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The impact of thiazide diuretics on bone mineral density and the trabecular bone score: the Rotterdam Study

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ABSTRACT

The decreased risk of osteoporotic fractures in thiazide diuretics (TD) users is possibly not only caused by an increase in bone mineral density (BMD), but by an increase in other determinants of bone strength as well, such as the trabecular bone score (TBS). To test this hypothesis, we studied the association between TD use and both lumbar spine BMD (LS-BMD) and lumbar spine TBS (LS-TBS) cross-sectionally in 6096 participants from the Rotterdam Study, as well as the association between TD use and bone turnover estimated by serum osteocalcin levels. We found that past and current use of TD were associated with an increase of LS-BMD ($\beta = 0.021$ g/cm^2 (95% CI: 0.006;0.036) and $\beta = 0.016$ g/cm^2 (95% CI: 0.002;0.031), respectively). Use of ≥1 defined daily dose (DDD) ($\beta = 0.028$, 95% CI: 0.010;0.046; p for trend within DDD of use <0.001) and use of >365 days ($\beta = 0.033$, 95% CI: 0.014;0.052; p for trend within duration of use <0.001) were positively associated with LS-BMD. No significant association between TD use and LS-TBS was observed. Mean serum osteocalcin levels were significantly different between users and non-users of TD (20.2 ng/ml (SD 8.3) and 22.5 ng/ml (SD 17.0), respectively, $p < 0.001$). Furthermore, linear regression analysis showed that the use of TD was associated with a 3.2 ng/l (95% CI: −4.4.; −2.0) lower serum osteocalcin level compared to non-use of TD, when adjusted for Rotterdam Study cohort, age, and sex. Our results may implicate that the decreased fracture risk in TD users is explained by increased bone mass rather than by improved bone microarchitecture. Alternatively, changes in bone microarchitecture might not be detected through TBS and more sophisticated techniques are possibly needed to study a potential effect of TD on bone microarchitecture.

1. Introduction

Thiazide diuretics are known to have a small but positive effect on bone mineral density (BMD) [1–8]. Furthermore, our research group demonstrated in 2003 that the use of thiazide diuretics was associated with a significantly reduced risk of hip fracture, which disappeared after four months of discontinuation of use [9]. Similarly, several other studies have shown a reduced risk of hip fractures as well as of other osteoporotic fractures when using thiazide diuretics [10–13].

Thiazide diuretics can affect bone through different mechanisms. These drugs were shown to directly stimulate osteoblast differentiation and bone formation [14]. This could result in an increase in serum osteocalcin, which is considered as a marker of osteoblast activity, bone formation, and bone turnover in general [15–17]. However, bone histomorphometric studies have presented evidence for a reduced bone resorption and markers of bone resorption such as N-telopeptide and of bone formation such as osteocalcin have been shown to be reduced, especially during the first six months of therapy with thiazide diuretics [6,18]. Furthermore, use of thiazide diuretics directly stimulates calcium uptake by the bones [19] and indirectly increases the calcium concentrations in the human body *via* calcium retention through the kidneys [20–22]. In addition, thiazide diuretics use has been associated

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with lower parathyroid hormone (PTH) levels, independently of serum calcium levels [23]. PTH plays an important role in skeletal homeostasis and lower levels of this hormone can lead to a decrease in bone remodeling [24].

BMD is an important determinant of bone strength [25] and fracture risk [26]. However, previous studies have shown that use of thiazide diuretics is associated with only a small increase in BMD and a much larger decrease in the risk of osteoporotic fractures, suggesting that this decrease is not only caused by an increase in BMD, but by an increase in other determinants of bone strength as well. This highlights the importance of measuring and studying determinants of bone strength other than BMD. Recently, the trabecular bone score (TBS), estimated from dual-energy X-ray absorptiometry (DXA) scan images, has been approved by the Food and Drug Administration (FDA) as a non-invasive technique for producing a metric that correlates with the trabecular microarchitecture of bones [27]. BMD and TBS are independent measures of bone strength [25]. In addition, TBS has been shown to be a predictor of fracture risk independently of both BMD and the Fracture Risk Assessment Tool (FRAX) and adjusting the FRAX score for TBS could also improve the assessment of fracture risk [28–30]. Thus, investigating the effect of thiazide diuretics on both BMD and TBS could provide new and important insights into the mechanism by which the decreased risk of osteoporotic fractures in thiazide diuretics users can be explained.

To the best of our knowledge, the effect of thiazide diuretics on TBS has not been studied before. In view of the frequent use of thiazide diuretics and the high prevalence of osteoporosis in the ageing population [31,32], it is important to evaluate the association between thiazide diuretics and several aspects of bone strength. Therefore, our objective was to investigate the association between thiazide diuretics use and both BMD and TBS as well as the association between thiazide diuretics use and bone formation estimated by serum osteocalcin levels, in a large population-based cohort study.

