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CLINICAL INVESTIGATION

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Effects of walnut consumption for 2 years on older adults' bone health in the Walnuts and Healthy Aging (WAHA) trial

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Abstract

Background: Nutritional strategies to maintain bone health in aging individuals are of great interest. Given the beneficial nutrient composition of walnuts, rich in alpha-linolenic (the vegetable n-3 fatty acid) and polyphenols, their regular consumption might be a dietary option to reduce age-related bone loss. We determined whether daily walnut consumption improves bone mineral density (BMD) and circulating biomarkers of bone turnover.

Methods: The Walnuts and Healthy Aging study (WAHA) is a two-center, parallel, randomized controlled trial evaluating the effect of a diet enriched with walnuts at ≈15% energy compared with a control diet for 2 years on age-related health outcomes in healthy men and women aged 63–79 years. Changes in BMD were a prespecified secondary outcome only at the Barcelona node of the trial, where 352 participants were randomized. Retention rate was 92.6%. Primary endpoints were 2-year changes in BMD at the spine and the nondominant femoral neck, determined by dual-energy X-ray absorptiometry (DXA). Secondary endpoints were 2-year changes in bone turnover biomarkers

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(adrenocorticotropic hormone, Dickkopf WNT signaling pathway inhibitor-1, osteoprotegerin, osteocalcin, osteopontin, sclerostin, parathyroid hormone, and fibroblast growth factor-23), which were quantified in 211 randomly selected participants.

Results: The walnut diet versus the control diet had no effect on 2-year changes in BMD at the spine (0.15% vs. 0.35%, $p = 0.632$) and femoral neck (−0.90% vs. −0.70%, $p = 0.653$), or on bone turnover biomarkers. Results were similar in participants treated or not with bone resorption inhibitors or those with or without osteoporosis/osteopenia at inclusion.

Conclusions: Compared with the usual diet, a diet enriched with walnuts at 15% of energy for 2 years failed to improve BMD or circulating markers of bone metabolism in healthy older people.

KEYWORDS

bone mineral density and turnover biomarkers, nutrition, randomized controlled trial, walnuts

INTRODUCTION

Adults aged >65 years represent almost 10% of the worldwide population and 20% of the population in Western countries.[1] Advances in medicine and technology have led to an aging population living longer, and successful and healthy aging has become a public health challenge.[2]

Bone mass decreases with age, particularly from age 65 years onward, when the normal accrual of bone mineral density (BMD) in the spine occurring in adulthood in men and after menopause in women begins to attenuate, while BMD decreases at the total hip and femoral neck in both sexes, thereby increasing the risk of osteoporotic fractures,[3] which is a major health concern.[4] Beyond established pharmacologic anti-resorptive and anabolic therapy, there is a pressing need to uncover lifestyle factors that might favor bone homeostasis and help prevent fragility fractures. In this sense, a growing body of evidence points to the benefits for bone health of balanced, nutrient-rich dietary patterns.[4–7] Therefore, nutritional strategies to maintain BMD and reduce the burden of osteoporotic fractures in aging populations are of great interest.

Nuts, an integral part of healthy plant-based diets, are energy-dense foods that possess a complex matrix of salutary nutrients and bioactive compounds, including protein, fiber, unsaturated fatty acids, non-sodium minerals, and phenolic compounds, and their consumption has been associated with many health benefits.[8] Among nuts, walnuts may have additional health effects because of their differential nutrient composition, as they contain ≈10% of energy as alpha-linolenic acid (ALA), the main vegetable polyunsaturated n-3 fatty acid (n-3PUFA), and are richer in polyphenols than any other nut type.[9] Also, walnuts have anti-inflammatory properties, reducing the circulating levels of pro-inflammatory cytokines,[10] while their regular consumption displaces nutrient intake in the usual diet toward a favorable nutrient profile.[11] Epidemiologic studies suggest that ALA intake may reduce the risk of hip fracture in older individuals,[12,13] although no effect on BMD was ascertained in one study.[12] In a small clinical trial, a diet rich in ALA from flaxseed and walnuts reduced a marker of bone resorption.[14] Experimental data point to a beneficial effect of polyphenols on bone remodeling,[15] and emerging clinical evidence

Key points

- Walnuts are rich in healthy nutrients, including alpha-linolenic acid, non-sodium minerals, and polyphenols, which might benefit bone health.
- This is the first randomized trial to evaluate the effect on bone health of supplementing older adults' diets with walnuts.
- A diet enriched with walnuts at 15% of energy for 2 years had no effect on bone mineral density in healthy older people.

Why does this paper matter?

