Original Article

Relationship between Osteoporosis, Multiple Fractures, and Egg Intake in Healthy Elderly

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Abstract

Context: The role of dietary patterns in the prevention of osteoporosis has been investigated in many studies, but few have examined the association between consumption of specific food and whole-body (WB) bone mineral density (BMD). Recent evidence suggests that whole eggs contain bioactive compounds that could have beneficial effects on BMD. BMD is also expressed as the T-score, which is used for the clinical diagnosis of osteoporosis and to evaluate the effectiveness of drugs. **Aims:** We conducted a study to assess the association between eggs consumption and bone density in a population of the elderly. **Settings and Design:** This cross-sectional study included 176 individuals of both genders and aged ≥65 years. **Subjects and Methods:** Egg intake was ascertained by a combination of dietary intake assessment, and a dual X-ray absorptiometry scan was performed to measure WB T-score. **Results:** In our study, among all the food groups and nondietary factors evaluated, we find a positive association between the WB T-score and egg consumption (B = 0.02; P = 0.02), gender (B = 0.85; $P < 0.001$), and body mass index (B = 0.04; P = 0.03). Multiple fractures were associated with the daily intake of eggs (B = –0.26; P = 0.02) and high-density lipoprotein-cholesterol (B = 0.09; P = 0.03). **Conclusions:** This study provides novel evidence of a positive link between whole egg consumption and bone health. If results observed in this study will be confirmed through future randomized controlled trials, whole eggs may represent a viable strategy to prevent osteoporosis and reduce the risk of fractures in the elderly.

Keywords: *Bone mineral density, egg, elderly, food, fractures*

Submitted: 07-Jul-2021
Revised: 14-Nov-2021
Accepted: 06-Dec-2021
Published: 20-Jan-2022

Introduction

An adequate nutritional status is essential for healthy aging,[1,2] especially to bone health. Numerous evidences suggest the association between specific dietary patterns and bone health,[3] particularly in elderly subjects.[4,5] The process of bone formation requires an adequate supply of nutrients which, either directly – by acting on bone – or indirectly – by preserving muscle mass – prevent bone loss.[6] The dietary interventions represent, thus, effective and safe public health strategies, particularly for elderly individuals that are at risk for polypharmacy due to multiple chronic diseases. Aging causes a significant reduction of whole-body (WB) bone mineral density (BMD),[7,8] as well as an increase in fractures risk.[9] Furthermore, a fracture not only adversely affects the quality of life[10] but also leads to an increased risk of mortality. Indeed, the mortality in the 1st month after hip fractures surgery is high, ranging between 6% and 11%[11] and between 20 and 30% during the 1st year.[12]

Currently, scientific evidence indicates that consuming high quantities of fruit, vegetables, whole grains,

Access this article online

Quick Response Code:

Website: www.jmidlifehealth.org

DOI: 10.4103/jmh.jmh_118_21

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How to cite this article: Pujia R, Ferro Y, Maurotti S, Mare R, Arturi F, Montalcini T, *et al*. Relationship between osteoporosis, multiple fractures, and egg intake in healthy elderly. J Mid-life Health 2021;12:287-93.

fish, and legumes improves BMD[6,13] and decreases the risk of bone fractures in the elderly.[13-17] Due to several evidences between the dietary cholesterol and cardiovascular mortality,[18,19] eggs consumption was demonized for a long time. Then to date, the effect of egg consumption on BMD and fractures in adults has never been studied.

Few studies suggest bone-preserving proprieties of egg proteins[20,21] as ovotransferrin (OVT).[20,21] Shang and Wu have identified the mechanism by which the OVT is able to inhibit the bone resorption process.[21] Human and *in vitro* studies[22,23] have suggested that carotenoids, which are present in eggs yolk,[24,25] could prevent bone loss.

Therefore, we conducted a study to assess the association between eggs consumption and bone density in a population of the elderly.

