a median of 15.0 years (IQR: 10.5-15.0). During this follow-up, 454 (26%) women experienced an incident fracture, and 241 (14%) died. BMD declined at the site of femoral neck with a median of -0.38 % / year (IQR: - 1.00 to 0.22), and increased slightly at the lumbar spine with a median of +0.22 % / year (IQR: -0.39 to 0.92).

Women were classified according to treatment as nBP (n=387), etidronate users (n=337), and NoRx (n=1019). All bisphosphonate users had several factors associated with poorer survival: significantly lower femoral neck BMD, weight, and more prior fractures than NoRx individuals. They also had several factors associated with “healthy users” such as better education, lifestyle habits (less smoking, more exercise and more vitamin D use) and less cardiovascular disease and diabetes (Table 1). To overcome this imbalance in characteristics, treated participants were matched 1:1 to non-treated participants based on a propensity score which included age, weight, femoral neck BMD, prior fractures education, lifestyle habits (smoking and exercise) and co-morbidities.

There was no difference in the prevalence of treatment across the nine study centres.

Etidronate versus matched Non treatment

Almost all etidronate users (327 out of 337) were matched 1:1 by propensity score to non treated participants. After matching, all baseline variables had a standardized mean difference less than 0.10, except age (not treated were older) and smoking (more in not treated) (Table

1). Follow-up time was comparable between etidronate and non-treatment groups [9.0 years (IQR, 6-13) for etidronate and 9 years (IQR, 6-12) for non-treatment, p=0.7)].

The number of incident fractures were similar between etidronate users and matched not users (53 (16%) for etidronate users vs 43 (13%) for non-users; adjusted HR 1.27 (95%CI, 0.71-2,25); p=0.42].

Mortality rates were similar for etidronate and NoRx groups [27 deaths/1000 person-years follow-up (95% CI, 16-26) and 23 deaths/1000 person-years follow-up (95% CI, 18-28) for etidronate and NoRx, respectively)] (Figure 3, Table 2).

Etidronate users had a significantly lower rate of bone loss than NoRx for femoral neck [difference 0.29%/year (95% CI, 0.06-0.52)], and lumbar spine [difference 0.56%/year (0.38-0.75)]. Individuals with the highest rate of femoral neck bone loss had increased risk of mortality compared to those with lower rate of bone loss (Figure 4B). By contrast a higher rate of bone loss at the site of lumbar spine was not associated with increased mortality risk. However, due to the non-significant effect of etidronate on survival, mediation analysis was not performed.

Nitrogen-Bisphosphonates versus matched Non Treatment

Of the 387 nitrogen-bisphosphonate users, 271 could be matched 1:1 to non-treated participants; all with 2 or more BMD measurements. After matching, the standardised mean differences between variables were less than 0.10 except for neurological conditions (more prevalent in treated), cancer (more prevalent in not treated) and smoking (more prevalent in not treated) (Table 1). Follow-up time after medication start was on average ~9 (IQR, 7 to 12) years for nBP users and ~7 (IQR, 5 to 10) years for non-treated.

The number of incident fractures was similar between treated and not treated (n=50 (18%) for nBP users and n=53 (20%) for non-users; p=0.74]. Fracture risk was similar between treatment group [adjusted HR 1.0 (95% CI, 0.53- 1.90)]. However, there was an interaction between treatment and baseline femoral neck BMD, and a subgroup analysis of individuals with a T-score ≤ -2.5 SD revealed a non-significant but clinical relevant fracture risk reduction associated with nBP use [adjusted HR 0.40 (95%CI, 0.12 -1.32)].

Mortality rates were significantly lower for nBP users (9 deaths/1000 person-years (95% CI, 5-13) compared to NoRx (17 deaths/1000 person-years (95% CI, 11-22); p=0.03 (Table 2, Figure 3). The rate of bone loss was significantly lower in the nBP group compared to matched not treated at both femoral neck (difference 0.64%/year (95% CI, 0.42 to 0.86); p<0.0001) and lumbar spine sites (difference 0.77%/year (95% CI, 0.56 to 0.98; p<0.0001). Moreover, a reduction in the rate of bone loss was associated with a significant reduction in mortality, particularly for the highest quartile of bone loss (Figure 4B). Cox proportional hazard analysis determined that individuals with accelerated rate of bone loss at the site of femoral neck (highest quartile of bone loss) had a ~ 2-fold higher mortality risk compared to those who were in the lower bone loss quartiles [HR 2.0 (95% CI, 1.1-3.6)] (Figure 4A). Bone loss at the site of lumbar spine was not associated with mortality risk.

