Bone Reports 13 (2020) 100737

Contents lists available at ScienceDirect

Bone Reports

journal homepage: www.elsevier.com/locate/bonr

# Effects of bendroflumethiazide on bone mineral density; results from the BONATHIAD randomized double-blind placebo-controlled cohort study

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ARTICLE INFO

*Keywords:*
Osteoporosis
Fractures
Bendroflumethiazide
RCT
QCT
VFA

ABSTRACT

*Purpose:* Thiazide diuretics (TD) may play a role in preventing osteoporosis. The objective was to investigate the effects of bendroflumethiazide in combination with bisphosphonates on bone mineral density, selected blood parameters, blood pressure, pulse, and muscle function.

*Methods:* Double-blinded, randomized, placebo-controlled interventional study in postmenopausal osteoporotic women over the age of 50 years consisting of four arms: 1) 24 weeks with bendroflumethiazide +24 weeks of washout, 2) 24 weeks with placebo +24 weeks of washout, 3) 48 weeks with bendroflumethiazide, or 4) 48 weeks with placebo. At inclusion, participants were on oral bisphosphonates. Intervention consisted of either bendroflumethiazide or placebo. Dual energy X-ray absorptiometry (DXA), vertebral fracture assessment (VFA), quantitative CT (QCT) and selected blood parameters were acquired at baseline and at 48 weeks and Timed-Up-and-Go, handgrip strength, blood pressure, pulse and balance additionally at 24 weeks.

*Results:* 139 postmenopausal Caucasian women over 50 years were randomized (mean age 64.7 years (SEM 0.6, range 51–79)). 109 (78%) completed the study. No difference in the effect of bendroflumethiazide on DXA, VFA, QCT, biochemistry or muscle function were found between the treatment arms.

*Conclusion:* Bendroflumethiazide for 24- or 48 weeks in combination with bisphosphonates does not improve bone mineral density, selected blood parameters or muscle function compared to placebo combined with bisphosphonates. Studies with longer treatment periods and more patients are needed to further characterize the effects of bendroflumethiazide on bone and subpopulations that might benefit from the treatment.

## 1. Introduction

Thiazide diuretics (TD) are a class of medications commonly used as first line treatment for uncomplicated hypertension (Wright et al., 2018). TD can influence bone mineral formation, osteoblast differentiation, and increase calcium retention in the kidneys(Alexander and Dimke, 2017; Dvorak et al., 2007). Consequently, it has been suggested that TD might play a role in preventing osteoporosis (Cheng et al., 2018), though the exact mechanism is not fully elucidated. Studies have reported higher bone mass in participants taking thiazides (Transbøl et al., 1982; Wasnich et al., 1983, 1995). TD have been shown to decrease the risk of hip fractures with 16–30% (Bokrantz et al., 2020; Felson et al., 1991; Puttnam et al., 2017; Rejnmark et al., 2005). Calcium retention induced by TD might lead to muscle function disturbances, such as sarcopenia, which is known to increase the risk of falls and fractures (Wong et al., 2019). Bisphosphonates are a commonly used antiresorptive treatment for osteoporosis. Co-occurrence of osteoporosis and hypertension is common. Hence, understanding the effects of thiazide diuretics on bone density, fracture risk, blood profile and possible interaction with bisphosphonates becomes increasingly desirable. The objective of the present study was to investigate the effects of the thiazide diuretic bendroflumethiazide on bone mineral density (BMD), measured using DXA and QCT in a population of postmenopausal women already using bisphosphonates. This allows for the comparison of the two bone measurement modalities. Additionally, the present study investigated the blood profile, and muscle function tests which includes Timed-Up-and-Go (TUG), handgrip strength and balance and orthostatic blood pressure difference.

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https://doi.org/10.1016/j.bonr.2020.100737
Received 16 October 2020; Accepted 20 November 2020
Available online 25 November 2020

## 2. Materials and methods

### 2.1. Study design and population

The **Bon**e **A**ssociation with **Thi**azide **D**iuretics (**BONATHIAD**) study was an investigator initiated double-blinded, randomized, placebo-controlled interventional study consisting of four arms. Participants were randomly assigned to receive either 1) 24 weeks with active +24 weeks of washout (24 wks active), 2) 24 weeks with placebo +24 weeks of washout (24 wks placebo), 3) 48 weeks with active (48 wks active), or 4) 48 weeks with placebo (48 wks placebo). The affiliation to either washout (group 1 and 2) or continuation of medication (group 3 and 4) was masked until 24 wks. Active consisted of a tablet containing 2.5 mg bendroflumethiazide and 573 mg potassium chloride in the form of Centyl® with potassium chloride (Leo Pharma A/S, Ballerup, Denmark). Placebo was a biologically inactive tablet containing starch and talc (JemoPharm A/S, Stege, Denmark). The active and placebo tablets were visually indistinguishable and administered orally. Participants were recruited from the osteoporosis clinic at Aalborg University Hospital in Denmark from July 2016 to March 2018. The study population included postmenopausal women over the age of 50 years with osteoporosis diagnosed using traditional dual energy x-ray absorptiometry (DXA) scans. All participants were treated with oral alendronate 70 mg once weekly prior to inclusion and throughout the study in accordance with Danish guidelines for osteoporosis treatment. The length of bisphosphonates treatment prior to inclusion for completed patients in the 24 wks active group was mean 2.0 years (SEM = 0.35, n = 22); the placebo 24 wks group mean was 2.0 years (SEM = 0.34, n = 17); the active 48 weeks group mean was 2.1 years (SEM = 0.40, n = 30), and the placebo 48 wks group mean was 2.2 years (SEM = 0.32, n = 24). The total group mean treatment time for patients who completed was 2.1 years (SEM = 0.18, n = 93). Unfortunately, data was not available for 16 patients. Exclusion criteria included past hip- or lumbar fractures, prior diagnosis of osteoporosis, or prior use of thiazide-, or anabolic bone treatments. A full list of inclusion and exclusion criteria can be seen in the supplemental appendix. All laboratory analyses were performed in an ISO 15189 and DANAK accredited laboratory.

