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Whole egg consumption and cortical bone in healthy children

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Abstract

Summary—Eggs contain bioactive compounds thought to benefit pediatric bone. This cross-sectional study shows a positive link between childhood egg intake and radius cortical bone. If randomized trials confirm our findings, incorporating eggs into children’s diets could have a significant impact in preventing childhood fractures and reducing the risk of osteoporosis.

Introduction—This study examined the relationships between egg consumption and cortical bone in children.

Methods—The cross-sectional study design included 294 9–13-year-old black and white males and females. Three-day diet records determined daily egg consumption. Peripheral quantitative computed tomography measured radius and tibia cortical bone. Body composition and biomarkers of bone turnover were assessed using dual-energy X-ray absorptiometry and ELISA, respectively.

Results—Egg intake was positively correlated with radius and tibia cortical bone mineral content (Ct.BMC), total bone area, cortical area, cortical thickness, periosteal circumference, and polar strength strain index in unadjusted models ($r = 0.144$ – 0.224 , all $P < 0.050$). After adjusting for differences in race, sex, maturation, fat-free soft tissue mass (FFST), and protein intakes, tibia

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Compliance with ethical standards

The Institutional Review Boards for Human Subjects at all study sites approved the study procedures. Informed assent and permission were obtained from each participant and their parent/guardian, respectively.

Conflicts of interest None.

relationships were nullified; however, egg intake remained positively correlated with radius Ct.BMC ($r = 0.138$, $P = 0.031$). Egg intake positively correlated with total body bone mineral density, BMC, and bone area in the unadjusted models only ($r = 0.119$ – 0.224 ; all $P < 0.050$). After adjusting for covariates, egg intake was a positive predictor of radius FFST ($\beta = 0.113$, $P < 0.050$) and FFST was a positive predictor of Ct.BMC ($\beta = 0.556$, $P < 0.050$) in path analyses. There was a direct influence of egg on radius Ct.BMC ($\beta = 0.099$, $P = 0.035$), even after adjusting for the mediator, FFST ($\beta = 0.137$, $P = 0.020$). Egg intake was positively correlated with osteocalcin in both the unadjusted ($P = 0.005$) and adjusted ($P = 0.049$) models.

Conclusion—If the positive influence of eggs on Ct.BMC observed in this study is confirmed through future randomized controlled trials, whole eggs may represent a viable strategy to promote pediatric bone development and prevent fractures.

Keywords

Cortical bone; Egg; Fat-free soft tissue; pQCT

Introduction

The majority of evidence supporting the role of nutrition on bone health has focused on individual nutrients, particularly calcium, vitamin D, and protein [1–7]. There is strong evidence for supplemental calcium [7] to increase bone mineral accrual in children, whereas findings are less consistent with respect to vitamin D [5, 6] or protein [8,9] supplementation and their effects on childhood bone accrual. The osteogenic effects of these individual nutrients may be improved when packaged together and consumed contemporaneously [7–9] or as whole foods [10, 11]. For example, in a prospective study of children aged 15–17 years, consuming at least two servings of dairy per day, in comparison to fewer than two servings per day, was associated with significantly higher mean BMC and bone area [10]. In older adults, greater yogurt consumption is related to higher bone mineral density and physical function scores [11]. However, with the exception of dairy foods [9], little data exist on the effects of whole foods on bone health.

Eggs contain nutrients that may benefit bone health including vitamin D and zinc and osteogenic bioactive components, lutein and zeaxanthin. Whole eggs have been examined in prior studies with respect to their beneficial effects on bone and fracture outcomes, but egg intake was only considered in food groupings and not independently. For example, Inose et al. showed greater Z-scores for tibia cortical speed of sound, a measure associated with cortical strength, in mothers who consumed more milk, dairy products, and eggs [12]. In older adults, intake of red meat, but not poultry or eggs, was associated with greater risk for skeletal fractures [13]. While these two adult studies suggest the bone-augmenting potential of eggs, it is difficult to ascertain whether and to what degree eggs contributed to these positive relationships given they were not studied in isolation.