Given the significant association between survival and both nBP-treatment and the annual rate of bone loss, a causal mediation analysis was performed to determine the proportion of mortality risk reduction mediated through a reduction in the rate of bone loss. The relationship between treatment and mortality not adjusted by hypothesised mediator (annual rate of femoral neck BMD loss) was 0.61 (95%, 0.39-0.96). However, after adjusting for the mediator, the relationship between treatment and mortality decreased and became non-significant [HR 0.74 (95%, 0.46-1.19)]. The proportion of mortality risk reduction mediated by a reduction in the rate of femoral neck bone loss was estimated at ~ 39% (95% CI, 7 - 84%) (Figure 2). Adjustment for the unbalanced covariates (SMD≥0.03) only marginally affected the causal relationship between treatment, annual rate of bone loss and survival (Figure 2). None of the baseline variables still unbalanced after matching, were significantly associated with mortality risk [age-adjusted HR: neurological conditions, 1.00 (95% CI, 0.06 – 16.00); p=0.89; cancer , 1.52 (0.62-3.77) and smoking 0.91 (95%CI, 0.31- 2.64)] and thus could not mediate the relationship between treatment and survival.

Sensitivity analysis

The participants who only had one BMD measurement (28% of not-treated and 41% of those treated) were additionally included in a sensitivity analysis. After matching, there were 487 nBP users matched 1:1 to non-users and 466 etidronate users matched 1:1 to non-users (Table S1). Similar to the primary analysis, several baseline variables remained unbalanced (SMD≥0.10) after matching (i.e. age, weight, diabetes). nBP treatment was associated with significant reduction in mortality risk after adjustment for all baseline variables excluding

rate of BMD loss (Table S2). Similar to the primary analysis, after adjustment for the rate of bone loss, the magnitude of the treatment effect on mortality risk reduction decreased. The mediation model indicated that the rate of bone loss, mediated ~ 45% of the mortality rate difference between treated and not treated [percent mediated: 45.2% (95% CI, 12.3-83.0%); p=0.01]. The findings of the sensitivity analysis confirmed the robustness of the primary analyses.

Etidronate use was not associated with lower mortality rates than the matched non-users (Table S2).

Discussion

Recent evidence suggests that treatment with bisphosphonates is associated not only with fracture risk reduction, but also with a reduction in mortality risk. However, the mechanism behind this association is not yet understood. In this observational study, nitrogen-bisphosphonates were associated with both a significantly lower mortality risk and a significantly lower rate of bone loss compared to non-treated individuals with similar baseline mortality risk. Individuals in the highest quartile of bone loss were twice as likely to die compared to those with lower rates of bone loss. Using mediation analysis to combine these findings, approximately 39% of the mortality risk reduction in the nitrogen-bisphosphonate group was found to be mediated through a reduction in the rate of bone loss. Although causality cannot be confirmed in an observational study, this statistical mediation analysis suggests that lower rates of bone loss are associated with mortality reduction in those on nitrogen bisphosphonates.

We have previously shown in the same cohort that nitrogen-bisphosphonates were associated with ~ 34% mortality risk reduction, while etidronate, a non-nitrogen bisphosphonate was not associated with any survival benefit (35). While this finding suggested the existence of a possible “true” survival benefit over the unavoidable “healthy bias” effect, it did not provide any explanation for this effect. The risk of future fracture investigated as potential mediator of mortality risk reduction, was not significantly reduced in treatment groups, perhaps due to a higher baseline fracture risk in treated versus not treated individuals. However, the existence of a survival benefit only in the group taking the most potent bisphosphonates, when analysed in a direct head-to-head comparison with non nitrogen bisphosphonate, together with the evidence from previous studies that bone loss is associated with increased mortality risk(25, 26) led to the hypothesis that bone loss reduction may contribute to the