Our study provides novel information on the association of diet with bone health in older individuals.

suggests that intake of polyphenol-rich beverages such as green tea and coffee is associated with lower fracture risk[15–17] while consumption of prunes, a food naturally enriched in polyphenols, helps preserve hip BMD.[18] Furthermore, pro-inflammatory cytokines are implicated in the pathogenesis of osteoporosis, as they promote bone resorption,[19] and there is suggestive evidence that increased plasma levels are associated with fragility fractures.[20,21]

No formal studies of exposure to nuts for outcomes of bone health have been conducted. Changes in BMD at the spine and femoral neck, assessed by dual-energy x-ray absorptiometry (DXA), was a prespecified secondary outcome of the Walnuts and Healthy Aging (WAHA) randomized controlled trial (RCT) testing the effects of walnut consumption on age-related outcomes in older people.[22] We hypothesized that, in comparison with a control diet, participants consuming a diet enriched with walnuts for 2 years would sustain less bone loss or experience more bone gain at the spine and proximal femur. We also examined the effect of the intervention on the incidence of fragility fractures and changes in circulating biomarkers of bone turnover.

MATERIALS AND METHODS

Study design and participants

WAHA is a two-center (Barcelona, Spain and Loma Linda, California), parallel, observer-blinded RCT designed to test the 2-year effects of a diet enriched with walnuts at ≈15% energy compared with a control diet on age-related diseases in cognitively healthy older people (ClinicalTrials.gov identifier NCT01634841). Although the parent WAHA study included two sites, the current analysis of bone health outcomes only includes participants from the Barcelona site due to availability of a certified technologist and a whole-body scanner GE-Lunar iDXA apparatus. Therefore, the candidates for the present study were the 352 participants randomized into the WAHA trial at the Barcelona site only.

A detailed protocol of the WAHA study has been published.[22] All participants were recruited between May 2012 and May 2014. Eligible candidates were women and men aged 63–79 years. Given that the primary outcomes of WAHA were age-related changes in cognitive function and retinal health, major exclusion criteria were previously diagnosed neurodegenerative disease; prior stroke, significant head trauma, or brain surgery; relevant psychiatric illness; major depression; cognitive decline or dementia with a score of <24 on the Mini-Mental State Examination; and advanced macular degeneration or eye-related conditions precluding ophthalmological evaluation. Other reasons for exclusion were excessive alcohol consumption; morbid obesity (BMI ≥ 40 kg/m^2); uncontrolled diabetes (HbA1c > 8%); uncontrolled hypertension (on-treatment blood pressure ≥ 150/100 mmHg); prior chemotherapy; allergy to walnuts; habitual consumption of nuts (>2 servings/week); or use of dietary supplements containing n-3PUFA.

Participants were randomly allocated to the control or intervention group through a computerized, web-based, random number table with stratification by gender and age range in a 1:1 ratio. Pairs of individuals (i.e., couples, household members, partners) entering the study were allocated to the same group using the same stratification criteria. Study clinicians and researchers were blind to participants' intervention group, except for the dietitians taking care of dietary assessments and walnut supply.

Participants in the walnut group were given packaged walnuts (30–60 g/day), equivalent to ≈15% of daily energy requirements, to incorporate into their habitual diet. Participants in the control group were asked to abstain from walnuts and to continue with their usual diet. Participants were scheduled for face-to-face meetings with the study dietitians every 2 months. The visits were aimed at assessing compliance, providing cues for retention, collecting data on tolerance, and delivering the packaged walnuts to those allocated to the active treatment group. Follow-up data on anthropometry, physical activity, and food consumption via 3-day food records, were obtained from all participants at the 6-, 12-, and 24-month visits. Compliance was assessed by directly questioning the participants and recount of empty packages. The WAHA study was conducted in accordance with the guidelines of the Declaration of Helsinki and was approved by the Ethics Committee of the center. All participants provided written informed consent before enrolment. At baseline and every 6 months during intervention, we collected clinical and medication data, measured anthropometric variables, and assessed physical activity with a validated version of the Minnesota Leisure Time Physical Activity Questionnaire.[23]

Bone mineral density determination by DXA scanning

BMD was assessed by DXA (Whole body scanner GE-Lunar iDXA, GE Healthcare, Madison, WI, USA) at inclusion and after intervention for 2 years. The participants were positioned in the center of the padded table for each scan. The appropriateness of patient's position was further assessed by the DXA software with an automatic detection system. For this study, scans were

performed using the default scan mode automatically selected by the DXA software. The GE Lunar Body Composition Software was used to obtain information on BMD and T-score of the lumbar spine (from L1 to L4) and nondominant femoral neck.[24] The T-score is automatically calculated by the software and is the number of standard deviations the BMD is below the average BMD of a healthy adult of the same sex, with NHANES as the reference cohort.[25] The precision error (coefficient of variation) for measurements was 1.1% and 0.7% for spine and femoral BMD, respectively. The same expert technologist performed all DXA procedures and scan analyses.

According to WHO criteria, osteoporosis was defined as a T-score ≤ -2.5 and osteopenia as a T-score between -1 and -2.5[26] from the femoral BMD. Self-reported nontraumatic fractures were also recorded during follow-up.

Laboratory analyses

At baseline and at 2 years, blood samples were obtained after an overnight fast. Aliquots of EDTA-plasma, serum, and red blood cells were stored at $-80°C$ until analyses. Thyroid hormones were measured with standard methods at the Biomedical Diagnostic Centre of the Hospital Clínic of Barcelona, Spain.