The ability of whole eggs to positively impact cortical bone may occur indirectly through effects on skeletal muscle mass. Skeletal muscle development precedes bone mass accrual during pubertal growth, and it is well documented that fat-free soft tissue (FFST) mass is the most important determinant of bone strength in youth [14, 15]. In young men, consumption

of 20 g of whole egg protein was shown to stimulate greater muscle protein synthesis following resistance exercise than in those not consuming whole egg protein [16]. Moreover, leucine, a primary amino acid in whole eggs, improved skeletal muscle synthesis in young adults [17]. Thus, it is possible that the amino acid and protein components of eggs may indirectly enhance bone strength through increased muscle mass.

The 2015 Dietary Guidelines for Americans de-emphasized the recommendation to limit cholesterol-rich foods, including eggs, which had been restricted in years past due to their perceived risk on cardiovascular health [18]. Reviews conducted by Shin et al. [19] and Griffin et al. [20] evaluated results from 16 and 12 studies, respectively, and provided the basis for the 2015 guidelines. These studies showed that egg consumption was not associated with hyperlipidemia, risk of cardiovascular disease, and cardiac mortality in the general population. Given that eggs are the least expensive source of high-quality protein per standard USDA serving, incorporating eggs into everyday diets may be a cost-effective strategy to benefit skeletal health in growing youth.

To our knowledge, studies investigating the associations between egg consumption and bone outcomes during the critical period of rapid pubertal growth have not been conducted. Therefore, the aim of this study was to cross-sectionally explore the relationships between whole egg intake and cortical bone parameters, total body bone outcomes, and biomarkers of bone turnover in children entering the early stages of puberty. A secondary aim was to determine if FFST has a mediating role on the relationship between egg intake and cortical bone.

Subjects and methods

Study design and participant characteristics

This study was a secondary analysis of baseline data obtained from a multi-site randomized controlled vitamin D clinical trial [21]. Study sites were in Georgia (University of Georgia [UGA]) and Indiana (Purdue University [PU] and Indiana University [IU]). Reported healthy participants who were free from chronic diseases ($N = 323$) were included in the parent study if they were between ages 9–13 years, in the early stages of puberty with self-reported sexual maturation ratings of 2 or 3 for breast development in females and genital development in males as described by Tanner [22], and self-reported non-Hispanic white or black race, with both sets of biological parents and grandparents all identifying as the same race. Exclusion criteria included menarche (for females), the presence of growth disorders/ chronic disease (e.g., cerebral palsy), or the use of medications (e.g., corticosteroids) known to influence bone metabolism. Power analyses were conducted to estimate the power of obtaining significant parameters when regressing outcome variables (FFST and Ct.BMC) on predicting variables. Based on the available sample size ($N = 323$), with a medium effect size of 0.30 and an alpha of 0.05, the expected statistical power for this cross-sectional study is greater than 0.99, which indicates a high probability to detect an existing effect in our data. Twenty-nine participants were excluded from this ancillary analysis due to incomplete diet records, as described below. The Institutional Review Boards for Human Subjects at all study sites approved the study procedures. Informed assent and permission were obtained from each participant and their parent/guardian, respectively.

Demographic and dietary assessment

With assistance from parents or guardians, 3-day diet records, a valid and reliable method for estimating energy and nutrient intakes in children [23–25], were completed at home on two weekdays and one weekend day. Records were analyzed by one trained registered dietitian nutritionist using Food Processor SQL version 9.7.3 (ESHA Research). Three-day diet records were used to estimate total whole egg intake (e.g., scrambled eggs, hard-boiled eggs, fried eggs) over 3 days, as well as mean daily energy (kcal) and mean daily protein (g) intakes. Egg consumption from mixed dishes was not quantified and therefore was not included in these analyses. Average measure (3-day) intraclass correlation coefficients (ICCs) were calculated in girls aged 6–10 years (N = 10), whose 3-day diet records were completed twice over 2 weeks and calculated for vitamin D, calcium, and energy (0.86).

Anthropometry

Height (to the nearest 0.1 cm) and body mass (to nearest 0.1 kg) were measured using a wall-mounted stadiometer and electronic scale, respectively. Body mass index (BMI, in kilograms per meter squared) was calculated, and sex- and age-specific percentiles were derived using a BMI percentile calculator [26]. Single-measure ICCs and test-retest coefficients of variation (CV) were determined previously in our lab for standing height (0.99 and 0.4%) and body weight (0.99 and 1.4%) in females aged 6–10 years (N= 10) who were measured twice over a 2-week period by the same researcher.