Subjects and Methods

Participants were recruited from February 2013 to August 2016, and this study was a secondary analysis of baseline data obtained from a study named: "Effect of the Mediterranean Diet on cognitive function in the elderly." This study was funded by the Italian Ministry of Health and the protocol was approved by the "Mater Domini" University Hospital ethics committee in Catanzaro (projects codes 2011.48). All individuals provided their informed consent to participate to the study. The investigation conforms to the principles outlined in the Declaration of Helsinki.

In this study, we enrolled elderly white people, of both genders, aged ≥65 years living in Calabria, southern Italy, through newspaper advertisements. We excluded all patients who presented clinical condition affecting bone metabolism (such as kidney, liver, thyroid, or parathyroids, rheumatic diseases, malabsorption syndromes, malignant tumors, or hematological diseases). We also excluded individuals whp took psychotropic drugs, glucocorticoids, estrogens, thyroid hormone, fluoride, calcitonin, or dietary supplements, as ascertained from their medical history.[26] Undergoing treatment for osteoporosis, hypertension, hyperlipidemia, and diabetes was not considered an exclusion criterion.

All participants underwent anthropometric measurements, biochemical evaluations, and a dual X-ray absorptiometry (DXA) scan to measure WB T-score. From the patient's clinical interview, we assessed smoking habits, the prevalence of bone fractures, and the use of drugs for hyperlipidemia, hypertension, diabetes, and osteoporosis including anti-osteoporotic agents, calcium, and Vitamin D supplementation.

Anthropometric measurements

All tests were performed after a 12 h overnight fast. Body weight was measured before breakfast with the subjects lightly dressed, subtracting the weight of clothes. Body weight was measured with a calibrated scale and height was measured with a wall-mounted stadiometer. Body mass index (BMI) was calculated with the following equation: Weight (kg)/height (m) ^{2}.

Biochemical evaluation

Venous blood was collected after fasting overnight into vacutainer tubes (Becton and Dickinson, Plymouth, England) and centrifuged within 4 h. Serum glucose, total cholesterol, high-density lipoprotein (HDL-C) cholesterol, triglycerides (TG), creatinine, and calcium were measured by chemiluminescent immunoassay on COBAS 8000 (Roche, Switzerland). Low-density lipoprotein (LDL-C) cholesterol level was calculated by the Friedewald formula. 25-hydroxy Vitamin D (25OH Vitamin D) was measured by radioimmunoassay (normal value 30-100 ng/mL). Quality control was assessed daily for all determinations.

Whole-body bone analysis

Total body bone measurement was assessed for each patient using a DXA-scan (Hologic QDR Inc., MA, USA). The bone area was calculated in centimeters squared (cm^2), BMC in grams (g), and BMD was calculated by dividing the BMC by the area and was expressed grams/cm^2.[15]

BMD was then expressed as the T-score defined as the number of standard deviation scores (SDs) from the healthy young adult mean, while the Z-score was the number of SDs in comparison to healthy subjects of the same age.[15] For WB DXA, osteoporosis was defined by a T-score <–2.35, osteopenia: T-score between − 2.35 and − 0.9, and normal BMD: T-score >–0.9.[27] The DXA instrument was calibrated daily in accordance with the manufacturer's recommendations. The *in vivo* precision was <1%.

Fractures assessment

Vertebral and nonvertebral fractures ascertainment (e.g., femur, forearm, humerus, wrist, or rib) was carried out through participants' interviews or the evaluation of their clinical records (when available). For each participant, we also estimated fractures number. Fractures in body regions not commonly associated with osteoporosis such as those of the fingers, toes, face, and skull, were not taken into account. High-energy fractures caused by severe trauma (e.g., motor vehicle accidents, a fall from a height higher than standing) or pathological fractures (e.g, prostate cancer, lung cancer, multiple myeloma, and breast cancer) were not taken

into account.[9,15] In addition, the participants with body fractures have been classified into two groups based on the presence of "multiple fractures." In particular, older people with a single fracture than those with more than one fracture.