Determination of alpha-linolenic acid in red blood cell membranes

Compliance with the intervention was estimated by determining 2-year changes in the proportion of alpha-linolenic acid (ALA) in red blood cell membranes, as described.[27]

Quantification of circulating biomarkers of bone turnover

For the quantification of soluble molecules related to bone homeostasis, the plasma samples of 211 participants were randomly selected (105 in the control group and 106 in the walnut group). The MILLIPLEX Human Bone Magnetic Bead Panel kit (ref. HBNMAG-51 K, Merck Millipore) was used to quantify Adrenocorticotropic Hormone (ACTH), Dickkopf WNT Signaling Pathway Inhibitor 1 (DKK1), Osteoprotegerin (OPG), Osteocalcin (OC), Osteopontin (OPN), Sclerostin (SOST), Parathyroid hormone (PTH), and Fibroblast growth factor 23 (FGF-23), according to the manufacturer's instructions.

Statistical analyses

Changes in BMD were a prespecified secondary outcome at the Barcelona node of the trial, where 352 subjects were randomized. Therefore, power calculation was performed post hoc with the ENE 3.0 statistical program (GlaxoSmithKline, Brentford, United Kingdom). Accepting an alpha risk of 0.05 and a beta risk of 0.2 in a two-sided test, and assuming a loss of 10% of participants, we would need 56 and 45 participants per group (final $n = 50$ and 40) to have 80% power to detect significant differences of $0.5 \pm 0.05\%$ in femoral and spine BMD, respectively, indicating that our study was powered to detect potential changes in BMD.

All analyses were performed using SPSS v. 29 (IBM Corp., Armonk, NY, USA). Descriptive data are presented as means (95% confidence intervals [CI]) for continuous variables and frequency or proportions (%) for categorical variables. Values for continuous variables were examined for normality before analyses. Baseline differences between groups were assessed by the Chi square for categorical variables and *t* tests or Mann–Whitney tests for parametric and nonparametric numerical variables, respectively. We evaluated within-group differences at 2 years in energy and nutrient intake, the ALA proportion in RBC membranes, BMD at the spine and nondominant femoral neck, and concentrations of biomarkers of bone turnover by paired *t*-tests. Changes in BMD were the primary outcome of this substudy of the WAHA trial, whereas changes in circulating bone markers were a secondary outcome. Between-group differences in changes after 2 years were assessed by multivariable linear regression analysis adjusting for the baseline value of the variable, sex, changes in physical activity, and changes in statins and bone resorption inhibitors. We evaluated differences in treatment effects according to sex, age at inclusion, baseline body mass index (BMI), educational level, presence of dyslipidemia and osteopenia/osteoporosis, in-trial treatment with bone resorption inhibitors or vitamin D/calcium supplements, and physical activity and proportion of ALA in red blood cells at the end of the trial. Subgroup analyses were performed with repeated-measures ANCOVA with three factors: time (baseline vs. 2 years) as repeated measure, group (control diet vs. walnut diet), and the two categories of each variable of interest. Associations between BMD and circulating molecules were assessed with Spearman's rank correlation coefficients. A two-tailed $p < 0.05$ was considered significant.

RESULTS

As depicted in Figure 1, after exclusion of dropouts, 326 participants were available for analyses ($n = 163$ per