Body composition and whole body bone measurements

Fat mass, percent fat, and FFST were assessed using dualenergy X-ray absorptiometry (DXA; Delphi-A, Hologic Inc. [UGA]; Lunar iDXA, GE Medical Instruments [PU]; and Hologic Discovery-W [IU]). Whole body bone mineral density (BMD), bone mineral content (BMC), and bone area were also assessed with DXA. At each study site, the same technician completed scans and performed analyses using instrument-specific software and protocols. ICCs were calculated for body composition in females, aged 5–8 years (N= 10), scanned twice at UGA over 7 days (all 0.98). As previously reported, DXA scanners at each testing site were cross-calibrated, and regression formulae were determined to the adjusted data [21, 27, 28].

Cortical bone measurements

Cortical bone was assessed using peripheral quantitative computed tomography (pQCT) at each study site using Stratec XCT 2000 machines (Stratec Medizintechnik GmbH, Pforzheim, Germany), as reported previously [27]. A single tomographic slice was taken at the tibia and radius 66% site relative to the distal growth plate. Subjects were positioned supine with their self-reported non-dominant leg and arm in the center of the gantry of the pQCT machine. A cortical bone phantom specific to the pQCT machine with known properties was scanned a minimum of 20 times on each scanner to ensure comparability of machines between each testing site. The variation in phantom measures differed by < 1% [27].

Cort mode 1 (threshold, 710 mg/cm³) was used to obtain cortical volumetric bone mineral density (Ct.vBMD, mg/cm³), cortical bone mineral content (Ct.BMC, mg/mm), and cortical

area (Ct.Ar, cm²) and to define the outermost edge of the bone. Peel mode 2 (threshold, 400 mg/cm³) was used to separate the cancellous and cortical bone compartments. Total bone area (Tt.Ar, mm²), cortical thickness (Ct.Th, mm), periosteal perimeter (Ps.Pm, mm), and endosteal perimeter (Es.Pm, mm) were also measured. This same threshold was used to calculate polar-strength strain index (pSSI, mm³), which is derived from Ct.vBMD, section modulus, and normal physiological bone density that is estimated at 12,000 mg/mm³ [27, 29, 30]. Using a F03F05 filer (contour mode 3 [threshold of – 100 mg/cm³] and peel mode 2 [threshold of 40 mg/cm³]), muscle cross-sectional area (MCSA) was measured. Five healthy females (ages 18–24 years) were scanned at the UGA site to determine test-retest reliability [31]. One-way random effects model and single-measure ICCs for all pQCT variables were $R = 0.97$. At the IU site, short-term precision for the pQCT scanning procedure on 30 healthy individuals scanned six times with interim repositioning showed root mean square coefficients of variation (RMS-CVs) of < 1% for bone density, mass, structure, and estimated strength measures and < 1.5% for mCSA [32].

Biochemical analyses

Blood and urine samples were collected in the morning following an overnight fast. All samples were prepared for storage and frozen in a < – 80 °C freezer until analyses. Reference controls (kits) and internal controls (in-house pooled samples) were included with each assay run for quality control. Repeat analyses were conducted when duplicate samples differed by > 10%. Serum osteocalcin (OC) and bone-specific alkaline phosphatase (BSAP) were assessed as measures of bone formation and were measured by ELISA (Quidel Corp., San Diego, CA). Urine N-terminal telopeptide (NTX) was assessed as a measure of bone resorption and was measured by ELISA (Ostex International, Seattle, WA). Mean interassay CVs for the bone turnover markers ranged from 4.1 to 8.0%. Serum 25(OH)D was assessed using a 2-step RIA (Diasorin). The inter- and intra-assay CV were 5.6 to 8.4% and 5.5 to 7.0%, respectively. Analytical reliability of 25(OH)D assays was further monitored through DEQAS (the Vitamin D External Quality Assessment Scheme).