Whole egg consumption and dietary intake assessment

A combination of 24-h recalls (24HRs) and of 7-day food records (7DFR) were used to estimate total egg intake (e.g., scrambled eggs, hard-boiled eggs, fried eggs) over 7 days. Whole egg consumption from mixed dishes was quantified and therefore was included in these analyses. The 24HR was performed via a face-to-face interview with a skilled dietician who used photos associated with a comprehensive food list. The 24HR required 15–20 min to complete for each individual. We also performed a 7DFR, in which each patient reported any ingredients, food, and drinks consumed, during 7 days before the clinical examination. All elderly ate freely and self-reported their intake. Before starting the study, each patient was trained by a dietician how various foods and beverages should be recorded. The portion sizes used were based on the typical or natural portion consumed (e.g., a slice of bread, one egg). When a typical serving size was not obvious, a commonly used portion size was selected (e.g., one cup). We also estimate the consumption of other major food groups, such as milk, cheeses, fish, meat, cereals, legumes, potatoes, vegetables, fruits, virgin olive oil, wine, soft drinks, animal fats/margarines, cookies, and cake/pies. Daily energy, carbohydrates, lipids, protein, and alcohol intake were calculated by the dietician using nutritional software MetaDieta 3.0.1 (Meteda Srl, San Benedetto del Tronto, Italy). In addition, the investigators performing the dietary assessment were blinded to the patients' clinical data.

Data analysis

Data were reported as mean ± SD. Based on the study of Coheley *et al.*[28] and Colica *et al.*,[15] to find a correlation between WB T-score and eggs consumption with an r equal to 0.20, with 80% study power for an alpha one-tailed of 0.05, 153 subjects are required.

Pearson's correlation was performed to identify the variables correlated with WB T-score, given that the continuous variables were normally distributed. In particular, we evaluated the correlation with WB T-score and the following variables: age, BMI, serum glucose, LDL-C, HDL-C, TG, creatinine, calcium, Vitamin D, and eggs consumption per day and other major food groups. We also analyzed the correlation with gender, smoking, and medications (diuretics, lipid-lowering, hypoglycemic, antiplatelet/anticoagulant, antiosteoporotic agents, calcium, and Vitamin D supplementation). In addition, stepwise multivariable linear regression analysis was performed to analyze the association between WB T-score and all the potentially confounding variables. Moreover, we also tested the correlation between all parameters and presence of bone multiple fractures in the older population. Finally, we performed a binary logistic regression analysis to evaluate the association between all of the potentially confounding variables (as independent variables), with multiple fractures serving as the dependent variable. In this analysis, multiple fractures have been classified as binary variables. The potential confounders were all those factors correlated with WB T-score and multiple fractures in the univariate analysis with a $P < 0.1$.

Significant differences were assumed to be present at $P < 0.05$ (two tailed). All comparisons were performed using SPSS 25.0 for Windows (IBM Corporation, New York, NY, USA).

Results

Table 1 shows the participants' demographic and clinical characteristics. A total of 176 elderly (64% female) were consecutively enrolled in the study. The mean age was 69 ± 4 years, mean BMI was 28.5 ± 4 kg/m^2, and WB T-score was –1.08 ± 1.3 SDs (which suggests the presence of osteopenia). However, 16% had osteoporosis [Table 1]. A total of 39 subjects had a history of fractures (22%), and the total number of fragility fractures was 50 (vertebrae, $n = 4$; femur, $n = 1$; rib, $n = 4$; wrist, $n = 13$; forearm/humerus, $n = 5$; other sites $n = 23$; data not shown). More than one fracture was reported in ten individuals. Table 2 shows the nutrients and food groups' intake of the population. About 56% of study participants consumed from 1 to 5 eggs/weeks, with an average weekly consumption one egg per week.

Table 3 shows that WB T-score correlated positively with daily egg consumption ($r = 0.16$; $P = 0.027$), while multiple fractures correlated negatively with egg intake ($r = –0.39$; $P = 0.014$) at Pearson's correlation.

WB T-score remained positively associated with egg intake (B = 0.02; P = 0.02) at regression analysis [Table 4], and at the logistic regression analysis, the condition of multiple fractures remained also associated with daily egg consumption (B = -0.26; P = 0.02) [Table 5].