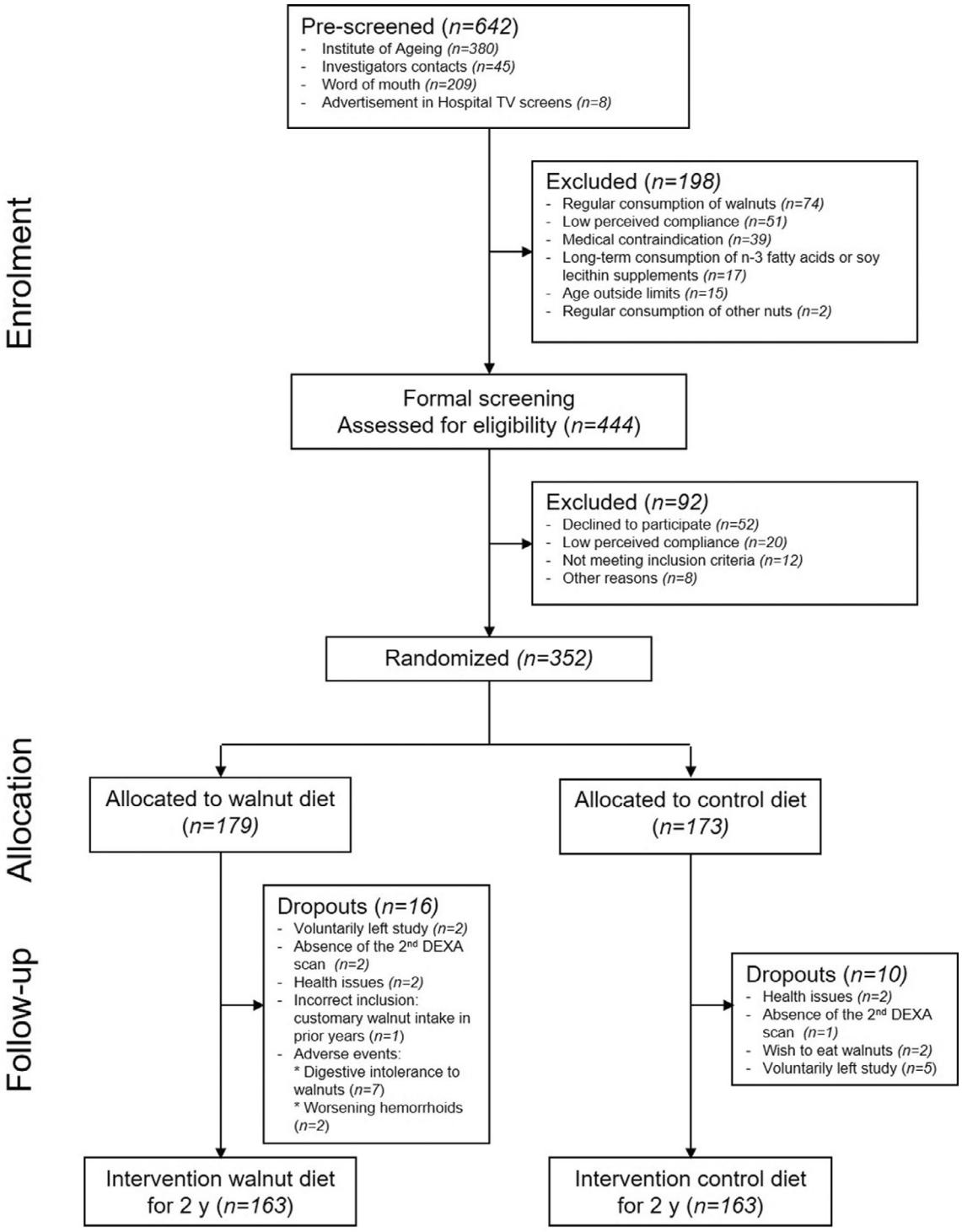

FIGURE 1 Flow diagram of the Walnuts and Healthy Aging (WAHA) study in the Barcelona Center.

group of intervention), of whom 220 were women and 106 were men. About 55% of study participants had osteopenia and 14% had osteoporosis. The baseline characteristics of participants completing the study were similar by intervention group (Table 1).

The nutrient composition of walnuts is described in Supplemental Table S1. Baseline energy and macronutrient intake and changes at 2 years by intervention group are presented in Supplemental Table S2. Participants allocated to the control group had no major energy or nutrient changes at 2 years, except for small increases in total carbohydrate, sugar, and fiber intake, and small reductions in total PUFA and linoleic acid intake. At the end of the trial, participants in the walnut group increased the intake of total energy,

TABLE 1 Baseline characteristics of the participants who completed the study by intervention group.

	Walnut diet ($n = 163$)	Control diet ($n = 163$)
Women—*n* (%)	111 (68)	109 (67)
Age, y	69.0 (68.5, 69.5)	68.6 (68.1, 69.1)
Smoking—*n* (%)		
Never smoker	120 (73.6)	115 (70.6)
Former smoker	36 (22.1)	39 (23.9)
Current smoker	7 (4.3)	9 (5.5)
Education—*n* (%)		
Basic (0–4 y)	14 (8.6)	2 (1.2)
Elementary (5.8 y)	59 (36.2)	56 (34.4)
Secondary (9–12 y)	47 (28.8)	42 (25.8)
Post-secondary (>12 y)	43 (26.4)	63 (38.7)
Height—cm	161.4 (160.2, 162.7)	160.8 (159.5, 162.1)
Weight—kg	70.1 (68.2, 71.8)	71.2 (69.2, 73.2)
Body mass index (kg/m^2)	26.8 (26.2, 27.4)	27.5 (26.9, 28.1)
Waist circumference (cm)	97.4 (95.8, 99.1)	99.4 (97.5, 101.1)
Hypertension—*n* (%)	95 (58.3)	85 (52.1)
Type-2 diabetes—*n* (%)	16 (9.8)	19 (11.7)
Dyslipidemia—*n* (%)	82 (50.3)	91 (55.8)
Use of statins—*n* (%)	27 (16.6)	36 (22.1)
Osteoporosis—*n* (%)	28 (17.2)	19 (11.7)
Osteopenia—*n* (%)	85 (52.1)	98 (60.1)
Use of bone resorption inhibitors—*n* (%)	15 (9.2)	13 (8.0)
Use of vitamin D/ calcium supplements—*n* (%)	19 (11.7)	21 (12.9)
Physical activity (MET-min/d)[a]	3045 (2733, 3357)	2916 (2617, 3215)

Note: Data are means (95% CI) or *n* (%).
[a]Physical activity is expressed in MET-min/d, minutes/day at a given metabolic equivalent level (units of energy expenditure in physical activity, 1 MET-min roughly equivalent to 1 Kcal).

soluble fiber, total fat, total PUFA, linoleic acid, and n-3 PUFA at the expense of ALA, reflecting the nutrient composition of walnuts. In the walnut arm, there was also a reciprocal decrease in intake of total carbohydrate and sugar. These changes translated into significant differences in comparison with the changes observed in the control diet arm. Intake of phytosterols and total polyphenols also increased in the walnut group compared with the control group (Supplemental Table S3). In addition, at 2 years, the percentage of ALA in RBC more than doubled in the walnut group compared with the control group (Supplemental Figure S1), supporting good compliance with the intervention.