2. Materials and methods

2.1. Study design and population

This cross-sectional analysis was conducted in individuals who participated in the Rotterdam Study, an ongoing prospective population-based cohort study. The design and rationale of the Rotterdam Study have been described elsewhere in detail [33]. In brief, the Rotterdam Study originated in 1990 and was designed to investigate chronic diseases in the elderly. The study started with 7983 participants aged 55 years and older, living in Ommoord, a suburb of Rotterdam, The Netherlands. This original cohort (RS-I) was extended with a second cohort (RS-II) in 2000 and a third cohort (RS-III) in 2006, adding 3011 (aged ≥55 years) and 3932 (aged ≥45 years) participants, respectively. This resulted in a total study population of 14926 participants aged 45 years and older. All participants were examined at baseline and asked to participate in follow-up examinations every 3–4 years. For this analysis, we studied participants from the fourth visit of RS-I (RS-I-4, 2002–2004), the second visit of RS-II (RS-II-2, 2004–2005), and the first visit of RS-III (RS-III-1, 2006–2008) for whom there was both a LS-BMD and a LS-TBS measurement available. In total, 6601 participants gave written informed consent to participate in the study. Ever users of bisphosphonates and current users of loop diuretics were excluded from the study. Ever users of bisphosphonates were excluded because previous literature suggests that the effect of bisphosphonates might persist for years after discontinuation of use [34]. Loop diuretics have been suggested to influence BMD, however, to the best of our knowledge, literature about the persistence of the effect after discontinuation of use is lacking. Therefore, we only excluded current users of loop diuretics. The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). The Rotterdam Study Personal Registration Data collection is filed with the Erasmus MC Data Protection Officer under registration number EMC1712001. The Rotterdam Study has been entered into the Netherlands National Trial Register (NTR; www.trialregister.nl) and into the WHO International Clinical Trials Registry Platform (ICTRP; www.who.int/ictrp/network/primary/en/) under shared catalogue number NTR6831. All participants provided written informed consent to participate in the study and to have their information obtained from treating physicians.

2.2. Assessment of thiazide diuretics use

Information about thiazide diuretics exposure was obtained through linkage with the pharmacies in Ommoord which use one shared computer network. The drug exposure period was calculated by dividing the total number of units per dispensed prescription by the prescribed daily number of units. The daily dose was expressed in 'defined daily dose' (DDD), as defined by the World Health Organization (WHO) [35]. When a renewed prescription was filled within seven days after ending the previous one, it was considered as one continuous episode of drug use. The number of days between the last episode of drug use and the date of the DXA scan was used to determine the time a participant was unexposed to the drug prior to undergoing the DXA scan. We defined three different groups of users: current users, past users, and never users. If the date of the performed DXA scan was within the drug exposure period or when the participant had a drug exposure period within 120 days prior to the performed DXA scan, the participant was classified as being a current user. This cutoff was set because previous literature has shown that the protective effect of thiazide diuretics on hip fracture, one of the most important types of osteoporotic fractures, disappears after four months of discontinuation of thiazide diuretics use [9]. If the date of the performed DXA scan was not within the drug exposure period but when the participant had a drug exposure period in the past not within 120 days prior to the performed DXA scan, the participant was classified as being a past user. Participants for whom the date of the performed DXA scan was not within the drug exposure period and who did not have a drug exposure period in the past were considered as never users. We used the following WHO's Anatomical Therapeutic Chemical codes for thiazide and thiazide-like diuretics: C03AA and C03BA; for the combination of thiazide diuretics and potassium-sparing agents: C03EA; for the combination of thiazide diuretics and beta blocking agents: C07BB; and for the combination of thiazide diuretics and ACE inhibitors: C09BA.

2.3. Measurement of BMD and TBS

As TBS is measured at the lumbar spine and in order to study BMD and TBS measurements of the same skeletal site, measurements of lumbar spine BMD (LS-BMD) and lumbar spine TBS (LS-TBS) were used in the current study. These measurements were carried out with DXA using a GE Lunar Prodigy densitometer (Lunar Radiation Corp., Wadison, WI). DXA scans were analyzed with GE lunar software for LS-BMD and with iNsight software version 4.0 for LS-TBS. LS-BMD values are expressed in g/cm^2. LS-TBS values are expressed as a score. LS-TBS was calculated as the slope of the log-log representation of a two-dimensional variogram, which is derived from gray-level differences on the DXA image. The higher the LS-TBS value, the higher the microstructural quality of the bones. The method of TBS measurement has been described elsewhere in detail [25]. As measurements of LS-TBS were not reliable when having a body mass index (BMI) above 37 kg/m^2, participants with a BMI above 37 were excluded.

2.4. Osteocalcin measurements

As measurements of osteocalcin were not available for RS-I-4 and

RS-II-2, osteocalcin measurements from the third visit of RS-I (RS-I-3, 1997–1999) and the first visit of RS-II (RS-II-1, 2000–2001) were used in the current analysis. In addition, measurements of osteocalcin from RS-III-1 were available and added to the analysis as well. In total, 8707 participants were included in the osteocalcin analysis. Blood samples used to measure serum osteocalcin levels were stored at −80 degrees Celsius and were only frozen and thawed once. Osteocalcin levels were measured in these blood samples by the Department of Clinical Chemistry of the Erasmus Medical Center using the Roche/Hitachi cobas e411/e601/e602, Elecsys 2010 and MODULAR ANALYTICS E170 analyzers (Roche Diagnostics, Indianapolis, IN, USA). Information about thiazide diuretics, bisphosphonates, and loop diuretics use was obtained in the same way as described above. For the osteocalcin analyses, the participant was classified as being a user if the date of the blood sampling was within the drug exposure period. This definition of users is different from the definition of current users in the other analyses, because a change in osteocalcin by thiazide diuretics is expected to occur rapidly whereas it takes time to increase the bone mineralization and microarchitecture. Every participant who was not classified as being a user, was classified as being a non-user. Current users of bisphosphonates and loop diuretics were excluded from the analysis.