Statistical analyses

Data were analyzed using SPSS version 21 (SPSS, Inc.) for the Mac OS X. Histograms were visually inspected for outliers and normal distribution. Distributions were classified as skewed or kurtotic if > 2.0 standard deviations (SDs). An outlier was detected when visually inspecting the histogram of 3-day egg consumption. Given that one participant reported consumption of 12 whole eggs during the 3-day dietary recall period, this participant was removed from our data. Because 3-day egg consumption, urine NTX, and serum OC each had positive skewed distributions, they were log-transformed (i.e., NTX) or square-root-transformed (i.e., 3-day egg consumption and OC) prior to analyses. Pearson's bivariate and partial correlations were conducted to determine the association between egg intake and whole body bone outcomes and cortical bone outcomes while adjusting for variance related to stage of sexual maturation, sex, race, FFST, and average 3-day daily total protein intake. Using Mplus software (version 7.31), path analysis was performed to examine the FFST-mediated relationship between egg intake and Ct.BMC. Indirect effects tests were tested using the product coefficient method [33]. Each of the abovementioned path models was

identified and included sexual maturation, race, and sex as covariates. A P value < 0.05 was considered statistically significant for all analyses.

Results

Participant characteristics

Descriptive participant characteristics are presented in Table 1. Approximately 30% of study participants consumed eggs during the 3-day dietary recording period ($n = 87$). Between-group differences were assessed by independent samples t tests. There were statistically significant differences in stages of sexual maturation between egg consumers and non-egg consumers (Table 1). Moreover, egg consumers were significantly taller and heavier and had greater FFST in comparison to the non-consumers. Total protein intake was higher in egg consumers; however, energy intake did not differ between the two groups.

Egg consumption and cortical bone outcomes

Egg consumption was positively associated with mid-tibia and mid-radius Ct.BMC, Tt.Ar, Ct.Ar, Ct.Th, PC, and pSSI (Table 2). After adjustments for stage of sexual maturation, sex, race, FFST, and average total protein intake, the relationships between egg consumption and mid-tibia cortical bone outcomes were nullified, but egg intake remained positively correlated with mid-radius Ct.BMC ($P = 0.031$, Table 2).

Egg consumption and total body outcomes

Egg consumption was positively associated with total body FFST in the unadjusted model ($r = 0.185$, $P = 0.001$) and remained positively associated with total body FFST when adjusting for the covariates, stage of sexual maturation, sex, race, and average total protein intake ($r = 0.126$, $P = 0.033$). Egg intake was positively associated with total body bone mineral density (BMD; $r = 0.181$, $P = 0.002$), bone mineral content (BMC; $r = 0.224$, $P < 0.001$), and bone area (BA; $r = 0.119$, $P = 0.043$) in the unadjusted model. After controlling for the covariates, all total body relationships were nullified.

Mediating role of FFST in the association between egg intake and Ct.BMC at the mid-radius

The path models presented in Fig. 1 represent the FFST-dependent relationship between egg intake and mid-radius Ct.BMC while controlling for race, sex, and stage of sexual maturation. Three-day egg intake was a positive predictor of FFST ($P = 0.027$) and FFST was a positive predictor of Ct.BMC ($P < 0.001$). After adjusting for the mediator, FFST, egg intake remained a positive predictor of Ct.BMC ($P < 0.050$). The test for an indirect effect was statistically significant ($P = 0.020$).

Egg consumption and bone biomarkers

Egg consumption was positively correlated with OC ($r = 0.170$, $P = 0.005$) and negatively correlated with both BSAP ($r = -0.084$, $P = 0.163$) and NTX ($r = -0.128$, $P = 0.033$). After controlling for race, sex, stage of sexual maturation, and average total protein intake, these relationships were nullified except for OC ($r = 0.120$, $P = 0.049$).

Discussion

The aim of this cross-sectional study was to determine whether whole egg intake was associated with cortical bone strength and biomarkers of bone turnover in healthy children. The secondary aim of this study was to determine whether FFST mediates the relationship between egg and bone. The primary finding was that egg intake was a positive predictor of mid-radius Ct.BMC and that the association between egg intake and Ct.BMC was partially mediated through FFST. With the exception of dairy foods, including a recent study using yogurt [9, 11, 34, 35], limited research exists regarding the connection between whole food consumption and bone outcomes in children. The present study is the first to assess the relationships between whole egg intake and radius and tibia cortical bone in children. The only other study that examined egg intake and bone strength used a composite measure of eggs and dairy intake, which showed positive linkages between tibia cortical speed of sound in adult females [12]. The degree to which eggs alone contributed to these positive relationships was not ascertained.