### 2.2. Randomization process

Simple randomization was performed at The Hospital Pharmacy of the North Denmark Region using https://www.randomizer.org. Subjects were assigned a randomization code and allocated to one of the study groups. The study medicine was prepared by The Hospital Pharmacy and dispensed in identical packages to ensure blinding. The packages had numbered labels, with each subject receiving the medication sequentially in ascending order. We assessed compliance with the study treatment by counting the number of non-administered tablets upon return from the participants.

Randomization codes and the corresponding contents were known only to the Pharmacy Department. Randomization codes and participants were unblinded to the investigators after the completion of the study.

### 2.3. Study visits

Dual energy x-ray absorptiometry (DXA), vertebral fracture assessment (VFA), quantitative computed tomography (QCT), Timed-Up-and-Go (TUG), handgrip strength, blood pressure, pulse, and balance were acquired at baseline and at 48 wks. TUG, handgrip strength, blood pressure, pulse and balance were furthermore acquired at 24 wks. The study setup is displayed in Fig. S1.

### 2.4. BMD measurement

Bone mineral density (BMD) was determined using a Hologic Horizon A DXA-scanner (Software Version 13.6.0.5:3; S/N 200084; Hologic, MA, USA). Further analysis was conducted in APEX System Software Version 5.5.3.1 (Neptune Systems, Morgan Hill, CA, USA). DXA-scans were performed by experienced laboratory technicians at the Endocrine laboratory at Aalborg University Hospital. Quality verification for 2017 at our facility resulted in the following estimates for variation for routine scan procedures: Least significance change (LSC), coefficient of variation (CV)% = 1.091% LSC = 3.02%; T-hip: CV% = 1.15%, LSC = 3.18%; FN: CV% = 1.77% LSC = 4.91%. BMD was measured using manufacturer-supplied standard methods over the lumbar spine (LS) L1-L4, the total hip (TH), and the femoral neck (FN), and results were reported as T-scores. The standard Hologic reference databases for Caucasian Danish women were used. BMD equipment underwent daily quality control and regular maintenance.

### 2.5. Vertebral fracture assessment

Immediately after BMD measurements by DXA, VFA was performed in the same session using the same equipment and analyzed using Genant's semiquantitative method (Genant et al., 1993). Assessments were performed independently by PV, who was blinded to BMD, biochemical data, and randomization groups. Fractures were considered significant if more than a 20% height reduction was present. Fractures were graded as follows: Grade I: 20% < height reduction ≤25%; Grade II: 25% < height reduction ≤40%; Grade III: height reduction >40%.

### 2.6. Quantitative computed tomography (QCT)

QCT images were acquired using the GE-Discovery 750 HD, 64 slice CT-scanner, 2012, software v. 40 (GE Healthcare, IL, USA.) and analysis were conducted in QCT PRO™ software v. 5.1.3 (Mindways Software, Austin, TX, USA). Image acquisition and analysis was conducted according to the manufacturer's recommendation. BMD was measured using manufacture supplied standard methods over the lumbar spine L1-L5 and the left femoral neck. Scanners were serviced 4 times a year, and internal consistency checkups were performed monthly.

### 2.7. Timed-Up-and-Go (TUG)

On a "one, two, three, go!" signal subjects were asked to start from a sitting position in an armchair (seat height approximately 43–47 cm), walk three meters, turn, walk back to the chair and sit down again. Time was recorded manually from the "go" signal and stopped when the subject was sitting in the chair again. The test was repeated two times and the fastest time selected for further analysis.

### 2.8. Handgrip strength

Isometric handgrip strength was measured using a hand dynamometer (NC70144, Procare.dk, Denmark). Each subject was instructed to perform maximal contraction force with their dominant hand (defined as their writing hand) in a seated upright position and the test hand pointing downwards, parallel with the trunk and unsupported. The test was repeated three times. The maximal strength of the three trials was used for further analysis. Values were provided as kg adjusted for BMI.

### 2.9. Blood pressure and pulse

Blood pressure and pulse were measured on the left arm both standing and sitting as a measure of orthostatic blood pressure changes.

### 2.10. Balance ability

Balance tests were performed with both legs close together on an elevated platform. In this study, the experiment was performed with open and closed eyes counted from 1 to 200 (forward) and with open

and closed eyes counting down the 7 table (backward) from a high number. Data was collected for 45 s. If participants lost balance the experiment was terminated.

### 2.11. Statistical analysis and figures

During the planning of the study, a power calculation was performed based on an expected increase in posterior–anterior BMD measured using DXA of 1% after 48 weeks. This increase was based on results by LaCroix et al. (2000). Based on the assumption of a standard error of 3%, risk of type-1-errors of 5% and type-2 errors of 10%, 70 subjects would be necessary in each of the 24- and 48 weeks subgroups. To account for dropouts or exclusions, we planned to include 88 patients in each subgroup (a total of 175 patients). Our previous study showed a decrease in fracture occurrence after the end of treatment which is why we included a 24 week washout period for two groups (Kruse et al., 2016a, 2016b).

The Shapiro-Wilk test was used as normality test. For descriptive analyses, parametric quantitative variables were expressed with means and standard error of the mean (SEM). Nonparametric quantitative variables were expressed with median and interquartile range.

To investigate the association between groups, one-factor analysis of variance (ANOVA) with post hoc Bonferroni was used. When comparing the evolution of quantitative variables at different time points ANOVA with repeated measures, mixed effects model or a paired *t*-test was used. Missing or incomplete data were not included. All statistical analysis was performed in GraphPad Prism v8.00 and Excel 365. A p-value $\leq 0.05$ was deemed significant. Figures were made in Adobe Photoshop® CC 2019 (Adobe, Park Avenue, San Jose, CA, USA).