No significant between-group changes in anthropometric variables or thyroid hormones were observed at the end of the trial (Supplemental Table S4).

During the study, 14 of the 326 participants (4.3%) reported nontraumatic vertebral fractures, with similar frequency per group (8 in the walnut arm and 6 in the control arm). No hip or long bone fractures were reported.

Primary outcome: Changes in BMD

At inclusion, BMD and T-scores at the spine and femoral neck were similar between groups. After 2 years of intervention and after multivariable adjustment, small increases in spine BMD and T-score and decreases in femoral BMD and T-score were observed in both groups, with no between-group differences (Table 2).

Subgroup analyses of BMD

Subgroup analyses are presented in Table 3. Changes in spine BMD by group were similar irrespective of sex, age, BMI, or physical activity, and indistinctly of being or not under bone resorption inhibitors or under vitamin D or calcium supplements in both interventions. Small increases in spine BMD were observed in participants in the control group with higher education, those without dyslipidemia, or those without spine osteopenia/osteoporosis. No intervention group–subgroup–time interactions were observed.

Femoral BMD significantly decreased in women compared with men in both groups ($p < 0.001$). However, no sex–group–time interactions were observed (Table 3). Significant decreases in femoral BMD were also noticed in participants without femoral osteopenia/osteoporosis in both groups; again, no significant interactions were observed. Significant decrements of femoral BMD occurred after the walnut diet in participants aged 68 years or older and in those with higher education, dyslipidemia, or higher physical activity. In the control group, femoral BMD decreased in participants who did not receive vitamin D or calcium supplements. No significant intervention group–subgroup–time interactions were observed in any case.

For both spine and femoral BMD, the effects did not vary by changes in the red blood cell proportion of ALA

TABLE 2 Baseline values and 2-year changes in bone mineral density in completers by randomization group.

	Walnut diet ($n = 163$)	Control diet ($n = 163$)	p
BMD Spine (g/cm^2)			
Baseline	1.07 (1.04, 1.09)	1.08 (1.06, 1.11)	
Changes, unadjusted	0.005 (−0.004, 0.015)	0.004 (−0.002, 0.010)	0.891
Changes (%)	0.152 (−0.413, 0.717)	0.348 (−0.229, 0.924)	0.632
Changes, adjusted[a]	0.002 (−0.004, 0.007)	0.004 (−0.002, 0.011)	0.259
T-Score Spine			
Baseline	−1.07 (−1.30, −0.84)	−0.91 (−1.12, −0.70)	
Changes, unadjusted	0.019 (−0.313, 0.351)	0.015 (−0.314, 0.344)	0.985
Changes, adjusted[a]	0.009 (−0.314, 0.331)	0.012 (−0.306, 0.330)	0.113
BMD Femoral neck (g/cm^2)			
Baseline	0.92 (0.89, 0.94)	0.93 (0.90, 0.95)	
Changes, unadjusted	−0.005 (−0.013, 0.002)	−0.006 (−0.012, −0.001)	0.794
Changes (%)	−0.903 (−1.544, −0.262)	−0.704 (−1.297, −0.112)	0.653
Changes, adjusted[a]	−0.010 (−0.014, −0.006)	−0.006 (−0.014, 0.001)	0.350
T-Score Femoral neck			
Baseline	−0.88 (−1.03, −0.73)	−0.89 (−1.04, −0.74)	
Changes, unadjusted	−0.041 (−0.258, 0.176)	−0.010 (−0.260, 0.239)	0.854
Changes, adjusted[a]	−0.077 (−0.294, 0.171)	−0.003 (−0.254, 0.248)	0.451

Note: Data are means (95% CI). BMD indicates bone mineral density. T-scores were generated using peak reference data from young, healthy adults of the same sex. p values for the comparison between interventions from the multivariable regression analyses.

[a]Adjusted by baseline value of the variable, sex, changes in physical activity, and changes in statins and bone resorption inhibitors.

above or below median values attained at 2 years, which is a reliable marker of compliance with the walnut intervention.

Secondary outcome: Changes in circulating biomarkers of bone turnover

Baseline plasma concentrations of circulating biomarkers of bone turnover were similar between intervention groups. At the end of the trial, no within- or between-group changes in bone turnover markers were detected (Table 4). No correlations existed between BMD and any of the measured markers (data not shown).