mortality risk reduction observed with nitrogen bisphosphonates. The present study confirmed the survival benefit of nitrogen-bisphosphonates in a subset of individuals, in whom rate of bone loss could be reliably assessed through multiple bone mineral density tests. This study also confirmed the associations between nitrogen-bisphosphonates and bone loss reduction, previously demonstrated in this cohort (36). An accelerated rate of bone loss was independently associated with a ~ 2-fold increased mortality risk. Importantly, the addition of bone loss to the model of nitrogen bisphosphonates and survival lessened this association. According to the statistical definition of mediation, a reduction in the rate of bone loss is a partial mediator of the relationship between nitrogen-bisphosphonates and survival because: 1) nitrogen-bisphosphonates are associated with a reduction in the rate of bone loss; 2) bone loss significantly predicts mortality risk; 3) the relationship between nitrogen-bisphosphonates and survival was diminished and became not significant after adjusting for bone loss and 4) bone loss remained significantly associated with survival in the final model.

Using a causal mediation analysis, it was estimated that ~ 39% of the mortality risk reduction associated with nitrogen-bisphosphonates was mediated by a reduction in the rate of bone loss. In contrast with nitrogen-bisphosphonate, etidronate use was associated with a weaker effect on bone loss reduction and was not associated with mortality risk reduction.

Notably, this mediation effect of bone loss was site specific. This difference is probably driven by the spurious increase in lumbar spine BMD due to degenerative disease that occurs in the aging population. In the current study, the trajectories of BMD change at the two bone sites were divergent, with loss experienced only at the femoral neck site, but increase in lumbar spine BMD, consistent with osteoarthritic lesions in the lumbar spine that increase with age. Thus lumbar spine BMD change was not a predictor of mortality risk.

In the current study, the trajectories of BMD change at the two bone sites were divergent, with loss experienced only at the femoral neck site, consistent with osteoarthritic lesions which increase with age. Due to the same reasons lumbar spine BMD change, was not a predictor of mortality risk.

The association between femoral neck bone loss and mortality risk has been previously demonstrated in both individuals with and without fracture (25, 26). The mechanism behind this association is most likely multifactorial. The high bone turnover associated with bone loss may lead to release of heavy metals from bone(37), which subsequently predisposes to cardio-vascular risk(38). Several papers have reported bone loss as a component of the geriatric frailty syndrome characterised by deterioration in physical function and activity with an increased propensity for falling, fracture and mortality risk(39-41). It has been proposed that the mechanism behind the geriatric syndrome may be the presence of a chronic low inflammatory state which subsequently leads to bone loss, disability, and increased fracture risk and mortality. Chronic inflammation is characterised by overproduction of cytokines such as IL-1, Il-6, TNF- alpha which not only perpetuate the inflammatory state, but also activate bone resorption and inhibition of bone-building mechanisms(42). Bisphosphonates, besides their anti-resorptive effect on bone also have anti-inflammatory properties(43). In vitro studies indicated that these agents impair macrophage differentiation and promote macrophage cytotoxicity and apoptosis. In vivo, nitrogen-bisphosphonates induces a pro-inflammatory effect in the short-term, but chronic administration may suppress pro-inflammatory cytokines. A recent study reported that treatment with bisphosphonates for one year was associated with a significant reduction in plasma IL-6, IL-17 and IL-23 compared to controls (44).

Besides the anti-inflammatory effect discussed above, some evidence suggests that bisphosphonates may have an effect on the immune system. In a recent study, treatment with zoledronic acid was associated with activation of gamma delta T-cells, which are involved in the immune defence against infection(45). Furthermore, in a post-hoc analysis of the potential mediators of the mortality risk reduction observed in patients treated with zoledronic acid following hip fracture, treatment was associated with a significant reduction in deaths due to pneumonia, despite a similar incidence of the condition(24). Notably, in a recent RCT of zoledronic acid in women with osteopenia, treatment was associated with a significant reduction in the incidence of cancer [HR 0.67 (95% CI, 0.50 – 0.89)](17).