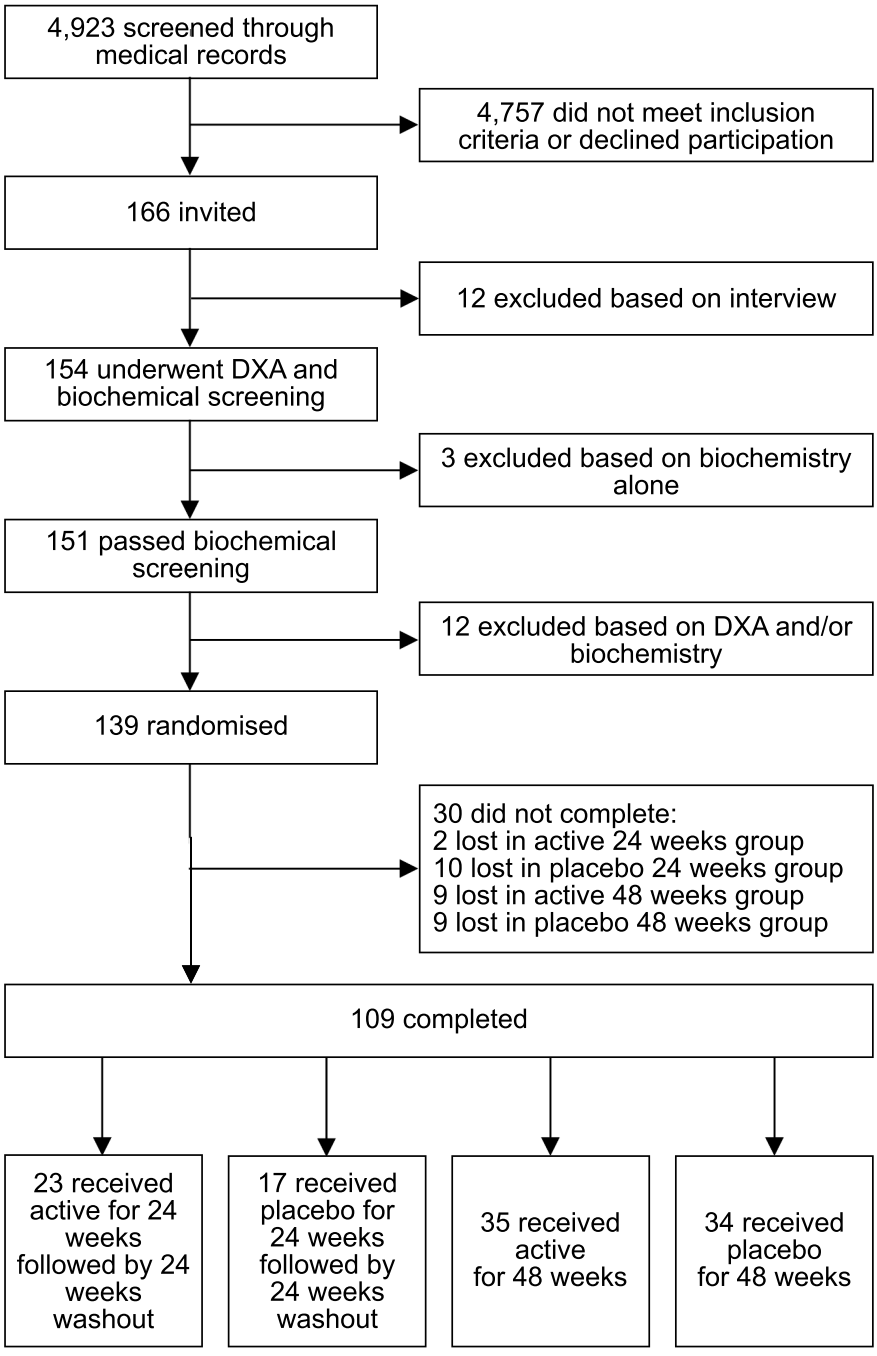


**Fig. 1.** 1 or 1.5 column, color: black/white. Flow chart of the participants.

### 2.12. Endpoints

#### 2.12.1. Primary endpoints

The primary endpoints were absolute changes in BMD as measured by DXA at the lumbar spine (LS), total hip (TH), and femoral neck (FN) between baseline and 48 wks.

#### 2.12.2. Secondary endpoints

Changes in volumetric BMD and cortical width by QCT at the LS and the FN between baseline and 48 wks.

Changes in plasma(p)-potassium, p-sodium, p-ionized calcium, p-total albumin corrected calcium, blood urea nitrogen (BUN), p-magnesium, hemoglobin A1 (HbA1c), p-creatinine, and 24-h urinary calcium between baseline and 48 wks.

Change in TUG in seconds measured between baseline, 24- and 48

**Table 1**
Demographic, clinical characteristics and measurements of the patients at baseline. Additional information can be found in the Supplementary Appendix. Participants were randomly assigned to receive either 1) 24 weeks with active +24 weeks of washout, 2) 24 weeks with placebo +24 weeks of washout, 3) 48 weeks with active, or 4) 48 weeks with placebo. Active consisted of 2.5 mg bendroflumethiazide and 573 mg potassium chloride. Placebo was visually indistinguishable and contained starch and talc. Standard error of the mean (SEM). Dual X-ray absorptiometry (DXA). Bone mineral density (BMD). Quantitative computed tomography (QCT). Lumbar spine (LS). Total hip (TH). Femoral neck (FN).