2.5. Assessment of covariables

Information on bisphosphonates, loop diuretics, oral glucocorticoids and estrogen replacement therapy (ERT) use was obtained in the same way as information about thiazide diuretics was obtained. Ever use of bisphosphonates was defined as having at least one current or past drug exposure period. Current use of loop diuretics, oral glucocorticoids and ERT was defined in the same way as current use of thiazide diuretics, in order to be consistent in the definitions of use. Information on alcohol intake, smoking, and physical activity was acquired using home interviews. Alcohol intake was measured continuously in g/day and categorized into four categories, based on quantiles of use: none, low, medium, and high. Smoking was expressed categorically as never, past, and current smokers. BMI was calculated by dividing the weight in kilograms by the height in meters squared. Physical activity was expressed in total metabolic equivalent (MET) hours per week. Ascertainment methods for diabetes mellitus (DM), stroke, and coronary heart disease (CHD) have been previously described in detail [36–38]. In short, history of DM, stroke, and CHD were assessed during the baseline home interviews and verified by reviewing medical records. Subsequently, DM, stroke, and CHD were assessed during follow-up in the Rotterdam Study using information from general practitioners' records, hospital records, and lab measurements of serum glucose for DM. Serum 25(OH)D concentration, serum calcium, serum sodium, serum potassium, serum magnesium, and serum phosphate were measured in blood samples by the Department of Clinical Chemistry of the Erasmus Medical Center using standard methods. Season of blood sample collection was divided in two categories based on possible sunlight exposure: 1) autumn and winter and 2) spring and summer.

2.6. Statistical analyses

Continuous variables were expressed as means and standard deviations (SD), while categorical variables were expressed as frequency and percentage or valid percentage. Univariable and multivariable linear regression were used to examine the relationship of the use of thiazide diuretics with LS-BMD and LS-TBS. Furthermore, categories were created based on the mean defined daily dose (DDD) of thiazide diuretics to study the effect of dosage of thiazide diuretics on LS-BMD and LS-TBS. This resulted in two categories: <1 DDD and ≥ 1 DDD. To study the effect of the duration of thiazide diuretics use, three different categories of use were created: 1–120 days, 120–365 days, and >365 days. The higher cut-off of 365 days was chosen because of its clinical relevance: it has been shown that the use of thiazide diuretics for >365 days was associated with a reduced risk of osteoporotic fractures [9,13] and that the protective effect of thiazide diuretics on femur fractures is largest among those using the medication for >365 days [39]. In addition, it has been shown that the protective effect of thiazide diuretics on hip fracture disappears after four months of discontinuation of use [9], which could imply the existence of an adaptation phase of bone with a duration of four months. Another explanation might be that the changes in bone caused by the start or the discontinuation of thiazide diuretics will only be detectable after four months. Subsequently, we hypothesized that the effect of thiazide diuretics on bone will only appear after four months of thiazide diuretics use and therefore, the lower cut-off of 120 days was chosen. In both the DDD and the duration analyses, never use of thiazide diuretics was used as the reference category. Subsequently, both univariable and multivariable linear regression was used to examine the relationship of the DDD and duration of medication use with LS-BMD and LS-TBS. For all analyses, we used four models. The first model was adjusted for Rotterdam Study cohort, age, and sex, whereas the second model was additionally adjusted for BMI, serum 25(OH)D concentration, serum calcium, serum sodium, serum potassium, serum magnesium, serum phosphate, alcohol intake, smoking, and diabetes mellitus. The third model was additionally adjusted for LS-TBS in the models where LS-BMD was the outcome, and *vice versa*. In addition, in a fourth model, we investigated if additional adjustment for season of serum 25(OH)D measurement, corticosteroids use, ERT use, physical activity, stroke, and CHD significantly changed the results. A p for trend was calculated across both the 3 categories of duration of use and the two categories of DDD of use, and the reference category.

In case of a significant association between thiazide diuretics use and LS-BMD or LS-TBS, we tested interactions of thiazide diuretics use with age, sex, and BMI. Interaction terms were considered to be significant when the p for interaction was below 0.10.

Serum osteocalcin levels for current users and non-users of thiazide diuretics were expressed as means and SD. Linear regression analysis was used to examine the relationship of the use of thiazide diuretics with serum osteocalcin levels. The first model was unadjusted and the second model was adjusted for Rotterdam Study cohort, age, and sex. In a third model we investigated if additional adjustment for serum 25(OH)D concentration, corticosteroids use, and ERT use significantly changed the results.

Multiple imputation was performed to impute missing values in the covariables, using the 'Multivariate Imputation by Chained Equations' package in R [40]. The number of imputed datasets was based on the average percentage of missing values per variable [41]. The average percentage of missing values in the total population was 3.2%. Rounding to a value which is a multiplication of 5 generated a total number of 5 imputed datasets. The number of iterations was increased with 5 at a time until convergence was achieved. With the exception of the characteristics of the study population, results are reported for imputed data.

A two-sided p-value below 0.05 was considered as statistically significant. Data were analyzed using R version 3.4.4 (R Foundation for Statistical Computing, Vienna, Austria) and SPSS Statistics for Windows, Version 24.0 (IBM, Armonk, NY, USA).