The positive relationship between whole egg intake and radius cortical bone observed in the current study could be related to the protein component of eggs, as cross-sectional [34–37] and prospective [4, 38–40] studies in children support the role of dietary protein on bone. For example, Bounds et al. [39] showed that protein intake in children is a positive predictor of total body BMC. Alexy et al. [4] reported that dietary protein intake over 4 years during growth is associated with increased diaphyseal bone strength, specifically increases in PC, Ct.Ar, Ct.BMC, and pSSI. Randomized controlled trials have not been conducted and are needed to confirm the results of the cross-sectional and prospective protein studies.

Beyond protein, components of eggs that may benefit bone health include nutrients, such as vitamin D and zinc and bio-active components, including lutein and zeaxanthin. There is moderate evidence supporting a role of vitamin D for improving bone outcomes in children and adolescents [7], and zinc supplementation in children has been shown to increase bone formation [41]. Lutein and zeaxanthin, bioactive components present in eggs, have known anti-inflammatory effects [42]. These dietary factors may mediate a potential benefit of egg consumption on bone geometry, as the pro-inflammatory cytokines tumor necrosis factor alpha, interleukin-6, and C-reactive protein have a negative effect on bone strength [43, 44]. Assessments of biomarkers of inflammation in future studies can provide additional insight into the anti-inflammatory roles of whole eggs and bone outcomes.

In addition to a direct effect of whole eggs on bone, it is possible that eggs could improve bone strength through actions on lean body mass. In our path model, FFST mediated the relationship between egg intake and Ct.BMC. This novel finding is supported by previous studies showing that protein and leucine stimulate muscle protein synthesis [17, 45] and skeletal muscle mass is a strong determinant of pediatric bone mass [14, 15, 46]. The significant positive relationships between 3-day egg intakes and FFST mass reported in the current study are supported by a short-term intervention study by Candow and colleagues [45] who showed that independent of source, young adults who consumed a protein supplement during resistance training had increases in lean tissue mass and strength vs. those who consumed an isocaloric placebo during resistance training ($P < 0.050$). Our data

showing that FFST mass is a significant predictor of radius Ct.BMC is in agreement with Crabtree et al. [47] who demonstrated that among school children aged 5–18 years living in the UK, lean body mass was the strongest predictor of BMC at the total body and lumbar spine. In a recent review, the authors reaffirmed the importance of FFST mass as a strong positive predictor of cortical bone geometry [46].

Though our data showed a positive association between egg intake and radius Ct.BMC, we did not find significant relationships between egg consumption and mid-tibia cortical bone outcomes and total body bone outcomes after adjusting for the covariates. Measures of areal bone mineral density may underestimate the strength of the bone given it does not provide information regarding trabecular and cortical bone properties. While there are no studies investigating egg intake on cortical bone outcomes, the site-specific differences reported in the present study are consistent with pediatric physical activity investigations. For example, Jackowski et al. [48] did not show a difference between recreational gymnastic, an activity known to exert positive influences on bone mineral accrual during growth, or control, on bone mineral content and bone area at the tibia in children aged 4–12 years. However, increased bone mineral content and area were seen at the distal radius in those children who participated in recreational gymnastics. Thus, it is plausible that mechanical loading associated with compression forces on the lower limbs with weight bearing masked any potential effects of gymnastic, or in the present study, egg intake, on tibia outcomes. It is also possible that the null findings of egg consumption on tibia bone outcomes were associated with weight bearing masking effects. Future intervention trials should account for site-specific effects of diet and load bearing, including physical activity, when assessing the relationship between egg intake and bone outcomes.