| Parameter | Active 24 weeks | Placebo 24 weeks | Active 48 weeks | Placebo 48 weeks |
|---|---|---|---|---|
| | Baseline Mean, (SEM), [range] | Baseline Mean, (SEM), [range] | Baseline Mean, (SEM), [range] | Baseline Mean, (SEM), [range] |
| Demographics and clinical characteristics | | | | |
| Age, yr | 63.7, (1.5), [53–78] | 65.0, (1,3), [57–77] | 65.1, (1.0), [51–77] | 64.9, (1.1), [51–79] |
| Age of menopause, yr | 48.9, (1.0), [39–56] | 48.7, (1.1), [39–57] | 48.0, (1.0), [33–56] | 48.0, (0.9), [38–56] |
| Body Mass Index, kg/m$^2$ | 23.3, (0.6), [19.2–28.6] | 24.5, (0.7), [19.0–28.6] | 23.5, (0.5), [19.0–30.2] | 25.8, (0.8), [19.3–38.0] |
| Comorbidities and additional diagnoses, n (%) | | | | |
| Medication for diabetes | 0 (0) | 0 (0) | 0 (0) | 1 (3) |
| Medication for hypokalemia | 1 (4) | 0 (0) | 0 (0) | 0 (0) |
| Medication for hyperkaleamia | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Medication for hypokaleamia | 1 (4) | 0 (0) | 0 (0) | 1 (3) |
| Medication for hyponatriæmia | 2 (9) | 0 (0) | 1 (3) | 1 (3) |
| Medication for hypomagnesiæmi | 0 (0) | 0 (0) | 4 (11) | 1 (3) |
| Medication for hypermagnesiæmi | 0 (0) | 0 (0) | 0 (0) | 1 (3) |
| Medication for hypercholesterolæmia | 1 (4) | 0 (0) | 6 (17) | 1 (3) |
| Medication for hypercalcemia | 0 (0) | 0 (0) | 0 (0) | 2 (6) |
| Medication for hypertension | 2 (9) | 4 (24) | 4 (11) | 4 (12) |
| Medication for pain | 1 (4) | 2 (12) | 3 (9) | 5 (12) |
| Thyroid dysfunction | 2 (9) | 0 (0) | 4 (11) | 1 (3) |
| Past fracture (not in lumbar or hip region) | 2 (9) | 2 (12) | 0 (0) | 0 (0) |
| Past or present malignant disease | 2 (4) | 0 (0) | 5 (14) | 3 (9) |
| None | 6 (26) | 3 (18) | 7 (20) | 5 (15) |
| Imaging measurements | | | | |
| DXA LS-BMD, g/cm$^2$ | 0.73, (0.015) | 0.77, (0.21) | 0.78, (0.019) | 0.76, (0.012) |
| DXA LS-T-score | −2.9, (0.1) | −2.5, (0.2) | −2.5, (0.2) | −2.7, (0.1) |
| DXA TH-BMD, g/cm$^2$ | 0.72, (0.017) | 0.72, (0.020) | 0.74, (0.010) | 0.74, (0.014) |
| DXA TH-T-score | −1.8, (0.1) | −1.9, (0.7) | −1.7, (0.08) | −1.7, (0.1) |
| DXA FN-BMD, g/cm$^2$ | 0.60, (0.015) | 0.60, (0.024) | 0.61, (0.012) | 0.61, (0.014) |
| DXA FN-T-score | −2.2, (0.1) | −2.2, (0.20) | −2.1, (0.1) | −2.1, (0.1) |
| QCT LS-BMD, mg/cm$^3$ | 92.01, (6.18) | 76.26, (3.83) | 81.37, (2.74) | 79.34, (3.79) |
| QCT LS-T-score | −2.9, (0.2) | −3.5, (0.2) | −3.3, (0.1) | −3.4, (0.2) |
| QCT FN-BMD, mg/cm$^3$ | 0.72, (0.20) | 0.70, (0.024) | 0.72, (0.014) | 0.72, (0.018) |
| QCT FN-T-score | −1.8, (0.2) | −1.9, (0.2) | −1.7, (0.1) | −1.7, (0.2) |
| Bloodtests | | | | |
| P-Potassium (K), mmol/l | 3.9, (0.07) | 4.0, (0.05) | 3.9, (0.04) | 3.9, (0.05) |
| P-Sodium (Na), mmol/l | 140.5, (0.6) | 140.2, (0.5) | 140.7, (0.3) | 140.4, (0.5) |
| P-Calcium ionized (Adjusted for pH 7.4), mmol/l | 1.2, (0.007) | 1.2, (0.01) | 1.2, (0.007) | 1.2, (0.01) |
| P-Total albumin corrected calcium, mmol/l | 2.4, (0.02) | 2.4, (0.02) | 2.4, (0.01) | 2.4, (0.02) |
| Blood urea nitrogen, mmol/l | 0.3, (0.01) | 0.3, (0.02) | 0.2, (0.008) | 0.3, (0.01) |
| P-Magnesium, mmol/l | 0.9, (0.02) | 0.8, (0.02) | 0.9, (0.01) | 0.8, (0.008) |
| Hemoglobin A1c, mmol/mol | 34.8, (0.7) | 33.7, (0.5) | 35.3, (0.4) | 35.0, (0.6) |
| P-Creatinine, μmol/l | 65.0, (2.0) | 67.0, (2.7) | 64.8, (1.7) | 67.7, (1.7) |
| Blood pressure, handgrip strength and timed up and go | | | | |
| Timed-Up-and-Go, seconds | 6.7, (0.3) | 7.0, (0.3) | 6.6, (0.23) | 7.6, (0.45) |
| Handgrip strength, kPa | 26.7, (0.9) | 25.3, (1.3) | 26.9, (1.1) | 25.3, (1.2) |
| Systolic blood pressure. Delta value between standing and sitting. mmHg | −4.6, (1.9) | −3.1, (2.0) | −1.2, (1.8) | −3.1, (2.3) |
| Diastolic blood pressure. Delta value between standing and sitting, mmHg | 4.9, (1.1) | −1.0, (5.3) | 5.5, (1.1) | 5.5, (1.2) |
| Pulse. Delta value between standing and sitting, beats/min | 7.5, (1.0) | 8.9, (1.4) | 7.9, (0.9) | 7.9, (1.1) |
| Balance | | | | |
| Eyes Open Forward, counts | 95.0, (4.1) | 85.0, (3.3) | 85.5, (3.8) | 77.9, (3.1) |
| Eyes Closed Forward, counts | 94.1, (4.5) | 84.8, (3.1) | 81.3, (3.6) | 76.4, (2.8) |
| Eyes Open Backwards, counts | 13.1, (1.2) | 13.8, (1.0) | 12.2, (1.1) | 12.6, (1.5) |
| Eyes Closed Backwards, counts | 12.7, (1.2) | 14.3, (1.1) | 13.0, (0.9) | 11.8, (1.4) |

wks.

Absolute change in the difference between handgrip strength in kPa between baseline, 24- and 48 weeks.

Change in the difference between standing and sitting systolic- and diastolic blood pressure in mmHg and pulse in beats/min between baseline, 24- and 48 weeks.

Change in the difference between eyes open forward (EOF), eyes closed forward ECF, eyes open backwards (EOB), eyes closed backwards (ECB) measured in counts between baseline, 24- and 48 weeks.

Safety endpoints are listed in the supplemental appendix.

## 3. Results

### 3.1. Demographics of the study subjects

The study flow chart is shown in Fig. 1. A total of 139 post-menopausal Caucasian women over 50 years were randomized to participate in this clinical trial. 109 (78%) completed the study. In total, 23 participants were assigned to 24 wks active, 17 to 24 wk. placebo, 35 to 48 wk. active, and 34 to 48 wk. placebo. Adherence to study medication among participants in the 24 wks active group was median 96% (IQR = 42%–100%), the 24 wks placebo group median was 99% (IQR = 95%–100%), the 48 wks active group median was 96% (IQR = 51%–99%), and the 48 wks placebo group median was 97% (IQR = 91%–99%).