3. Results

3.1. Population characteristics

The flowchart of the study population is shown in Fig. 1. A total number of 6640 participants were eligible for the current study as there was both a LS-BMD and a LS-TBS measurement available. Of the total study population, 41 participants were excluded because they did not provide written informed consent, resulting in an eligible study population of 6599 participants. Subsequently, 503 participants were excluded because of ever use of bisphosphonates and/or current use of

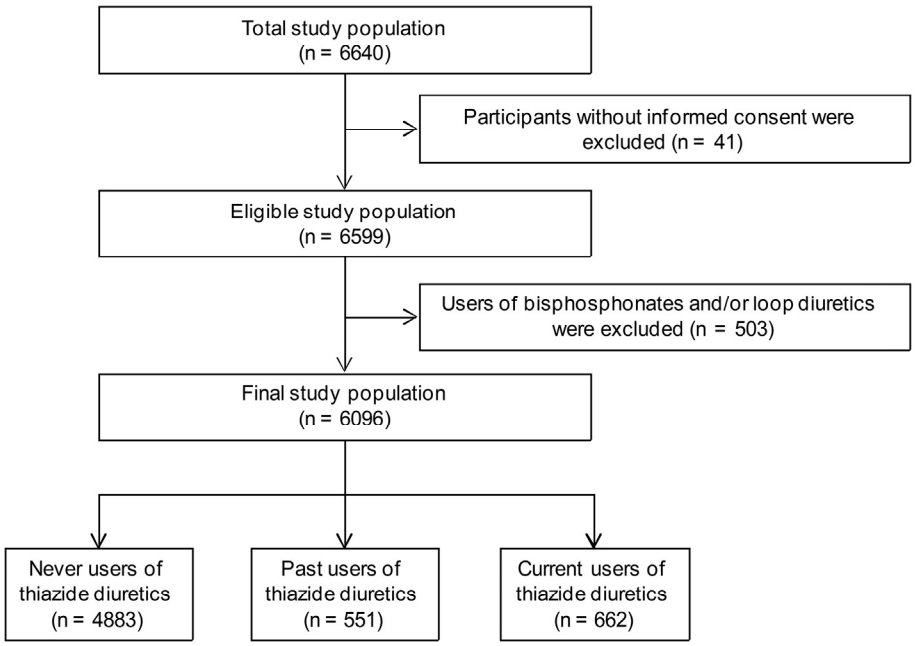

Fig. 1. Flowchart of the study population. Of the total population of 6640 eligible participants, 41 were excluded because of no informed consent, resulting in an eligible study population of 6599 participants. Subsequently, 503 participants were excluded because of ever use of bisphosphonates and/or current use of loop diuretics. This resulted in a final study population of 6096 participants. This population could be further divided into 4883 never users, 551 past users and 662 current users of thiazide diuretics.

loop diuretics, resulting in a final study population of 6096. This population could be further divided into 4883 never users, 551 past users, and 662 current users of thiazide diuretics.

Characteristics of the final study population as well as the characteristics of never, past, and current users of thiazide diuretics, before imputation, are shown in Table 1. Mean age of the total study population at the time of the performed DXA scan was 65.5 years and 56.2% were female. A total of 662 participants (10.9%) were current users of thiazide diuretics.

3.2. Association between thiazide diuretics use and LS-BMD

Table 2 shows the association between the use, dosage, and duration of use of thiazide diuretics and LS-BMD. The use of thiazide diuretics was found to be positively associated with LS-BMD in the fully adjusted model (model 3). Past use of thiazide diuretics was associated with a 0.021 g/cm^2 (95% CI: 0.006;0.036) higher LS-BMD value, while current use of thiazide diuretics was associated with a 0.016 g/cm^2 (95% CI: 0.002;0.031) higher LS-BMD value, both compared to never use of thiazide diuretics.

When taking dosage into account, the use of <1 DDD of thiazide diuretics was associated with a decrease in LS-BMD of 0.0002 g/cm^2 (95% CI: -0.021;0.021) compared to never users in the fully adjusted model (model 3), although this difference was not statistically significant. Conversely, the use of ≥ 1 DDD of thiazide diuretics was positively and significantly associated with LS-BMD ($\beta = 0.028$, 95% CI: 0.010;0.046, model 3). A significant trend within the DDD of use was found (p for trend = 0.004). Analysis of the duration of thiazide diuretics use showed a positive association between thiazide diuretics use and LS-BMD when using the medication for >365 days ($\beta = 0.033$, 95% CI: 0.014;0.052). In addition, a significant trend within the duration of use was found (p for trend <0.001).

Additionally adjusting the analyses for season of serum 25(OH)D measurement, corticosteroids use, ERT use, physical activity, stroke, and CHD did not change the results (data not shown).

3.3. Association between thiazide diuretics use and LS-TBS

Results of the linear regression analyses of the use, dosage, and duration of use of thiazide diuretics and LS-TBS are shown in Table 3. No statistically significant result was found, neither in the model adjusted for Rotterdam Study cohort, age, and sex only, nor in the fully adjusted models. Additionally adjusting for season of serum 25(OH)D measurement, corticosteroids use, ERT use, physical activity, stroke, and CHD did not change the results (data not shown).

3.4. Interaction terms

As a significant association between the use of thiazide diuretics and LS-BMD was found, interaction terms of age, sex and BMI with thiazide diuretics were tested. None of these interaction analyses showed a statistically significant interaction with thiazide diuretics (data not shown).