DISCUSSION

In this prespecified analysis of the WAHA RCT, a diet supplemented with walnuts at 15% of energy for 2 years compared with a control diet had no significant effect on fragility fracture risk, BMD at the spine and proximal femur, or on the circulating markers of bone turnover in healthy older people. The effects on BMD were not modified by sex, age, BMI, physical activity, other factors related to bone health, or enrichment of red blood cell membranes with ALA, an objective biomarker of walnut consumption. These findings were contrary to our hypothesis that walnuts would favor bone health due to their beneficial composition, with high contents of ALA, the vegetable n-3PUFA, non-sodium minerals, and polyphenols,[9] in addition to their anti-inflammatory properties.[10]

Experimental, epidemiological, and clinical studies have suggested moderate associations between bone health and the intake of specific macronutrients such as protein, micronutrients like vitamins C and D, and calcium and other non-sodium minerals.[4,5] Supplemental vitamin D, however, failed to reduce fracture risk in the large, 5.3-year Vitamin D and Omega-3 Trial (VITAL)[28] or to influence BMD in a 2-year ancillary VITAL study.[29] RCTs with supplements of marine n-3PUFA have uncovered a modest benefit for BMD, but small studies using ALA-rich foods such as walnuts and flaxseed reported contradictory results, with no effects on BMD and small reductions in bone resorption markers.[30,31] There is also evidence of benefit for bone health from cohort studies and clinical trials of polyphenol-rich foods[16–18] and dietary patterns such as the Mediterranean diet.[4,5,28–31]

TABLE 3 Subgroup analyses. Absolute 2-year changes (g/cm^2) in spine and femoral neck bone mineral density by intervention group.

Spine BMD					
	Walnut diet ($n = 163$)		**Control diet ($n = 163$)**		
Subgroup	***n***	**Absolute change (95% CI) g/cm^2**	***n***	**Absolute change (95% CI) g/cm^2**	***p* for interaction**
Sex					
Women ($n = 220$)	111	−0.001 (−0.008, 0.005)	109	0.001 (−0.005, 0.008)	0.297
Men ($n = 106$)	52	0.020 (−0.008, 0.048)	54	0.010 (−0.004, 0.023)	
Baseline age					
≤68 years ($n = 175$)	81	0.016 (−0.002, 0.033)	94	0.002 (−0.006, 0.011)	0.056
>68 years ($n = 151$)	82	−0.005 (−0.013, 0.004)	69	0.007 (−0.003, 0.016)	
Baseline BMI					
≤27 kg/m^2 ($n = 167$)	84	0.001 (−0.007, 0.006)	83	0.002 (−0.007, 0.012)	0.475
>27 kg/m^2 ($n = 159$)	79	0.012 (−0.007, 0.030)	80	0.006 (−0.003, 0.015)	
Education					
≤12 years ($n = 220$)	120	0.007 (−0.005, 0.020)	100	−0.001 (−0.008, 0.007)	0.110
>12 years ($n = 106$)	43	0.001 (−0.012, 0.012)	63	0.012 (0.001, 0.023)	
Dyslipidemia					
Yes ($n = 173$)	82	−0.003 (−0.012, 0.005)	91	−0.001 (−0.01, 0.007)	0.678
No ($n = 153$)	81	0.014 (−0.003, 0.032)	72	0.011 (0.002, 0.019)	
Spine osteoporosis/osteopenia					
Yes ($n = 185$)	96	−0.001 (−0.008, 0.007)	89	−0.002 (−0.01, 0.006)	0.917
No ($n = 141$)	67	0.014 (−0.007, 0.036)	74	0.012 (0.002, 0.022)	
Bone resorption inhibitors					
Yes ($n = 28$)	15	0.008 (−0.011, 0.027)	13	−0.008 (−0.025, 0.008)	0.987
No ($n = 298$)	148	0.007 (−0.005, 0.019)	150	0.006 (−0.001, 0.013)	
Vitamin D or Calcium supplements					
Yes ($n = 40$)	19	−0.008 (−0.025, 0.008)	21	−0.007 (−0.034, 0.019)	0.903
No ($n = 286$)	144	0.007 (−0.004, 0.018)	142	0.006 (−0.001, 0.012)	
Physical activity at 2 years					
≤2440 MET-min/d ($n = 178$)	90	0.009 (−0.009, 0.260)	88	0.005 (−0.004, 0.013)	0.703
>2440 MET-min/d ($n = 148$)	72	0.002 (−0.006, 0.010)	76	0.004 (−0.006, 0.015)	
Proportion of alpha-linolenic acid in red blood cells at 2 years					
≤Median (0.27%) ($n = 164$)	78	0.006 (−0.012, 0.025)	86	0.002 (−0.007, 0.011)	0.470
>Median (0.27%) ($n = 162$)	85	0.005 (−0.004, 0.013)	77	0.007 (−0.002, 0.016)	
Femoral BMD					
	Walnut diet ($n = 163$)		**Control diet ($n = 163$)**		
Subgroup	***n***	**Absolute change (95% CI) g/cm^2**	***n***	**Absolute change (95% CI) g/cm^2**	***p* for interaction**
Sex					
Women ($n = 220$)	111	−0.012 (−0.019, −0.005)	109	−0.010 (−0.018, −0.003)	0.418
Men ($n = 106$)	52	0.001 (−0.005, 0.007)	54	0.002 (−0.004, 0.008)	
Baseline age					
≤68 years ($n = 175$)	81	−0.003 (−0.016, 0.011)	94	−0.006 (−0.012, 0.001)	0.730