Characteristics of the subjects at baseline are listed in Table 1. An additional list of baseline demographic and clinical characteristics can be seen in Supplemental Table S1. In summary, the mean age of the study population was 64.7 years (yrs) (SEM = 0.6, range = 51–79 yrs) and the average age of menopause was 48.3 yrs. (SEM = 0.5, range = 33–57 yrs). The mean height was 163.4 cm (SEM = 0.5, range = 150.5–176 cm) and the average weight was 65.0 kg (SEM = 1.0, range = 46.6–105 kg).

**Table 2**

Δ-value of results from DXA and QCT between baseline and 48 weeks. Additional information including *Z*-scores and BMC values are available in the Supplementary Appendix.

Participants were randomly assigned to receive either 1) 24 weeks with active +24 weeks of washout, 2) 24 weeks with placebo +24 weeks of washout, 3) 48 weeks with active, or 4) 48 weeks with placebo. Active consisted of 2.5 mg bendroflumethiazide and 573 mg potassium chloride. Placebo was visually indistinguishable and contained starch and talc

a (active 24 weeks compared to placebo 24 weeks, b (active 24 weeks compared to active 48 weeks), c (placebo 24 weeks compared to placebo 48 weeks), d (active 48 weeks compared to placebo 48 weeks). Bold and underlined indicates statistical significance.

Standard error of the mean (SEM). Number (n). Dual X-ray absorptiometry (DXA). Bone mineral density (BMD). Quantitative computed tomography (QCT). Lumbar spine (LS). Total hip (TH). Femoral neck (FN).

| Parameter | Active 24 weeks | | Placebo 24 weeks | | Active 48 weeks | | Placebo 48 weeks | | P-score of Δ value |
|---|---|---|---|---|---|---|---|---|---|
| | Δ Value, (SEM), percent change | P-score | Δ Value, (SEM), percent change | P-score | Δ Value, (SEM), percent change | P-score | Δ Value, (SEM), percent change | P-score | |
| DXA LS-BMD, g/cm$^2$ | 0.015, (0.0075), 2.7% | 0.0639 | 0.015, (0.0087), 2.6% | 0.1054 | 0.020, (0.0060), 2.5% | **0.0019** | 0.016, (0.0055), 1.3% | **0.0069** | a≥ 0.9999<br>b≥ 0.9999<br>c≥ 0.9999<br>d≥ 0.9999 |
| DXA LS-T-score | 0.1, (0.07) | 0.0707 | 0.1, (0.08) | 0.1326 | 0.2, (0.06) | **0.0035** | 0.2, (0.05) | **0.0051** | a≥ 0.9999<br>b≥ 0.9999<br>c≥ 0.9999<br>d≥ 0.9999 |
| DXA TH-BMD, g/cm$^2$ | 0.0081, (0.0035), 1.4% | **0.0343** | 0.012, (0.0039), 1.4% | **0.0098** | 0.0066, (0.0096), 1.4% | **0.0010** | 0.011, (0.0028), 1.3% | **0.0007** | a≥ 0.9999<br>b≥ 0.9999<br>c≥ 0.9999<br>d = 0.8579 |
| DXA TH-T-score | 0.07, (0.03) | **0.0156** | 0.09, (0.03) | **0.0063** | 0.05, (0.02) | **0.0023** | 0.1, (0.02) | **0.0005** | a≥ 0.9999<br>b≥ 0.9999<br>c≥ 0.9999<br>d = 0.5164 |
| DXA FN-BMD, g/cm$^2$ | 0.0086, (0.0043), 1.7% | 0.0639 | 0.016, (0.0034), 3.3% | **0.0002** | −0.00035, (0.0046), −0.07% | 0.9411 | 0.012, (0.0040), 1.6% | **0.0071** | a = 0.3194<br>b = 0.1747<br>c = 0.5391<br>d = 0.0273 |
| DXA FN-T-score | 0.07, (0.04) | 0.1064 | 0.1, (0.03) | **0.0003** | −0.003, (0.04) | 0.9306 | 0.1, (0.04) | **0.0074** | a≥ 0.9999<br>b = 0.8895<br>c≥ 0.9999<br>d = 0.0835 |
| QCT LS-BMD, mg/cm$^3$ | −3.64, (2.68), −4.0% | 0.1964 | −2.08, (2.46), −2.7% | 0.4129 | 3.26, (1.63), 4.1% | 0.0560 | −3.55, (2.01), −4.4% | 0.0895 | a≥ 0.9999<br>b = 0.1101<br>c≥ 0.9999<br>d = 0.0446 |
| QCT LS-T-score | −0.1, (0.1) | 0.1930 | 0.07, (0.2) | 0.7363 | 0.002, (0.2) | 0.9928 | −0.1, (0.08) | 0.0841 | a≥ 0.9999<br>b≥ 0.9999<br>c≥ 0.9999<br>d≥ 0.9999 |
| QCT FN-BMD, mg/cm$^2$ | 0.011, (0.019), 1.4% | 0.5716 | 0.0093, (0.0096), 1.4% | 0.3490 | 0.0059, (0.010), 0.8% | 0.5631 | 0.0022, (0.018), 1.4% | 0.9052 | a≥ 0.9999<br>b≥ 0.9999<br>c≥ 0.9999<br>d≥ 0.9999 |
| QCT FN-T-score | 0.09, (0.2) | 0.5792 | 0.08, (0.08) | 0.3516 | 0.05, (0.09) | 0.5652 | −0.07, (0.1) | 0.5623 | a≥ 0.9999<br>b≥ 0.9999<br>c≥ 0.9999<br>d≥ 0.9999 |

### 3.2. DXA, QCT and VFA

Baseline results from DXA and QCT is seen on Table 1. Table 2 shows the %-change values for each group. The full comparison including individual BMC can be found on Supplemental Table S2.

#### 3.2.1. DXA

A significant difference in the delta value between baseline and week 48 was observed for FN-BMD between the 48 wks active (−0.00035 g/cm$^2$, SEM = 0.0046, −0.07%) and 48 wks placebo group (0.012 g/cm$^2$, SEM = 0.0040, 1.6%), (p = 0.0273). No other significant difference in delta values between other groups or DXA indices were found.