3.5. Serum osteocalcin levels in users and non-users of thiazide diuretics

Of the 8707 eligible participants, 64 were excluded because they did not provide written informed consent and another 325 of current bisphosphonate and/or loop diuretics use, resulting in a total population of 8318 participants. The mean level of serum osteocalcin was 20.2 ng/ml (SD 8.3) in users of thiazide diuretics and 22.5 ng/ml (SD 17.0) in non-users of thiazide diuretics ($p < 0.001$). Linear regression analyses showed that the use of thiazide diuretics was associated with a 2.3 ng/ml (95% CI: -3.5;-1.1) lower serum osteocalcin level in the unadjusted model and with a 3.2 ng/ml (95% CI: -4.4.;-2.0) lower serum osteocalcin level when adjusted for Rotterdam Study cohort, age, and sex, both compared to non-use of thiazide diuretics (Table 4, Fig. 2). Additionally adjusting for serum 25(OH)D concentration, corticosteroids use, and ERT use, and removing the outliers did not significantly change the results (data not shown).

4. Discussion

In this cross-sectional analysis of 6096 participants from the Rotterdam Study, past and current use of thiazide diuretics were significantly associated with an increase in LS-BMD. Use of ≥ 1 DDD and use of thiazide diuretics for >365 days were positively and significantly associated with LS-BMD. In addition, a significant trend within the DDD and duration of use was found as well. On the other hand, no significant association between the use, the DDD, and the duration of use of thiazide diuretics and LS-TBS was found. Furthermore, analyses regarding the association between thiazide

Table 1
Characteristics of the study population.

	Total population (n = 6,096)	Never users of thiazide diuretics (n = 4,883)	Past users of thiazide diuretics (n = 551[a])	Current users of thiazide diuretics (n = 662[a])
General characteristics				
Female sex, n(%) (n= 6,096)	3,423 (56.2)	2,639 (54.0)	353 (64.1)	431 (65.1)
Age at DXA scan, years (n = 6,096)	65.5 ± 10.3	64.6 ± 10.2	69.9 ± 10.0	68.5 ± 10.0
Alcohol use, n(valid %) (n = 5,276)				
No alcohol use	572 (10.8)	419 (10.0)	87 (17.5)	66 (11.5)
Light drinking	774 (14.7)	621 (14.8)	66 (13.3)	87 (15.2)
Moderate drinking	2,687 (50.9)	2,137 (50.8)	246 (49.5)	304 (53.0)
Heavy drinking	1,243 (23.6)	1,028 (24.4)	98 (19.7)	117 (20.4)
Smoking, n(valid %) (n = 6,021)				
Never smoker	1,806 (30.0)	1,400 (29.0)	179 (33.0)	227 (34.8)
Former, non-smoker	3,036 (50.4)	2,204 (49.8)	297 (54.8)	335 (51.4)
Current smoker	1,179 (19.6)	1,023 (21.2)	66 (12.2)	90 (13.8)
Physical activity, hours/week (n = 5,215)	78.0 ± 52.6	77.1 ± 53.3	84.6 ± 49.0	79.5 ± 50.0
Comorbidities and medication use				
Diabetes mellitus, n(valid %) (n = 5,632)	544 (9.7)	386 (8.5)	64 (12.9)	94 (15.4)
Stroke, n(valid %) (n = 5,788)	68 (1.2)	45 (1.0)	15 (2.9)	8 (1.3)
CHD, n(valid %) (n = 5,702)	232 (4.1)	174 (3.8)	28 (5.5)	30 (4.8)
Oral corticosteroid use, n(%) (n = 6,096)	153 (2.5)	119 (2.4)	20 (3.6)	14 (2.1)
Estrogen replacement therapy use, n(%) (n = 6,096)	175 (2.9)	138 (2.8)	20 (3.6)	17 (2.6)
Measurements				
BMI, kg/m^2 (n = 6,036)	27.1 ± 3.6	26.8 ± 3.5	28.2 ± 3.7	28.6 ± 3.7
LS-BMD, g/cm^2 (n = 6,096)	1.144 ± 0.205	1.140 ± 0.205	1.156 ± 0.204	1.162 ± 0.205
LS-TBS (n = 6,096)	1.319 ± 0.103	1.324 ± 0.103	1.299 ± 0.103	1.305 ± 0.101
Systolic blood pressure, mmHg (n = 6,075)	142 ± 22	140 ± 21	153 ± 24	152 ± 23
Diastolic blood pressure, mmHg (n = 6,075)	81 ± 11	81 ± 11	83 ± 12	84 ± 12
Blood measurements				
Season of blood collection, summer and spring, n(valid %) (n = 5,874)	2,733 (46.5)	2,197 (46.6)	256 (48.8)	280 (44.4)
Serum 25(OH)D, ng/ml (n = 5,086)	58.97 ± 27.42	60.15± 27.56	54.78 ± 26.87	53.41 ± 25.78
Serum calcium, mg/dl (n = 5,772)	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	2.5 ± 0.1
Serum sodium, mmol/L (n = 5,669)	142 ± 2	142 ± 2	142 ± 3	142 ± 3
Serum potassium, mmol/L (n = 5,666)	4.4 ± 0.3	4.4 ± 0.3	4.3 ± 0.4	4.2 ± 0.4
Serum magnesium, mmol/L (n = 5,657)	0.85 ± 0.06	0.85 ± 0.06	0.84 ± 0.06	0.83 ± 0.06
Serum phosphate, mmol/L (n = 5,655)	1.11 ± 0.16	1.11 ± 0.16	1.12 ± 0.17	1.11 ± 0.16

Data are presented as number (%), number (valid %), or mean ± standard deviation. Values are shown for non-imputed data. For variables with missing data, valid % is given.
[a] Percentage of past users = 9.0%, percentage of current users = 10.9%
Abbreviations: DXA = dual-energy X-ray absorptiometry; CHD = coronary heart disease; BMI = body mass index; LS-BMD = lumbar spine bone mineral density; LS-TBS = lumbar spine trabecular bone score.