Discussion

This study investigated the relationship between the consumption of whole eggs and bone health in older men and women. We find a positive association

Table 1: Participants' demographic, anthropometric, and clinical characteristics

Variables	Mean±SD
Age (years)	69±4
BMI (kg/m^2)	28.5±4
Glucose (mg/dL)	103±26
Creatinine (mg/dL)	0.83±0.2
TC (mg/dL)	197±42
TG (mg/dL)	115±56
HDL-C (mg/dL)	58±15
LDL-C (mg/dL)	125±35
Calcium (mg/dL)	9.5±0.3
25OH Vitamin D (ng/mL)	27.7±13
Whole body bone analysis	
WB area (cm^2)	1898±243
WB BMC (g)	2005±445
WB BMD (g/cm^2)	1.046±0.1
WB T-score (SDs)	−1.08±1.3
WB Z-score (SDs)	−0.09±0.9
Prevalence	
Gender, females (%)	64
Smokers (%)	12
Lipid-lowering agents (%)	33
Diuretics (%)	25
Antiplatelet/anticoagulant drugs (%)	36
Oral hypoglycemic agents/Insulin (%)	14
Osteoporosis (%)	16
Body fractures (%)	22
Multiple fractures (%)	26
Antiosteoporotic agents and calcium, Vitamin D supplementation (%)	9

Values are expressed as mean±SD. BMI: Body mass index, TC: Total cholesterol, TG: Triglycerides, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, WB: Whole body, BMC: Bone mineral content, BMD: Bone mineral density, SD: Standard deviation

between the consumption of eggs and WB bone mass assessed by DXA-scan. We also find an inverse association between the eggs consumption and occurrence of multiple fractures in the subgroup with preceding fractures.

With the exception of studies on dairy products[16,17] and foods containing soy,[18] there is a scarcity of data in the literature regarding the whole foods intake and osteoporosis.

This study was the first to evaluate the relationship between the whole egg intake and bone health in the elderly. Only one previous study carried out in children explored a similar relationship.[28] This study, a daily consumption of whole eggs was positively associated with the mineral content at radius as well as serum osteocalcin, a key bone formation marker.[28]

Table 2: Characteristics of assessment nutrients and food groups

Nutrients	Mean±SD
Energy intake (kcal/die)	1846±405
Carbohydrates (g/die)	215±57
Lipids (g/die)	70±20
Protein (g/die)	76±20
Alcohol (g/die)	7±11
Food groups	
Cereals (g/die)	197±79
Legumes (g/die)	18±20
Potatoes (g/die)	19±23
Vegetables (g/die)	274±132
Fruit (g/die)	339±174
Milk (g/die)	122±104
Cheeses (g/die)	62±50
Eggs (g/die)	11±10
Meat (g/die)	78±46
Fish (g/die)	60±50
Wine (g/die)	73±111
Soft drinks (g/die)	21±62
Virgin olive oil (g/die)	34±12
Animal fats/margarines (g/die)	0.94±4.1
Cookies (g/die)	10±17
Cakes/pies (g/die)	36±31

SD: Standard deviation

Our results are very exciting for several reasons. Foods contain variable combinations of nutrient and nonnutrient components such as antioxidants and phytochemicals. Evidence from experimental and prospective cohort studies underlines the additive and synergistic effects of multiple factors from different foods on disease risk that is of greater extent than a nutrient alone.[29] We thus investigated the role of whole eggs rather than an egg component. Moreover, studies on food or dietary pattern could better facilitate the translation of knowledge to the general population compared to studies investigating the role of molecules alone.

A standard egg contains almost all the vitamins and is one of the best sources of choline vitamin.[30] It has been suggested that the mean intake of choline is inadequate in the European population.[30] A low concentration of plasma free choline is associated with low BMD in humans,[31] while habitual intake of choline was positively associated with high BMD in both the middle-aged men and elderly women[32] [Table 6 – molecular mechanisms and effects on bone health of some active compounds contained in eggs].