TABLE 3 (Continued)

Femoral BMD					
	Walnut diet ($n = 163$)		Control diet ($n = 163$)		
Subgroup	n	Absolute change (95% CI) g/cm^2	n	Absolute change (95% CI) g/cm^2	p for interaction
>68 years ($n = 151$)	82	−0.008 (−0.015, −0.001)	69	−0.007 (−0.016, 0.003)	
Baseline body mass index					
≤27 kg/m^2 ($n = 167$)	84	−0.006 (−0.013, 0.001)	83	−0.007 (−0.014, 0.001)	0.917
>27 kg/m^2 ($n = 159$)	79	−0.005 (−0.018, 0.008)	80	−0.006 (−0.014, 0.001)	
Physical activity at 2 years					
≤2440 MET-min/d ($n = 178$)	90	0.001 (−0.001, 0.001)	88	−0.007 (−0.017, 0.003)	0.225
>2440 MET-min/d ($n = 148$)	72	−0.012 (−0.021, −0.003)	76	−0.005 (−0.010, −0.001)	
Years of education					
≤12 years ($n = 220$)	120	−0.003 (−0.012, 0.006)	100	−0.006 (−0.012, 0.001)	0.988
>12 years ($n = 106$)	43	−0.012 (−0.023, −0.001)	63	−0.008 (−0.017, 0.001)	
Dyslipidemia					
Yes ($n = 173$)	82	−0.009 (−0.017, −0.002)	91	−0.005 (−0.010, 0.001)	0.946
No ($n = 153$)	81	−0.001 (−0.014, 0.011)	72	−0.008 (−0.019, 0.002)	
Femoral osteoporosis/osteopenia					
Yes ($n = 157$)	78	−0.001 (−0.015, 0.012)	79	−0.004 (−0.011, 0.003)	0.771
No ($n = 169$)	85	−0.009 (−0.016, −0.003)	84	−0.009 (−0.017, −0.001)	
Bone resorption inhibitors					
Yes ($n = 28$)	15	−0.008 (−0.021, 0.004)	13	−0.010 (−0.021, 0.001)	0.926
No ($n = 298$)	148	−0.005 (−0.013, 0.003)	150	−0.006 (−0.012, 0.001)	
Vitamin D or Calcium supplements					
Yes ($n = 40$)	19	0.001 (−0.011, 0.012)	21	−0.006 (−0.017, 0.005)	0.973
No ($n = 286$)	144	−0.006 (−0.014, 0.002)	142	0.007 (−0.012, −0.001)	
Physical activity at 2 years					
≤2440 MET-min/d ($n = 178$)	90	0.001 (−0.001, 0.001)	88	−0.007 (−0.017, 0.003)	0.225
>2440 MET-min/d ($n = 148$)	72	−0.012 (−0.021, −0.003)	76	−0.005 (−0.010, −0.001)	
Proportion of alpha-linolenic acid in red blood cells at 2 years					
≤Median (0.27%) ($n = 164$)	78	−0.003 (−0.015, 0.008)	86	−0.006 (−0.015, 0.003)	0.610
>Median (0.27%) ($n = 162$)	85	−0.007 (−0.016, 0.002)	77	−0.007 (−0.013, 0.002)	

Note: Data are means (95% CI). *P* values for interaction were obtained for each variable of interest in repeated-measures ANCOVA with three factors: time (baseline vs. 2 years) as a repeated measure, group (walnut diet vs. control diet), and the two categories of each variable of interest.

Nuts are another whole food with a salient content of beneficial nutrients and bioactive compounds underlying manifold health effects.[8] However, in the WAHA RCT conducted in older individuals, sizeable amounts of walnuts daily for 2 years failed to influence bone health. These results concur with data from three RCTs examining the effects of a Mediterranean diet supplemented with mixed nuts on bone-related variables in older individuals, whereby no effect was ascertained on fragility fractures[32] or biomarkers of bone turnover.[33,34] Still, our

TABLE 4 Baseline values and 2-year changes in soluble molecules related to bone metabolism in completers by randomization group.