It is most likely that other mechanisms are involved in the mortality risk reduction associated with nitrogen-bisphosphonates. There is a clear epidemiological association between bone loss and arterial calcification (46) that suggest common pathways, although the mechanism is not fully understood. Data from randomised controlled studies suggest a reduction of cardio-

vascular mortality risk in patients treated with risedronate and zoledronate (24, 47). Bisphosphonates have also been associated with a reduced risk of myocardial infarction in a case-control study of hip and vertebral fractures(48) and in patients with rheumatoid arthritis(49). In the RCT of zoledronic acid in women with osteopenia, treatment was associated with a non-significant reduction in the incidence of myocardial infarction and stroke (17). Furthermore, in a recent RCT comparing romososumab and alendronate, the incidence of severe cardio-vascular events was higher in the romososumab than alendronate groups, despite a similar cardiovascular risk at baseline(50). As romososumab was not associated with increased cardiovascular events in the previous and larger FRAME trial (romososumab versus placebo), it is possible that the increased cardiovascular events observed in romososumab versus alendronate may be due to a reduction in cardiovascular events for alendronate. However, in a recent meta-analysis of RCTs, bisphosphonates were not associated with a reduction in major cardio-vascular events although these events were not formally adjudicated (51). Thus there is still uncertainty about any of these potential mechanisms.

This study has several strengths. CaMOS had a large number of bisphosphonate users in comparison to other population based studies (over 40% of women)(18). This relatively large number of bisphosphonate users permitted an analysis of bisphosphonates according to their class, as well as adjustment for a large set of risk factors. The long follow-up permitted an investigation of the role of bone loss on mortality risk and its role in mediating mortality reduction in individuals treated with bisphosphonates. However, there are some limitations. Treatment was not randomly allocated and thus part of the observed association between treatment and survival could be related to confounding. The treated group had significantly higher baseline fracture risk (i.e. lower BMD, and weight, more prior fractures), and had a different co-morbidity profile to the non-treated group. In order to counteract these selection biases, this study used propensity score matching based on both baseline variables, which, similar to randomisation, is designed to produce groups with similar baseline risk, while acknowledging that there still may remain unknown confounders. The technique resulted in groups with similar baseline characteristics, albeit few exceptions. However, none of the imbalanced factors, were associated with mortality risk. Nevertheless, these factors may reduce the generalisability of these findings. Lastly, mediation analysis findings need to be interpreted through the light of its limitations. Mediation analysis has been developed to test a

causal association. However, causality cannot be proven in an epidemiological study. In this context, our analyses have only demonstrated that the data aligns with the proposed causal hypothesis.

In summary, in this long-term prospective population based study, nitrogen-bisphosphonate use in women was associated with both better survival and a significant reduction in the rate of bone loss. Bone loss was significantly associated with increased mortality risk with those in the highest quartile of bone loss being twice as likely to die compared to those who did not lose bone. Using a mediation analysis approach, approximately 39% of the difference in mortality rates between treatment groups was found to be related to the greater bone loss in the not treated versus the treated groups.

In conclusion, this study offers a plausible explanation for the association between potent anti-resorptive medication and survival. Future mechanistic studies into osteoporosis, anti-resorptive treatment and survival are warranted.

Figure legends

Figure 1 Flow chart of study participants

Figure 2 Mediation models with path coefficients: a-regression model for treatment and rate of femoral neck bone loss; b-Cox's model for rate of bone loss and survival; c'-Cox's model for treatment and survival adjusted for rate of femoral neck bone loss; c-Cox's model for treatment and survival not adjusted for rate of femoral neck bone loss; All models were adjusted for baseline characteristics with SMD≥0.3 (Table 1)

Figure 3 Kaplan-Meier survival curves for nitrogen-bisphosphonates vs matched no treatment and etidronate vs no treatment

Figure 4 Hazard ratios of mortality for quartiles of femoral neck bone loss in nitrogen-bisphosphonates and no treatment (A) and etidronate and no treatment (B).

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Table 1 Baseline characteristics of female participants according to medication use