However, eggs are an interesting carrier of biologically active pigments as carotenoids. Several studies supported the hypothesis that a high carotenoids or β-carotene intake might be potentially associated with a low risk of hip fracture[22,33] [Table 6].

Table 3: Univariate analyses - factors correlated with the WB T-score and multiple fractures

WB T-score	Gender	BMI	Glucose	Creatinine	Diuretics	Eggs	Virgin olive oil	Soft drinks
r	0.284	0.186	0.195	0.241	0.230	0.166	0.145	0.136
P	<0.001	0.014	0.011	0.002	0.003	0.027	0.056	0.072

Multiple fractures	Age	HDL-C	Antiosteoporotic agents, calcium, Vitamin D supplementation	Eggs
r	−0.278	0.308	0.396	−0.392
P	0.086	0.068	0.013	0.014

WB: Whole body, BMI: Body mass index, HDL-C: High-density lipoprotein cholesterol, *r*: Relationship, *P*: Probability value

Table 4: Multivariable linear regression analysis - Whole egg consumption and other factors associated with WB T-score

Dependent variable					CI 95%	
WB T-score	*B*	SE	β	*P*	LL	UL
Gender (male)	0.856	0.192	0.329	<0.001	0.476	1.235
IMC	0.048	0.023	0.158	0.034	0.004	0.093
Eggs	0.020	0.009	0.166	0.026	0.002	0.038

WB T score excluded variable: Glucose, creatinine, diuretics, virgin olive oil, and soft drinks. WB: Whole body, *B*: Unstandardized coefficients, SE: Standard errors, *P*: Probability value, CI: Confidence interval, LL: Lower limit, UL: Upper limit

Table 5: Logistic regression analysis-Whole egg consumption and other factors associated with "multiple fractures" as binary variable (individuals with one fracture vs. those with more than one fracture)

Dependent variable					CI 95%	
Multiple fractures	*B*	SE	*P*	OR	LL	UL
HDL-C	0.095	0.044	0.032	1.100	1.008	1.200
Eggs	-0.265	0.122	0.029	0.767	0.604	0.973

Excluded variables: Age and antiosteoporotic agents, calcium, and Vitamin D supplementation. *B*: Unstandardized coefficients, SE: Standard errors, *P*: Probability value, OR: Odds ratio, CI: Confidence interval, LL: Lower limit, UL: Upper limit

The proteins present in the egg are also important on the bone health. It is known that the water-soluble proteins of the egg yolk stimulate the proliferation and differentiation of osteoblasts and inhibit the bone resorption activity induced by the tumor necrosis factor-α.[20] In particular, phosvitin, egg yolk protein, is able to induce the expression of type I collagen and osteocalcin during the differentiation of osteoblasts,[20] and it is also able to inhibit parathyroid hormone-induced osteoclastogenesis.[24] Furthermore, OVT, the main protein component of egg white, inhibits the activation of the pathways of the nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB) and of the mitogen-activated protein kinase.[21] In addition, it mediates the maturation of osteoclasts, and down-regulates numerous proteins involved in osteoclastogenesis[21] leading to apoptosis.[21]

Egg is mainly composed of polyunsaturated fatty acids, which are able to stimulate osteoblastogenesis and inhibit bone resorption[34] as well as regulate inflammation during the bone remodeling process[35] [Table 6].

All together, these evidences confirm our findings on the beneficial effect of consuming eggs on bone.

Furthermore, recent guidelines do not limit the intake of eggs.[36] A recent meta-analysis found no association between egg consumption and risk of cardiovascular disease in three large US cohorts.[37]

Considering the high nutritional value of eggs and their economic accessibility, whole egg intake represents an interesting strategy in preventing bone loss and fractures and, if confirmed through future randomized controlled trials, whole eggs could be considered as a functional food for the maintenance of bone health in elderly subjects.

The strengths of this study rely on the enrolment of individuals of both genders. Furthermore, we tested the association for multiple factors, including medications.

However, some limitation needs to be addressed. First, given the cross-sectional nature of the study, there is no evidence of a temporal relationship between exposure and outcome.