	Walnuts ($n = 163$)	Control ($n = 163$)	p
ACTH (pg/mL)			
Baseline	5.9 (3.5, 8.3)	5.2 (3.3, 7.1)	
Change	−0.44 (−2.72, 1.84)	0.20 (−2.99, 3.40)	0.793
DKK1 (ng/mL)			
Baseline	248 (198, 298)	247 (201, 292)	
Change	−7.2 (−58, 44)	5.3 (−54, 64)	0.951
OPG (ng/mL)			
Baseline	518 (441, 594)	493 (443, 544)	
Change	31 (−16, 78)	0.35 (−53, 54)	0.188
OC (ng/mL)			
Baseline	16.9 (6.8, 27)	13.9 (7.2, 21)	
Change	−1.22 (−12.7, 10.2)	2.21 (−8.8, 13.2)	0.448
OPN (ng/mL)			
Baseline	6.5 (4.2, 8.9)	8.0 (4.4, 11.6)	
Changes	0.94 (−0.87, 2.76)	1.14 (−1.44, 3.73)	0.936
SOST (ng/mL)			
Baseline	1.53 (1.24, 1.83)	1.50 (1.25, 1.75)	
Changes	0.11 (−0.02, 0.24)	0.07 (−0.33, 0.18)	0.167
PTH (pg/mL)			
Baseline	94 (73, 115)	92 (73, 111)	
Changes	12.5 (−4.9, 30)	−17.4 (−37, 2.2)	0.054
FGF 23 (pg/mL)			
Baseline	50 (41, 60)	61 (46, 76)	
Changes	6.4 (−0.37, 13.3)	1.83 (−26, 29)	0.701

Note: Data are means (95%CI). ACTH indicates Adrenocorticotropic hormone; DKK1, Dickkopf WNT Signaling Pathway Inhibitor-1; OPG, Osteoprotegerin; OC, Osteocalcin; OPN, Osteopontin; SOST, Sclerostin; PTH, Parathyroid hormone; and FGF-23, Fibroblast Growth Factor 23. *P* values for the comparisons between interventions by multivariable regression analyses adjusted by the baseline value of the variable, sex, changes in physical activity, and changes in statins and bone resorption inhibitors.

study with walnuts is the first formal RCT investigating the exposure to nuts exclusive of any other dietary changes for effects on bone health.

Our study has limitations. First, the original study was designed to assess changes in cognitive function and retinal health,[22] and our results are derived from a secondary analysis in a subsample of one-half of the total study participants. The study is limited to participants from Barcelona. We do not know whether results would differ if walnuts were added to a different regional diet. Second, the WAHA cohort is composed of healthy older people; therefore, the results do not generally apply to younger individuals or older populations in poor health. We also do not know whether a diet enriched in walnuts during other life phases (e.g., during childhood or adolescence or peri-menopause) would show greater benefit. Third, the trial's 2-year duration may be too short a timeline to detect changes in fracture rates or BMD. Fourth, walnut supplementation resulted in increased energy intake compared with the control diet, which might have a potential influence on the outcomes of the study; however, 2-year weight changes were insignificant and similar in the two groups. Fifth, at 4.3% in 2 years, the incidence of self-reported nontraumatic fractures was low and no lateral DXA scans of the spine were obtained to detect new asymptomatic fractures. Nevertheless, Spain has one of the 10-year lowest probabilities of major osteoporotic fracture in men and women worldwide.[35] The circulating biomarkers of bone health selected in the study are not the common markers assessed in the clinical setting. However, given that neither changes in BMD nor in the selected biomarkers were observed, we would not expect significant changes in common markers of bone turnover. Finally, the good health status of participants

might have attenuated the BMD changes, which, at a mean accrual of 0.125% per year at the spine and a mean decline of −0.40% per year in the proximal femur, were lower than expected.[36]

Our study also has strengths. First, it is an observer-blinded RCT performed in a relatively large sample representative of the noninstitutionalized older population. Second, there was an excellent retention rate for a 2-year trial (almost 93%), as well as good compliance with the intervention. Finally, we used DXA to determine BMD, which is one of the most accurate and reliable methods to measure bone status in humans, and the same expert technologist obtained the scans at baseline and 2 years.

In summary, in this substudy of the WAHA RCT, we found that, compared with a control (usual) diet, daily dietary supplementation with walnuts at 15% of energy for 2 years had no clinically important benefit to fracture risk, BMD at the spine and proximal femur, or markers of bone turnover in healthy older people. Given the overall beneficial health effects of walnuts,[9,37] it is safe to recommend their consumption while considering that substantial amounts daily neither benefit nor harm bone health.

AUTHOR CONTRIBUTIONS

JS, ER, and GC-B designed research; CO-P, MC, AS-V, MS-M, IR, SV-P, MD, EO, SR, and GC-B conducted research; CO-P, ER, and GC-B analyzed the data and performed statistical analysis; ER and GC-B wrote the paper; ER and GC-B had primary responsibility for final content. All authors have read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Supplemental Table S1. Nutrient composition of walnuts (per 45 g serving).
Supplemental Table S2. Energy and macronutrient intake at baseline and 2-y changes in completers by randomization group.
Supplemental Table S3. Intake of non-nutrient bioactive compounds, minerals and vitamins at baseline and 2-y changes by randomization group.
Supplemental Table S4. Baseline values and 2-year changes in anthropometric characteristics and thyroid function by intervention group.
Supplemental Figure S1. 2-year changes in the proportion of alpha-linolenic acid in red blood cells by intervention allocation.

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