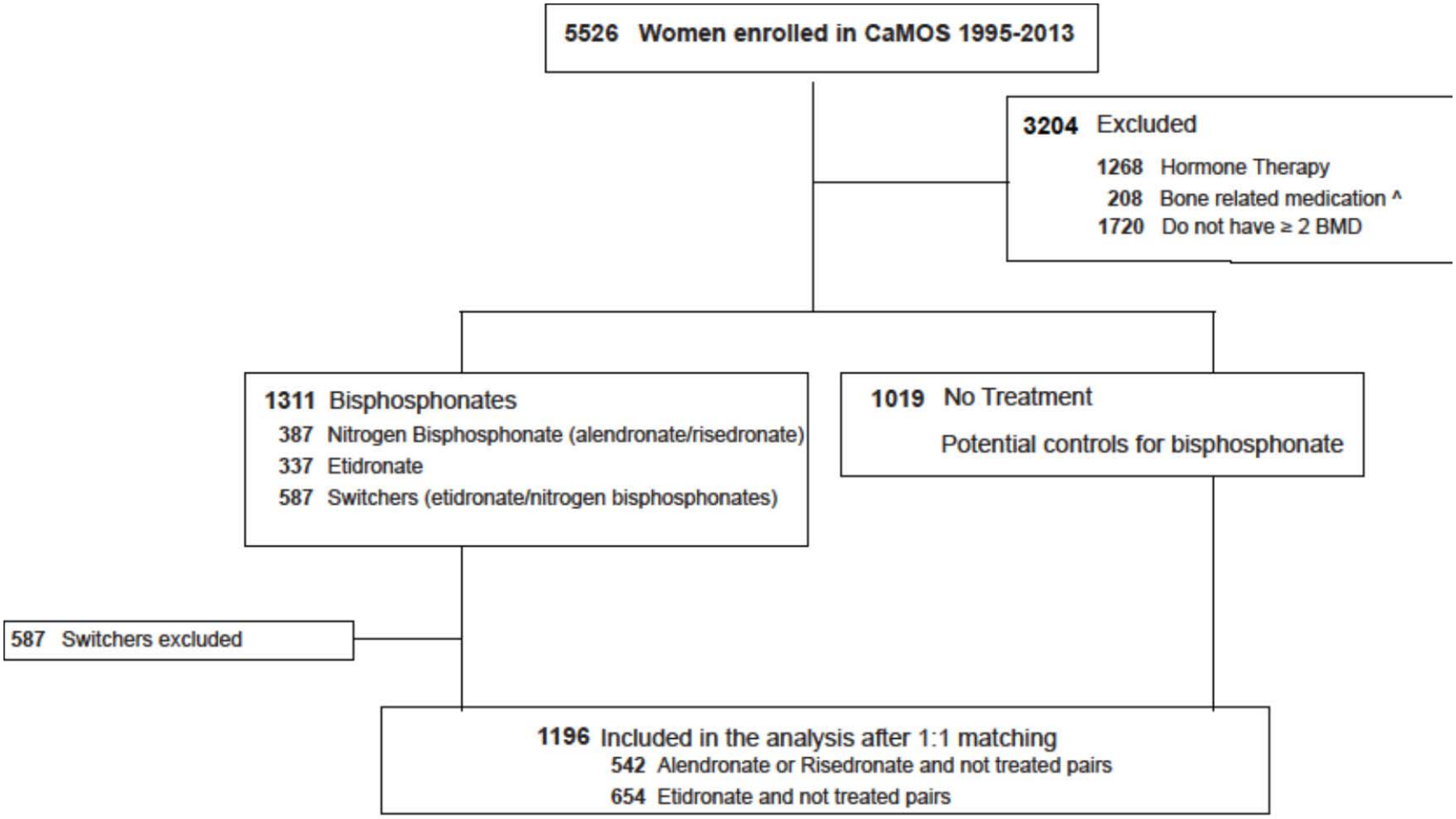
Characteristics	**Nitrogen Bisphosphonates and No Treatment**					
	Unmatched			**Matched**		
	Nitrogen Bisphosphonate	No Treatment	SMD[a]	Nitrogen Bisphosphonate	No Treatment	SMD[a]
Number	387	1019		271	271	
Age[b], yrs	64.8 (8.2)	65.6 (8.2)	-0.09	65.2 (8.1)	65.1 (8.1)	0.01
Weight[b], kg	64.7 (11.2)	73.6 (14.1)	**-0.720**	66.4 (11.4)	67.0 (11.8)	-0.02
Higher education[c]	114 (29.5)	214 (21)	**0.178**	75 (28.3)	70 (25.8)	-0.06
Prior fracture[c]	115 (29.7)	261 (25.6)	0.090	83 (31.32)	80 (28.78)	0.010
FN BMD[1,b], g/cm^2	0.64 (0.09)	0.73 (0.11)	**-0.850**	0.66 (0.09)	0.66 (0.11)	-0.06
LS BMD[2,b], g/cm^2	0.82 (0.13)	0.97 (0.15)	**-0.972**	0.85 (0.13)	0.85 (0.13)	-0.02
Co-morbidities[c]						
Heart disease	18 (4.7)	71 (7.0)	**-0.169**	11 (4.2)	13 (14.8)	0.02
Diabetes	11 (2.8)	83 (8.1)	**-0.212**	9 (3.4)	14 (5.17)	-0.09
Neurological	10 (2.6)	27 (2.7)	-0.023	8 (3.0)	3 (1.1)	**0.15**
Respiratory	28 (7.2)	79 (7.8)	0.058	22 (9.36)	21 (8.9)	0. 009
Cancer	19 (4.9)	67 (6.6)	0.034	10 (3.77)	17 (6.27)	**-0.10**
Life style factors[c]						
Exercise	253 (65.4)	585 (57.4)	0.166	171 (64.53)	169 (62.36)	0.03