Despite our method is well validated and it is considered a reliable methods for determining food consumption,[38] we cannot exclude an overestimation or underestimation of egg consumption.[39] Finally, we did not assess bone turnover markers; however, there is a large daily variation in the serum concentration of these markers, especially in patients with fractures,[40] which may limit their usefulness. Our results generate hypotheses for future investigations and provide valuable information on the relationship between egg consumption and bone health in the elderly.

Conclusion

This cross-sectional study provides evidence of a positive link between whole egg consumption and WB-BMD. If confirmed through future randomized controlled trials, considering the high nutritional value

Table 6: Molecular mechanisms and effects on bone health of some active compounds contained in eggs

Active compounds	Research model (*in vitro*, *in vivo*, or human study)	Mechanisms	Main effects
Ovotransferrin	Mouse macrophage RAW 264.7	Ovotransferrin inhibited osteoclasts differentiation and the calcium-phosphate resorptive ability via the suppression of RANKL-induced NF-κB-enhancer of activated B cells and MAPK signaling pathways Ovotransferrin-induced apoptosis of matured osteoclasts	Inhibited osteoclastogenesis[21] Promoted osteoclasts apoptosis[21]
Yolk water-soluble proteins	MC3T3-E1 cells line	Yolk water-soluble proteins increased cell proliferation, collagen content, and ALP activity	Promoted cell proliferation and differentiation[20]
	Bone marrow cells from male mice	Yolk water-soluble proteins abolished TNF-α induced TRAP formation and activity	Inhibited osteoclastogenesis[20]
Phosvitin	MC3T3-E1 cells line	Phosvitin induces the expression of type I collagen and osteocalcin during osteoblast differentiation	Promoted bone mineralization[20]
	Mouse calvarial bone organ culture models	Phosvitin inhibited PTH-induced osteoclastic bone resorption and promoted new osteoid/bone formation	Inhibited osteoclastogenesis[24]
Total carotenoids, lycopene, and β-carotene	Human osteoblast-like cell Saos-2	Lycopene results in higher WNT/β-catenin and phERK1/2 protein than control. RUNX2 and COL1A mRNA increases, while RANKL mRNA decreases	Promoted cell differentiation, collagen production, and calcification process[22] Suppressed resorption[22]
	Postmenopausal women whit osteopenia	/	Prevented bone loss and reduction of bone alkaline phosphatase[22]
	Women and men aged ≥50 years	/	Reduction of the risk of hip fracture[33]
Choline	Middle-aged men and elderly women	/	Positive association between intakes of choline and BMD[32]
Polyunsaturated fatty acids	RAW 264.7 osteoclast differentiation model	PUFAs inhibited RANKL-induced osteoclast formation in a dose-dependent manner	Stimulated osteoblastogenesis and decreased bone resorption[34]
	6 weeks old female rats	ω-3 fatty acid DHA increased whole-body BMD compared with control animals	
	4 weeks old rats	Animals in the ω-3 given group demonstrated a 60% reduction in osteoclast number and an 80% decrease in bone resorption when compared with control animals	
	Animal studies	Polyunsaturated fatty acids have anti-inflammatory effects that protect bone (reduction IL-1β, IL-6, IL-11, TNF-α)	Regulation of inflammation markers[34]

NF-Kb: Nuclear factor κ-light chain, MAPK: Mitogen-activated protein kinase, ALP: Alkaline phosphatase, TRAP: -resistant acid phosphatase, TNF-α: Tumor necrosis factor-α, PTH: Parathyroid hormone, RUNX2: Runt-related transcription factor 2, COL1A: Collagen type I alpha, RANKL: Receptor activator of nuclear factor κB ligand, BMD: Bone mineral density, IL: Interleukin

of eggs, their economic accessibility on the marketplace, and recent dietary guidelines, the incorporation of whole eggs into the diet of the elderly could have a significant public health impact, including osteoporosis prevention and fracture risk reduction.

Acknowledgments

We would like to thank all the participants for their valuable time and contributions to this study. This study was supported by a grant from the Italian Ministry of Health (GR 2009-1567179).

Financial support and sponsorship

Nil.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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