Table 2
Univariable and multivariable linear regression analyses of the use of thiazide diuretics, dosage, and duration of thiazide diuretics use, and LS-BMD (in g/cm^2) (n = 6,096).

	Model 1	Model 2	Model 3
	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)
Thiazide diuretics use			
Never users (n = 4,883)	Reference	Reference	Reference
Past users (n = 551)	0.044 (0.026;0.061)[a]	0.025 (0.008;0.042)[a]	0.021 (0.006;0.036)[a]
Current users (n = 662)	0.047 (0.031;0.062)[a]	0.020 (0.004;0.036)[a]	0.016 (0.002;0.031)[a]
Dosage of thiazide diuretics			
Never users (n = 4,883)	*Reference*	*Reference*	*Reference*
Current users (n = 662)			
< 1 DDD (n = 274)	0.029 (0.005;0.052) [a]	0.004 (-0.019;0.028)	-0.0002 (-0.021;0.021)
≥ 1 DDD (n = 388)	0.059 (0.039;0.079)[a]	0.031 (0.011;0.051)[a]	0.028 (0.010;0.046)[a]
P for trend	<0.001[a]	0.003[a]	0.004[a]
Duration of thiazide diuretics use			
Never users (n = 4,883)	*Reference*	*Reference*	*Reference*
Current users (n = 662)			
1-120 days (n = 256)	0.014 (-0.010;0.039)	-0.012 (-0.037;0.012)	-0.008 (-0.030;0.014)
120-365 days (n = 53)	0.059 (0.007;0.111)[a]	0.037 (-0.013;0.088)	0.025 (-0.021;0.070)
>365 days (n = 353)	0.069 (0.047;0.091)[a]	0.042 (0.020;0.063)[a]	0.033 (0.014;0.052)[a]
P for trend	<0.001[a]	<0.001[a]	<0.001[a]

Model 1: adjusted for Rotterdam Study cohort, age and sex; model 2: additionally adjusted for BMI, serum 25(OH)D concentration, serum calcium, serum sodium, serum potassium, serum magnesium, serum phosphate, alcohol intake, smoking, systolic blood pressure, diastolic blood pressure and, diabetes mellitus; model 3: additionally adjusted for LS-TBS. [a] $p < 0.05$.
Abbreviations: LS-BMD = lumbar spine bone mineral density; CI = confidence interval; DDD = defined daily dose; BMI = body mass index; LS-TBS = lumbar spine trabecular bone score.

Table 3
Univariable and multivariable linear regression analyses of the use of thiazide diuretics, dosage, and duration of thiazide diuretics use, and LS-TBS (n = 6,096).

	Model 1	Model 2	Model 3
	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)
Thiazide diuretics use			
Never users (n = 4,883)	*Reference*	*Reference*	*Reference*
Past users (n = 551)	0.005 (-0.003;0.013)	0.005 (-0.003;0.013)	-0.001 (-0.008;0.006)
Current users (n = 662)	0.006 (-0.002;0.013)	0.004 (-0.003;0.012)	-0.0002 (-0.007;0.007)
Dosage of thiazide diuretics			
Never users (n = 4,883)	*Reference*	*Reference*	*Reference*
Current users (n = 662)			
< 1 DDD (n = 274)	0.006 (-0.005;0.013)	0.005 (-0.006;0.016)	0.004 (-0.006;0.014)
≥ 1 DDD (n = 388)	0.005 (-0.004;0.017)	0.004 (-0.006;0.013)	-0.003 (-0.011;0.005)
P for trend	0.17	0.33	0.66
Duration of thiazide diuretics use			
Never users (n = 4,883)	*Reference*	*Reference*	*Reference*
Current users (n = 662)			
1-120 days (n = 256)	-0.003 (-0.015;0.008)	-0.005 (-0.017;0.007)	-0.002 (-0.013;0.009)
120-365 days (n = 53)	0.014 (-0.010;0.038)	0.013 (-0.011;0.037)	0.005 (-0.016;0.026)
>365 days (n = 353)	0.010 (0.0005;0.020) [a]	0.009 (-0.001;0.019)	-0.00001 (-0.009;0.009)
P for trend	0.04[a]	0.08	0.95

Model 1: adjusted for Rotterdam Study cohort, age and sex; model 2: additionally adjusted for BMI, serum 25(OH)D concentration, serum calcium, serum sodium, serum potassium, serum magnesium, serum phosphate, alcohol intake, smoking, systolic blood pressure, diastolic blood pressure and, diabetes mellitus; model 3: additionally adjusted for LS-BMD. [a] $p < 0.05$.
Abbreviations: LS-TBS = lumbar spine trabecular bone score; CI = confidence interval; DDD = defined daily dose; BMI = body mass index; LS-BMD = lumbar spine bone mineral density.

Table 4
Linear regression analyses of the use of thiazide diuretics and serum osteocalcin levels (in ng/ml) (n = 8318).