Smoking	41 (10.6)	142 (14)	-0.121	27 (10.19)	41 (15.13)	**-0.14**
	Etidronate and No Treatment					
	Unmatched			**Matched**		
	Etidronate	No Treatment	SMD[a]	Etidronate	No Treatment	SMD[a]
Number	337	1019		327	327	
Age[b], yrs	67.7(8.0)	65.6 (8.2)	0.276	67.7 ((8.1)	68.6 (8.6)	**-0.10**
Weight[b], kg	66.5 (12.2)	73.6 (14.1)	-0.484	66.5 (12.1)	66.4 (10.9)	0.006
Higher education[c]	67 (19.9)	214 (21)	-0.03	67 (20.3)	57 (17.4)	0.08
Prior fracture[c]	98 (29.8)	261 (25.6)	0.093	98 (29.4)	102 (31.2)	-0.03
FN BMD[1,b], g/cm^2	0.64 (0.09)	0.73 (0.11)	-0.879	0.64 (0.09)	0.64 (0.09)	-0.02
LS BMD[2, b], g/cm^2	0.86 (0.13)	0.97 (0.15)	-0.760	0.86 (0.13)	0.87 (0.12)	-0.05
Co-morbidities[c]						
Heart disease	31 (9.2)	71 (6.9)	0.0236	30 (9.1)	23 (7.0)	0.08
Diabetes	17 (5.1)	83 (8.1)	-0.256	17 (5.2)	24 (7.3)	-0.09
Neurological	14 (4.1)	27 (2.7)	0.057	14 (4.3)	11 (3.4)	0.05
Respiratory	32 (10.7)	79 (7.7)	0.097	32 (11.0)	32 (11.7)	0.00
Cancer	24 (7.1)	67 (6.5)	0.064	23 (7.0)	23 (7.0)	0.00
Life style factors[c]						
Exercise	205 (60.8)	585 (57.4)	0.081	199 (60.9)	183 (56.0)	0.10
Smoking	38 (11.3)	142 (14)	-0.074	36 (11.0)	50 (15.3)	**-0.130**