#### 3.2.2. QCT

A significant difference in the delta values between baseline and week 48 was observed for LS-BMD between 48 wks active (mean = 3.26 mg/cm$^3$, SEM = 1.63, 4.1%), compared to 48 wks placebo (mean = −3.55 mg/cm$^3$, SEM = 2.01, −4.4%), (p = 0.0446). None of the QCT BMD measurements were statically different between baseline and week 48 and no other significant differences between the delta values for any of the groups were found.

#### 3.2.3. VFA

A total of 2 patients (24 wks placebo = 1, 48 wks placebo = 1) had vertebral fractures as assessed by VFA. Both were present at baseline, and no new fractures had occurred at the end of the study. Both had a single vertebral fracture. Both fractures were grade 1.

### 3.3. Blood tests

Baseline results from blood tests can be found in Table 1. Table 3 shows the delta values for each group. The full dataset can be seen on Supplemental Table S3. In summary, we observed no significant difference in the delta values between any of the groups.

### 3.4. TUG, handgrip strength, and blood pressure- and pulse difference

Baseline results from TUG, handgrip strength and blood pressure can be found on Table 1. The full dataset can be found on Supplemental Table S4. No significant differences were observed in delta values between any of the treatment groups.

### 3.5. Balance ability

Full balance values can be seen on Table S5. The 48 wks active group experienced a larger increase in the difference between baseline and 48 weeks in EOF (mean = 9.5 s, SEM = 3.8, p = 0.0350) and ECF (mean = 10.8, SEM = 3.5, p = 0.0399) compared to the 24 wks active group (mean = −5.1, SEM = 2.6 and mean = −4.1, SEM = 2.9, respectively). This development, however, was not significantly different from development in the placebo groups.

### 3.6. Adverse events

A total of 7 serious adverse events were reported throughout this trial. Neither fatal events nor suspected fatal events due to study treatment was seen. A list of reported serious symptoms/adverse events can be found in the Supplementary Appendix.

## 4. Discussion

This study tested whether bendroflumethiazide in combination with bisphosphonates affected BMD, fracture risk, selected blood parameters, TUG, handgrip strength, blood pressure difference, pulse, and balance ability.

For a majority of the DXA indices an anticipated increase in BMD was observed. For FN-DXA BMD a small decrease in the delta value for the 48 wks active group was observed which was significantly different compared to the delta value for the 48 wks placebo group. Previous studies have found the regular use of TD to be associated with increased BMD (Bauer et al., 1993; Morton et al., 1994; Wasnich et al., 1983). A randomized study by Bolland et al. including 122 postmenopausal women with osteoporosis found a significant increase of 0.9% in total body density after four years of treatment compared with placebo ($p \leq 0.001$). However, in the same study no significant between-groups differences in femoral neck and lumbar spine was observed (Bolland et al., 2007). Arrabal-Polo et al. found that adding a thiazide diuretic in addition to bisphosphonate treatment resulted in improved BMD after 2 years of treatment pointing to an additive effect. When comparing the bisphosphonate group with the combination groups this increase was from −1.3 to −0.6 for hip BMD T-score, −1.5 to −1 for FN-BMD T-score and −2.2 to −1.4 for LS-BMD T-score (Arrabal-Polo et al., 2013). In a 3-year randomized, double-blinded trial among 320 (205 women, 115 men) normotensive patients LaCroix et al. found a 1.04% increase in anterior posterior spine density, and 0.92% increase in hip density compared to placebo. No change in total-body BMD was observed (LaCroix et al., 2000). Given our study's relatively short treatment period of 48 weeks compared to other studies one could speculate that a longer treatment period may lead to a larger positive increase in BMD. This is further substantiated by our previous study which showed that continued use of thiazides is more important for fracture prevention than dose and lifetime accumulation, and that fracture risk is decreased with increasing length of thiazide use duration (Kruse et al., 2016a, 2016b).

As far as we know, this was the first study to the use QCT to measure BMD after bendroflumethiazide treatment. QCT images are a superior modality due to the three-dimensional character of the images, which allows for volumetric BMD measurement of the trabecular vertebral bone. This also circumvents the effects of extraosseous calcification known to artificially raise DXA spine BMD measurements (Yu et al., 2012). We found an increase in LS-BMD of 4.1% in the active 48 wks group which was significantly different from the delta value of the placebo 48 wks group. Interestingly, however, none of the QCT measurements for any of the treatment arms were significantly changed between baseline and 48 weeks, which contrasts the DXA measurements.

Concerning fracture prevention, several studies found 30–50% reductions in the hip fracture risk in patients on thiazide therapy (Felson et al., 1991; Puttnam et al., 2017; Rejnmark et al., 2005). A study conducted on 376,061 Medicare beneficiaries found that TD reduced the risk of all fractures (HR = 0.85, 95% CI = 0.76–0.97). A prospective cohort study conducted on 7891 individuals of 55 years or older found that the risk of hip fractures was significantly reduced after only 1 year of continuous thiazide use with a hazard ratio of 0.46 (95% CI, 0.21 to 0.96) (Schoofs et al., 2003). However, a systematic review of published cohort studies found no effect of thiazides on the risk of osteoporotic fracture (Wang et al., 2019). In this study, we found no difference in fracture risk between treatment and placebo groups measured using VFA, though the timeframe was short and the patient sample low in this aspect. It should be considered that both drug-induced hyper- and hypotension can lead to falls and subsequent fractures. Consequently, tight regulation of the blood pressure is important. We consequently also tested whether bendroflumethiazide had any effect on the blood pressure- and pulse difference, however no difference was observed.

Inappropriate diuretic usage can lead to an increase in fall risk due to hypovolemia and electrolyte disturbances; affecting muscle function (Wehling, 2013). TD are known to cause disturbances in electrolytes, most importantly hyponatremia and hypokalemia (Arampatzis et al., 2013; Clayton et al., 2006; Rejnmark et al., 2001), but also hypomagnesaemia (Kieboom et al., 2018). For the blood profile no differences in delta values between the groups were found.