	Model 1	Model 2
	Beta (95% CI)	Beta (95% CI)
Use of thiazide diuretics	−2.3 (−3.5;−1.1)	−3.2 (−4.4.;−2.0)

Model 1: unadjusted; model 2: adjusted for Rotterdam Study cohort, age, and sex.

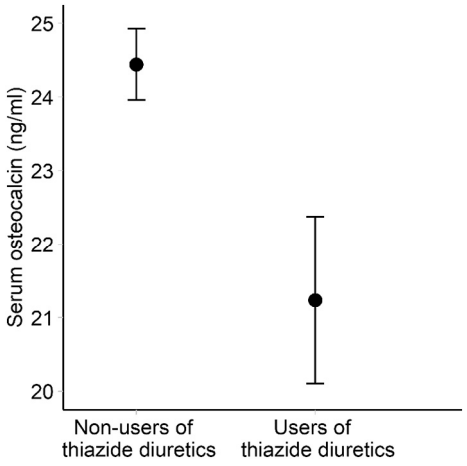

Fig. 2. Serum osteocalcin levels in ng/ml in users and non-users of thiazide diuretics, adjusted for Rotterdam Study cohort, age, and sex (estimate with standard error).

diuretics use and serum osteocalcin levels showed that the use of thiazide diuretics was significantly associated with lower serum osteocalcin levels.

The findings of our study regarding the association between current use of thiazide diuretics and LS-BMD are consistent with several previous studies which have shown a positive association between the use of thiazide diuretics and LS-BMD as well [1,5–7,43]. However, we also found an unexpected positive association between past use of thiazide diuretics and LS-BMD. We hypothesized that the effect of thiazide diuretics on LS-BMD would disappear after four months of discontinuation of use and our definition of current use of thiazide diuretics derived from this hypothesis. Our hypothesis was based on an important finding of a previous study of our research group, namely that the protective effect of thiazide diuretics on the incidence of hip fractures disappeared after four months of discontinuation of use [9]. As known, the risk of hip fracture is predominantly related to femoral neck BMD and not to LS-BMD. Nevertheless, we expected similar effects of thiazide diuretics on the BMD of different skeletal sites. Our finding could imply that the effect of thiazide diuretics on BMD lasts longer than their effect on the risk of osteoporotic fractures, but it is also possible that the effect of thiazide diuretics on BMD differs per skeletal site. Another explanation for this unexpected association could be the presence of residual confounding. In addition, as we found a significant association between thiazide diuretics use and LS-BMD, interaction terms of age, sex, and BMI with thiazide diuretics were tested. None of the tested interaction terms were statistically significant. This implies that the effect of thiazide diuretics on LS-BMD does not differ by age, sex, and BMI.

Furthermore, we found that the use of ≥1 DDD was positively and significantly associated with LS-BMD, while the use of <1 DDD was not. Similarly, when studying the effect of the duration of thiazide diuretics use on LS-BMD, a positive effect of thiazide diuretics on LS-BMD is only seen when using the medication for >365 days. Furthermore, we showed a significant trend within the DDD and duration of use as well, suggesting that the positive effect of thiazide diuretics on LS-BMD increases with dosage and time of thiazide diuretics use. Thiazide diuretics stimulate osteoblast differentiation, bone formation, and calcium uptake by the bones [19]. Higher dose and longer duration of thiazide diuretics use could stimulate those three processes to a greater extent, causing a larger increase in the LS-BMD value. To the best of our knowledge, we are the first showing this positive trend and further studies are needed to confirm this finding before implications for clinical practice can be made.

In order to study determinants of bone strength other than BMD, we investigated the association between thiazide diuretics use and bone microarchitecture, estimated by LS-TBS and measured using DXA. No statistically significant association between the use of thiazide diuretics

and LS-TBS was seen, neither overall nor when categorized according to DDD or duration of thiazide diuretics use. This suggests that the use of thiazide diuretics is not associated with changes in LS-TBS. However, another explanation for our findings could be that the measurement of LS-TBS by using DXA is not able to detect the small changes in bone microarchitecture caused by thiazide diuretics use and that more sophisticated techniques for measuring bone microarchitecture are needed. High-resolution peripheral quantitative computed tomography (HR-pQCT) is one of those techniques which has the potential to measure different aspects of bone quality, including bone microarchitecture.