*[a]**-SMD-**standardised mean difference; [b]-mean (sd); [c]-number (%);boldface represents variable unbalanced after matching (SMD>0.10); [1]-FN=femoral neck; [2]-LS=Lumbar spine L1-L4*

Table 2 Models of the association between treatment, mediator and survival. 1) treatment and bone loss, 2) treatment and survival unadjusted, and 3) adjusted for mediator

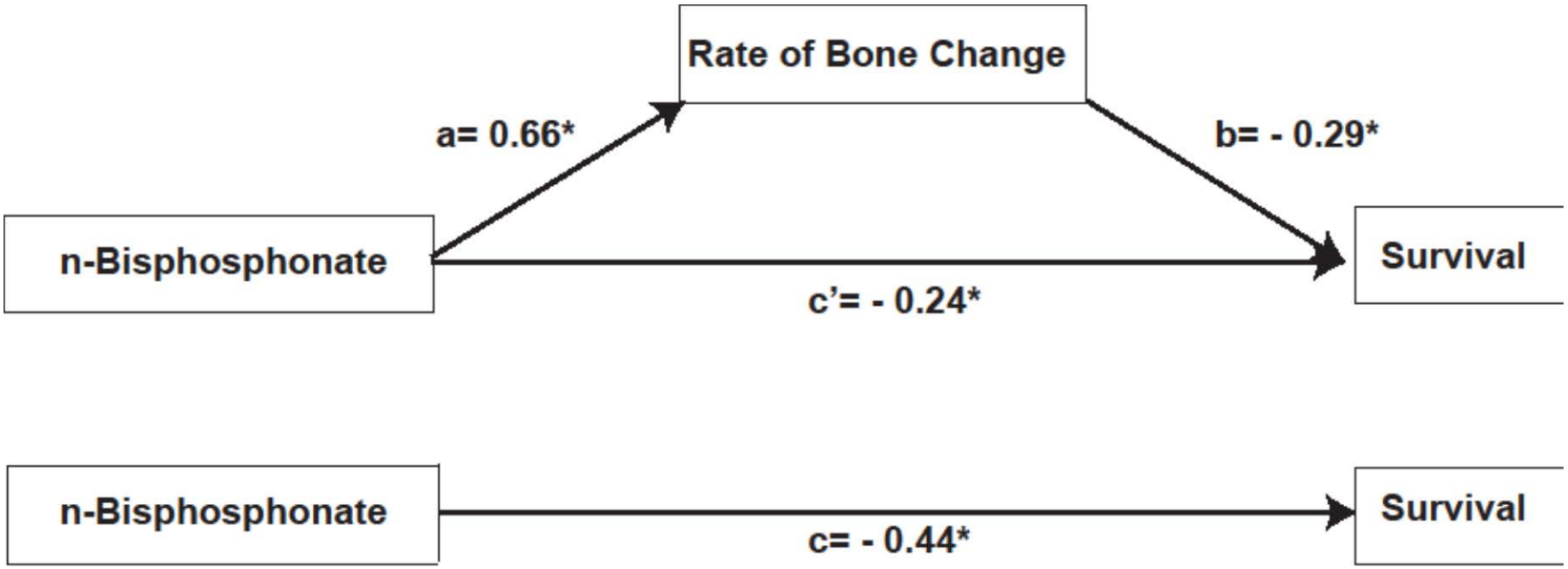
Models/outcomes	**Treatment**	**No Treatment**	**Treatment vs No treatment**	**p-value**
Nitrogen-Bisphosphonates and No Treatment				
1) Linear regression model of treatment (independent variable) and the rate of bone change (mediator)*	**Median (IQR)**	**Median (IQR)**	**Difference in annual rate**	**p-value**
% Annual rate of FN BMD change (median, IQR)	+0.11 (-0.52 to +0.87)	-0.56 (-1.17 to -0.06)	0.64 (0.42 to 0.86)	<0.0001
% Annual rate of LS BMD change (median, IQR)	+0.68 (-0.21 to +1.47)	-0.12 (-0.73 to +0.58)	0.77 (0.56 to 0.98)	<0.0001
2) Cox's model of treatment (independent variable) and survival (dependent variable)	**Mortality Rates (95% CI)**	**Mortality Rates (95% CI)**	**HR (95%CI)**	**p-value**
Treatment Unadjusted	8.89 (5.19-12.60)	16.70 (11.20-22.20)	0.61 (0.39-0.96)	0.03
3) Cox's model of treatment (dependent variable) and survival (independent variable) adjusted for bone change (mediator)			**HR (95%CI)**	**p-value**
Treatment multivariable adjusted + % annual rate FN BMD			0.74 (0.46-1.19)	0.17
Treatment multivariable adjusted + % annual rate LS BMD			0.61 (0.32-1.18)	0.14
Etidronate and No Treatment				
1) Linear regression model of treatment (dependent variable) and the rate of bone change (mediator)	**Median (IQR)**	**Median (IQR)**	**Difference in annual rate**	**p-value**
% Annual rate of FN BMD change (median, IQR)	-0.18 (-0.94 to + 0.53)	-0.58 (-1.16 to -0.05)	0.29 (0.06-0.52)	0.012
% Annual rate of LS BMD change (median, IQR)	0.56 (-0.12 to 1.47)	0.11 (-0.52 to 0.71)	0.56 (0.38- 0.75)	<0.0001
2) Cox proportional hazards model of treatment (dependent variable) and survival (outcome)	**Mortality Rates (95% CI)**	**Mortality Rates (95% CI)**	**HR (95%CI)**	**p-value**
Treatment Unadjusted	26.60 (15.50-25.70)	22.90 (17.50-28.30)	1.35 (0.86-2.11)	0.19
3) Cox's model of treatment (dependent variable) and survival (independent variable) adjusted for bone change (mediator)			**HR (95%CI)**	**p-value**
Treatment multivariable adjusted + % annual rate FN BMD			1.59 (0.97-2.61)	0.07
Treatment multivariable adjusted + % annual rate LS BMD			1.36 (0.85-2.17)	0.2