The positive relationship between egg consumption and the bone formation marker OC, following adjustments for key covariates, supports the mediation model and provides potentially some insight into mechanisms for the link between eggs and bone. To our knowledge, there are no prior studies examining the relationships between egg consumption and markers of bone turnover in children. A number of clinical studies have investigated the effects of protein intake on bone biomarkers and the findings are equivocal. For example, biomarkers of bone resorption and formation have been reported to decrease [49, 50], remain the same [51–53], or increase [54] following protein supplementation. It has been reported that milk protein supplementation in healthy women leads to a reduction in NTX and no change in BSAP or OC [55]. Further, since OC is not the ideal marker for bone formation and most likely better reflects overall bone turnover [56], a more specific marker of pediatric bone formation like N-terminal propeptide of type 1 procollagen should be assessed. Randomized controlled egg feeding trials will help to improve our understanding of the role of whole eggs on bone turnover.

The strength of this study is the utilization of path analysis statistical techniques to explore the mediation effects of FFST on the relationship of egg intake and Ct.BMC. However, when interpreting our results, certain aspects of the study should be considered. First, given its cross-sectional design, we cannot confirm causality when linking egg intake and bone. Second, participants self-reported their dietary intakes, which may have resulted in either over- or under-reporting of food consumption [57]. Though the 3-day dietary recall used in

our study is a valid method in children [23–25], another limitation of the study is our single-measure assessment of egg intakes. Given this study was a secondary analysis on baseline data from a randomized controlled trial and not originally intended to assess egg intake, more complete egg intake data, including egg intakes from mixed dishes, was not collected and is needed in future trials. Despite these limitations, our results are hypothesis generating and provide valuable insight into the relationship between egg consumption and musculoskeletal outcomes in children. Importantly, our results provide the basis to explore in clinical trials the viability of whole egg intake to increase bone strength and prevent childhood and adult fractures.

This cross-sectional study provides novel evidence of a positive link between whole egg consumption and cortical bone in healthy, black, and white children in the early stages of puberty, and we showed for the first time that FFST mediated these relationships. Considering the liberalization of egg intake recommendations in the 2015 Dietary Guidelines for Americans report, coupled with the affordability of eggs in the marketplace, greater incorporation of eggs into children's diets is feasible and could have a significant public health impact, including fracture risk reduction and osteoporosis prevention. Future randomized controlled trials are needed, however, to confirm the positive impact of egg intake on pediatric musculoskeletal development.

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References

1. Dibba B, Prentice A, Ceesay M, Stirling DM, Cole TJ, Poskitt EM (2000) Effect of calcium supplementation on bone mineral accretion in Gambian children accustomed to a low-calcium diet. *Am J Clin Nutr* 71:544–549
2. Moyer-Mileur LJ, Xie B, Ball SD, Pratt T (2003) Bone mass and density response to a 12-month trial of calcium and vitamin D supplement in preadolescent girls. *J Musculoskelet Neuronal Interact* 3:63–70 [PubMed: 15758367]
3. Ballard TL, Specker BL, Binkley TL, Vukovich MD (2006) Effect of protein supplementation during a 6-month strength and conditioning program on areal and volumetric bone parameters. *Bone* 38:898–904 [PubMed: 16364710]
4. Alexy U, Remer T, Manz F, Neu CM, Schoenau E (2005) Long-term protein intake and dietary potential renal acid load are associated with bone modeling and remodeling at the proximal radius in healthy children. *Am J Clin Nutr* 82:1107–1114 [PubMed: 16280446]
5. El-Hajj Fuleihan G, Nabulsi M, Tamim H, Maalouf J, Salamoun M, Khalife H, Choucair M, Arabi A, Vieth R (2006) Effect of vitamin D replacement on musculoskeletal parameters in school children: a randomized controlled trial. *J Clin Endocrinol Metab* 91:405–412 [PubMed: 16278262]
6. Viljakainen HT, Natri AM, Karkkainen M, Huttunen MM, Palssa A, Jakobsen J, Cashman KD, Molgaard C, Lamberg-Allardt C (2006) A positive dose-response effect of vitamin D supplementation on site-specific bone mineral augmentation in adolescent girls: a double-blinded randomized placebo-controlled 1-year intervention. *J Bone Miner Res* 21:836–844 [PubMed: 16753014]
7. Weaver CM, Gordon CM, Janz KF, Kalkwarf HJ, Lappe JM, Lewis R, O'Karma M, Wallace TC, Zemel BS (2016) The National Osteoporosis Foundation's position statement on peak bone mass development and lifestyle factors: a systematic review and implementation recommendations. *Osteoporos Int* 27:1281–1386 [PubMed: 26856587]