Bone is a dynamic tissue that is continuously remodeled in order to preserve its strength and integrity [44–46]. In the normal bone remodeling process, bone resorption is coupled to bone formation, ensuring that the resorbed bone is completely replaced by new bone [47]. Currently, several biochemical markers are available for the assessment of bone turnover [16]. In osteoporosis, the bone turnover rate is increased [16]. However, bone resorption and bone formation will be partly uncoupled, causing bone resorption to exceed bone formation [48]. Bone resorption will thus still be followed by bone formation in osteoporosis patients, only to a lesser extent, which results in an increase in both bone resorption and bone formation markers in osteoporosis. One of the important bone formation markers is osteocalcin [16] and the serum levels of this marker will be increased in osteoporosis patients. In the current study, we investigated the association between the use of thiazide diuretics and serum osteocalcin levels. Previous literature investigating the effect of thiazide diuretics use on serum osteocalcin levels has shown conflicting results. In 1993, it was reported that users of thiazide diuretics had lower levels of serum osteocalcin compared to non-users of thiazide diuretics [49]. In addition, a randomized controlled trial investigating the effect of hydrochlorothiazide on rates of bone loss in 320 men and women, showed a decrease in serum osteocalcin levels in subjects treated with thiazide diuretics [6]. In contrast, no significant change in serum osteocalcin levels was seen in a study of 50 postmenopausal women treated for 7 days with bendroflumethiazide compared to postmenopausal women treated with placebo [50]. Two years later, a study of the same research group was published, showing a dose-dependent increase in serum osteocalcin levels in users of bendroflumethiazide [51]. In our study, we found that users of thiazide diuretics had a significantly lower mean serum osteocalcin level compared to non-users of thiazide diuretics. Furthermore, linear regression analysis showed that the use of thiazide diuretics was significantly associated with lower serum osteocalcin levels after adjustment for Rotterdam Study cohort, age, and sex. As serum osteocalcin is a marker of bone formation, the lower serum osteocalcin levels found in users of thiazide diuretics imply a decrease in bone formation, which is probably following a decrease in bone resorption, indicating a decrease in bone turnover in general. However, important to note is that even though we found a significant difference in the serum osteocalcin levels between users and non-users of thiazide diuretics, the serum osteocalcin levels were still within the reference range for both groups. Nevertheless, we consider the statistically significant difference to be clinically relevant as well. The lower levels of serum osteocalcin in the users of thiazide diuretics provide evidence for a decrease in bone turnover in this group, providing a possible explanation for the increased BMD in the users of thiazide diuretics. In addition, the results could be relevant for clinical practice when choosing a treatment for patients with hypertension who also have osteoporosis. Based on the results of the current study, thiazide diuretics could be a good treatment option for hypertension in this situation, as it could also have a beneficial effect on the osteoporosis. Even though the serum levels of osteocalcin are in the reference range in both the users and non-users of thiazide diuretics, the difference between the two groups still provides evidence for a positive effect of thiazide diuretics on bone.

In this study, there was an unexpected finding, namely a significantly lower serum 25(OH)D concentration in users of thiazide diuretics compared to non-users. A possible explanation for this result may be found in the indication for prescribing thiazide diuretics. Thiazide diuretics are one of the cornerstones in the treatment of hypertension but are also used in the treatment of conditions related to volume-overload such as chronic kidney disease and heart failure [52]. Both chronic kidney disease and heart failure can lead to impaired mobility [53,54], which can cause a possible decrease in the serum 25(OH)D concentration due to a shortage of sunlight exposure in these patients.

Our study has a number of strengths and limitations. The main strength of our study is that we are the first to investigate the effect of thiazide diuretics, including dosage and duration of use, on multiple aspects of bone strength, namely BMD as well as TBS and bone turnover. Another strength is that we used a large prospective population-based cohort study with a high participation rate to investigate our research question, which limits the chance of selection bias and creates a high level of generalizability of our results to the general population. The Rotterdam Study is conducted in the elderly population, which is an important population of interest when performing studies of bone strength and osteoporosis. The main limitation of our study is the fact that our study is cross-sectional and therefore, we could not study changes in LS-BMD and LS-TBS over time. As a consequence, we were not able to establish a causal relationship between thiazide diuretics use and markers of bone strength. Second, potential misclassification of the exposure could have occurred in our study. Users of thiazide diuretics were identified using filling data at the pharmacies, which does not disclose whether the patient took the medication as prescribed and does not give information about treatment adherence. However, this potential misclassification of the exposure would probably be non-differential, as the probability of the exposure being misclassified is independent of the LS-BMD and LS-TBS values of the participants. Third, confounding by indication could be a possibility in our study, as thiazide diuretics are prescribed for hypertension and hypertension is associated with lower LS-BMD. However, we tried to address this confounding by indication by adjusting for both systolic and diastolic blood pressure. Fourth, the Rotterdam Study consists of a predominantly Caucasian population aged above 45 years of age, which could limit the generalizability of our results to other populations. Finally, measurements of serum PTH are not available in the Rotterdam Study. PTH plays an important role within skeletal homeostasis and previous research has shown that the use of thiazide diuretics was associated with lower PTH levels [23], which suggests that PTH may be important in explaining the effect of thiazide diuretics on bone.

5. Conclusion

The results from our study suggest that thiazide diuretics exert positive effects on LS-BMD but not on LS-TBS in the general population. This could imply that the reduced risk of osteoporotic fractures in thiazide diuretics users is explained by an increased BMD without improving bone microarchitecture. However, it is also possible that the measurement of LS-TBS is not able to detect small changes in bone microarchitecture and that more sophisticated techniques such as high-resolution peripheral quantitative computed tomography (HR-pQCT) are needed to investigate the association between thiazide diuretics use and bone microarchitecture. Furthermore, our study indicates that only a high dose and longer duration of thiazide diuretics use exert positive effects on LS-BMD. These results could be relevant for clinical practice when treating elderly individuals with both hypertension and osteoporosis.

CRediT authorship contribution statement

Anna C. van der Burgh: Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review &

editing.**Sadaf Oliai Araghi:**Conceptualization, Writing - review & editing.**M. Carola Zillikens:**Writing - review & editing.**Fjorda Koromani:**Writing - review & editing.**Fernando Rivadeneira:**Writing - review & editing.**Nathalie van der Velde:**Writing - review & editing.**Ewout J. Hoorn:**Writing - review & editing.**André G. Uitterlinden:**Writing - review & editing.**M. Arfan Ikram:**Writing - review & editing.**Bruno H. Stricker:**Conceptualization, Methodology, Writing - review & editing, Supervision.

Declaration of competing interest

All authors state that they have no conflicts of interest.

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