*Adjusted for all baseline variables with SMD≥ 0.03 (Table 1); a-mortality rates presented as death per 1000/ person-year



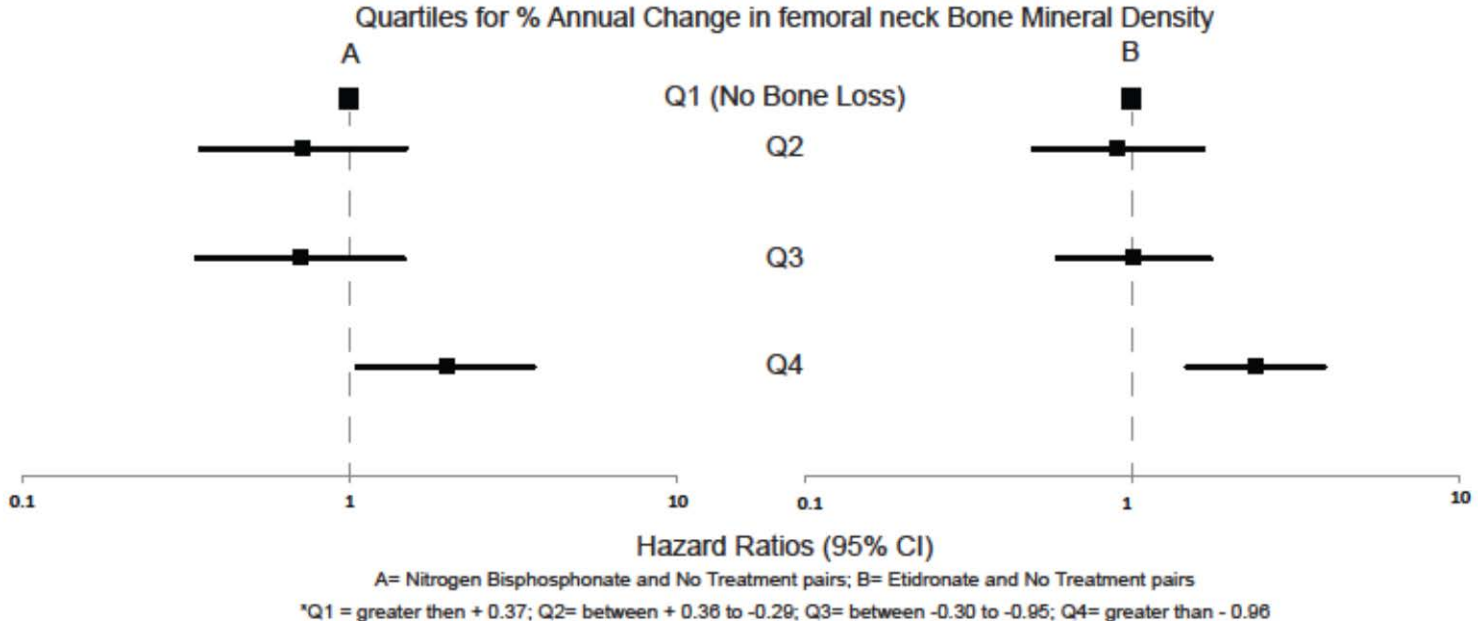
* The individual numbers do not add up to the total as some individuals may be listed more than once

^ The list of bone related medication not eligible includes: pamidronate, clodronate, calcitonin, SERMS, Hormone therapy and Tamoxifen



Proportion mortality mediated by bone loss reduction=39.1 (95%CI, 7.1-84.4) %

* all models were adjusted for all variables with SMD ≥ 0.03



A= Nitrogen Bisphosphonate and No Treatment pairs; B= Etidronate and No Treatment pairs

*Q1 = greater then + 0.37; Q2= between + 0.36 to -0.29; Q3= between -0.30 to -0.95; Q4= greater than - 0.96