In this study we used TUG, handgrip strength and balance tests as a

**Table 3**
Δ-value of blood tests, Timed-Up-and-Go, handgrip strength, blood pressure, pulse and balance between baseline and 48 weeks. Participants were randomly assigned to receive either 1) 24 weeks with active +24 weeks of washout, 2) 24 weeks with placebo +24 weeks of washout, 3) 48 weeks with active, or 4) 48 weeks with placebo. Weeks with active +24 weeks of washout, 2) 24 weeks with placebo +24 weeks of washout, 3) 48 weeks with active, or 4) 48 weeks with placebo. Active consisted of 2.5 mg bendroflumethiazide and 573 mg potassium chloride. Placebo was visually indistinguishable and contained starch and talc. Bold and underlined indicates statistical significance.

| Parameter | Active 24 weeks | | Placebo 24 weeks | | Active 48 weeks | | Placebo 48 weeks | | P-score of Δ value between baseline and 48 weeks |
|---|---|---|---|---|---|---|---|---|---|
| | Δ value, (SEM), percent change | P-score | Δ value, (SEM), percent change | P-score | Δ value, (SEM), percent change | P-score | Δ value, (SEM), percent change | P-score | |
| Blood tests | | | | | | | | | |
| P-Potassium (K), mmol/l | −0.1, (0.1), −2.6% | 0.3414 | 0.1, (0.06), 2.5% | 0.0730 | −0.1, (0.06), −2.6% | 0.1183 | −0.004, (0.07), −0.08% | 0.9573 | a = 0.3383<br>b≥ 0.9999<br>c≥ 0.9999<br>d≥ 0.9999 |
| P-Sodium (Na), mmol/l | 0.4, (0.6), 0.3% | 0.5218 | 0.2, (0.4), 0.1% | 0.5939 | −0.08, (0.6), −0.07% | 0.8823 | −0.1, (0.4), −0.4% | 0.7610 | a≥ 0.9999<br>b≥ 0.9999<br>c≥ 0.9999<br>d≥ 0.9999 |
| P-Calcium ionized (Adjusted for pH 7.4), mmol/l | −0.004, (0.01), −0.3% | 0.6748 | −0.02, (0.01), −0.8% | 0.1754 | −0.01, (0.007), −1.6% | 0.0689 | 0.008, (0.007), 0.8% | 0.3117 | a≥ 0.9999<br>b≥ 0.9999<br>c = 0.2652<br>d = 0.2052 |
| P-Total albumin corrected calcium, mmol/l | 0.0007, (0.03), 0.04% | 0.9799 | −0.02, (0.02), −0.6% | 0.4202 | 0.02, (0.02), 0.7% | 0.2506 | −0.003, (0.02), −0.4% | 0.8783 | a≥ 0.9999<br>b≥ 0.9999<br>c≥ 0.9999<br>d≥ 0.9999 |
| Blood urea nitrogen (BUN), mmol/l | 0.005, (0.009), 4.0% | 0.5647 | 0.006 (0.01), 2.4% | 0.3381 | 0.03, (0.007), 8.0% | **0.0010** | 0.005, (0.007), 1.8% | 0.5376 | a≥ 0.9999<br>b = 0.2725<br>c≥ 0.9999<br>d = 0.1098 |
| P-Magnesium (Mg), mmol/l | −0.02, (0.02), −2.4% | 0.3115 | 0.02, (0.01), 2.4% | 0.1159 | 0.009, (0.009), 1.2% | 0.3341 | 0.02, (0.006), 2.4% | **0.0047** | a = 0.1561<br>b = 0.2704<br>c≥ 0.9999<br>d≥ 0.9999 |
| Hemoglobin A1c (HbA1c), mmol/mol | 0.9, (0.6), 2.9% | 0.1405 | 1.0, (0.5), 3.0% | **0.0447** | 1.1, (0.3), 3.1% | **0.0020** | 1.4, (0.4), 4.1% | **0.0005** | a≥ 0.9999<br>b≥ 0.9999<br>c≥ 0.9999<br>d≥ 0.9999 |
| P-Creatinine, μmol/l | −2.2, (1.7), −3.4% | 0.2092 | 1.5, (1.5), 2.0% | 0.3299 | −1.2, (1.5), −1.8% | 0.4150 | 1.1, (1.4), 1.6% | 0.4291 | a = 0.6113<br>b≥ 0.9999<br>c≥ 0.9999<br>d = 0.9598 |
| 24-Hour urinary calcium, mmol/l | −0.07, (0.2), −2.2% | 0.7773 | 0.1, (0.2), 5.0% | 0.6468 | −0.4, (0.2), −16% | **0.0229** | 0.1, (0.2), 3.8% | 0.6556 | a≥ 0.9999<br>b = 0.9518<br>c≥ 0.9999<br>d = 0.1943 |
| Timed-Up-and-Go, handgrip strength, blood pressure, pulse and balance | | | | | | | | | |
| Timed-Up-and-Go, seconds | 0.22, (0.3), 3.3% | >0.9999 | −0.033, (0.2), −0.5% | >0.9999 | −0.11,(0.1), −1.7% | >0.9999 | −0.60, (0.2), −7.9% | 0.0768 | a = 0.7385<br>b = 0.3579<br>c≥ 0.9999<br>d≥ 0.9999 |
| Handgrip strength, kPa | 0.2, (0.5), 0.7% | >0.9999 | −1.1, (0.7), −4.5% | 0.4404 | −1.9, (0.4), −7.1% | **0.0007** | 3.1, (1.0), 7.9% | **0.0412** | a≥ 0.9999<br>b≥ 0.9999<br>c = 0.0989<br>d = 0.0800 |
| Orthostatic systolic blood pressure difference, mmHg | −1.0, (2.9), −22.7% | >0.9999 | 1.1, (5.0), 36.9% | >0.9999 | −0.3, (2.4), −22.7% | >0.9999 | −3.0, (1.9), −100.3% | 0.3897 | a≥ 0.9999<br>b≥ 0.9999<br>c≥ 0.9999<br>d≥ 0.9999 |
| Orthostatic diastolic blood pressure difference, mmHg | 2.2, (1.6), 45.2% | 0.5950 | −0.3, (2.9), −6.1% | >0.9999 | 0.1, (1.7), 1.4% | >0.9999 | −3.6, (2.1), −65.6% | 0.3034 | a≥ 0.9999<br>b≥ 0.9999<br>c = 0.9776<br>d = 0.4262 |
| Pulse. Delta value between standing and sitting, beats/min | 1.3, (1.7), 17.7% | >0.9999 | 0.2, (1.7), 2.1% | >0.9999 | −0.04, (1.4), −0.5% | >0.9999 | −0.5, (1.0), −6.4% | >0.9999 | a≥ 0.9999<br>b≥ 0.9999<br>c≥ 0.9999<br>d≥ 0.9999 |
| Balance | | | | | | | | | |
| Eyes open forward, seconds | −5.1, (2.6), −5.7% | 0.2196 | −1.9, (2.7), −2.2% | >0.9999 | 7.4, (2.4), 8.1% | 0.0191 | 9.5, (3.8), 10.9% | 0.0804 | a≥ 0.9999<br>**b = 0.0350**<br>c≥ 0.9999<br>d = 0.2506 |

(*continued on next page*)

**Table 3** (*continued*)

| Parameter | Active 24 weeks | | Placebo 24 weeks | | Active 48 weeks | | Placebo 48 weeks | | P-score of Δ value between baseline and 48 weeks |
|---|---|---|---|---|---|---|---|---|---|
| | Δ value, (SEM), percent change | P-score | Δ value, (SEM), percent change | P-score | Δ value, (SEM), percent change | P-score | Δ value, (SEM), percent change | P-score | |
| Eyes closed forward, seconds | −4.1, (2.9), −4.3% | 0.5146 | −1.0, (3.4), −1.2% | >0.9999 | 6.7, (2.6), 7.1% | 0.0556 | 10.8, (3.5), 14.1% | **0.0268** | a≥ 0.9999<br>**b = 0.0399**<br>c≥ 0.9999<br>d≥ 0.9999 |
| Eyes open backwards, seconds | 1.4, (0.8), 11.0% | 0.3329 | 1.7, (0.6), 12.3% | **0.0273** | 3.1, (0.7), 25.7% | **0.0012** | 1.2, (0.7), 9.5% | 0.3135 | a≥ 0.9999<br>b≥ 0.9999<br>c = 0.9296<br>d≥ 0.9999 |
| Eyes closed backwards, seconds | 1.6, (0.8), 13.0% | 0.1417 | 0.9, (0.7), 6.2% | 0.7348 | 2.9, (0.5), 22.4% | **0.0001** | 2.4, (0.6), 20.4% | **0.0098** | a≥ 0.9999<br>b≥ 0.9999<br>c≥ 0.9999<br>d≥ 0.9999 |

a (Active 24 weeks compared to placebo 24 weeks). b (Active 24 weeks compared to active 48 weeks). c (Placebo 24 weeks compared to placebo 48 weeks). d (Active 48 weeks compared to placebo 48 weeks). Standard error of the mean (SEM).

proxy for muscle function, mobility, walking ability, balance, and fall risk. For balance tests, the 48 wks active group saw an increase in EOF and ECF compared to the 24 wks active group which, contrarily, saw a decrease. However, no difference was observed compared to the placebo groups. For TUG and handgrip strength no difference in delta values between treatment arms were observed. To our knowledge, this is the first time these assessments have been included to test the efficacy of bendroflumethiazide in relation these parameters.

### *4.1. Strengths and limitations*

The double-blinded randomized controlled trial setup is the main strength of our study.

However, the study also has some limitations. Firstly, the study period was short compared to other studies. In future, longer studies might be needed to further characterize the potential improvement that bendroflumethiazide has on bone density. Secondly, we did not consider supplementary medication used by patients such as vitamin D or calcium supplements. Additionally, dietary patterns were not logged. Thirdly, we did not reach the number of patients specified in the power calculation due to dropout and patient exclusion. Finally, study participants included only Caucasian Danish women and therefore it is unclear whether the study findings can be generalized to other racial and ethnic groups. Most studies on the effects of TD on osteoporosis and fractures have been conducted on women and it has been suggested that women may be more susceptible to osteoporosis and fractures (Bokrantz et al., 2017). Therefore, other studies should be conducted to test the effect of bendroflumethiazide on additional subpopulations.

## 5. Conclusions

In conclusion, we found no clear evidence that thiazide therapy influences BMD in addition to oral bisphosphonates in postmenopausal women with osteoporosis. Further studies in larger patient cohorts are needed to further clarify the effect that thiazide diuretics may have on BMD. We furthermore encourage that the results provided herein are included in future metanalyses. The ageing of the population will constitute an increased disease burden due to fractures and thus effective prophylactic medications are desirable. The results presented in this study does not support using bendroflumethiazide as osteoporosis- or fracture treatment in addition to bisphosphonates. It is unclear whether combination treatment might have certain beneficial effect when used for longer than 48 weeks. Additional studies are also needed to stratify patients that may experience beneficial improvements of BMD and secondary parameters and those who may suffer from the usage of bendroflumethiazide.

## Ethical statement

All participants provided informed consent for participation. The present study was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki as revised in 2013 and was approved by the regional scientific ethics committee for the North Denmark Region with number: 66937 and by the Danish Medicines Agency with number: N-20150022. All procedures performed in studies involving human participants were in accordance with the ethical standards set by the institutional and national research committee. The study was registered with the EU Clinical Trials Register and designated the EudraCT number: 2015–001059-63. The study was monitored by the Center for Good Clinical Practice of the Aalborg- and Aarhus University Hospitals.

## Original publication statement

This is an original manuscript that has not been submitted for publication elsewhere.

## Funding

Funding was provided by The Obel Family Foundation. The Obel Family Foundation had no involvement in study design, collection, analysis and interpretation of data, writing, and in the decision to submit the article for publication.

## Consent to participate

Informed consent was obtained from all individual participants included in the study.

## Consent for publication

Not applicable.

## Availability of data and material

The data supporting the conclusions of this manuscript is available upon request.

## CRediT authorship contribution statement

CK and PV participated in the design of the study. CK, PV, and JSL

participated in monitoring the study. TE, PV and THP conducted the statistical analysis. TE, JSL, THP, and PV wrote the manuscript. All authors reviewed the final version of the manuscript and take full responsibility for the correctness of the trial, data, conduct, analyses used, and the reporting of the study.

## Transparency document

The Transparency document associated with this article can be found in online version.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

We are indebted to the indispensable technical assistance provided by Merete Christensen, Mette Brodersen, and Line Rosengren Kaldahl.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bonr.2020.100737.

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