8. Kontogianni MD, Melistas L, Yannakoulia M, Malagaris I, Panagiotakos DB, Yiannakouris N (2009) Association between dietary patterns and indices of bone mass in a sample of Mediterranean women. *Nutrition* 25:165–171 [PubMed: 18849146]
9. Okubo H, Sasaki S, Horiguchi H, Oguma E, Miyamoto K, Hosoi Y, Kim MK, Kayama F (2006) Dietary patterns associated with bone mineral density in premenopausal Japanese farmwomen. *Am J Clin Nutr* 83:1185–1192 [PubMed: 16685064]
10. Moore LL, Bradlee ML, Gao D, Singer MR (2008) Effects of average childhood dairy intake on adolescent bone health. *JPediatr* 153:667–673 [PubMed: 18701115]
11. Laird E, Molloy AM, McNulty H, Ward M, McCarroll K, Hoey L, Hughes CF, Cunningham C, Strain JJ, Casey MC (2017) Greater yogurt consumption is associated with increased bone mineral density and physical function in older adults. *Osteoporos Int* 28:2409–2419 [PubMed: 28462469]
12. Inose T, Takano T, Nakamura K, Kizuki M, Seino K (2006) Tibial cortical bone properties of preadolescents and their mothers in an urban area associated with lifestyle: a longitudinal study. *Acta Paediatr* 95:276–282 [PubMed: 16497636]
13. Zeng FF, Fan F, Xue WQ, Xie HL, Wu BH, Tu SL, Ouyang WF, Chen YM (2013) The association of red meat, poultry, and egg consumption with risk of hip fractures in elderly Chinese: a case-control study *Bone* 56:242–248 [PubMed: 23816759]
14. Baptista F, Barrigas C, Vieira F, Santa-Clara H, Homens PM, Fragoso I, Teixeira PJ, Sardinha LB (2012) The role of lean body mass and physical activity in bone health in children. *J Bone Miner Metab* 30:100–108 [PubMed: 21732232]
15. Hamrick MW (2012) The skeletal muscle secretome: an emerging player in muscle-bone crosstalk. *Bonekey Rep* 1:60 [PubMed: 23951457]
16. Moore DR, Robinson MJ, Fry JL, Tang JE, Glover EI, Wilkinson SB, Prior T, Tarnopolsky MA, Phillips SM (2009) Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. *Am J Clin Nutr* 89:161–168 [PubMed: 19056590]
17. Glynn EL, Fry CS, Drummond MJ, Timmerman KL, Dhanani S, Volpi E, Rasmussen BB (2010) Excess leucine intake enhances muscle anabolic signaling but not net protein anabolism in young men and women. *J Nutr* 140:1970–1976 [PubMed: 20844186]
18. DeSalvo KB, Olson R, Casavale KO (2016) Dietary guidelines for Americans. *JAMA* 315:457–458 [PubMed: 26746707]
19. Shin JY, Xun P, Nakamura Y, He K (2013) Egg consumption in relation to risk of cardiovascular disease and diabetes: a systematic review and meta-analysis. *Am J Clin Nutr* 98: 146–159 [PubMed: 23676423]
20. Griffin JD, Lichtenstein AH (2013) Dietary cholesterol and plasma lipoprotein profiles: randomized-controlled trials. *Curr Nutr Rep* 2:274–282 [PubMed: 24466502]
21. Lewis RD, Laing EM, Hill Gallant KM, Hall DB, McCabe GP, Hausman DB, Martin BR, Warden SJ, Peacock M, Weaver CM (2013) A randomized trial of vitamin D(3) supplementation in children: dose-response effects on vitamin D metabolites and calcium absorption. *J Clin Endocrinol Metab* 98:4816–4825 [PubMed: 24092833]
22. Tanner JM (1975) The measurement of maturity. *Trans Eur Orthod Soc*:45–60 [PubMed: 1072163]
23. Crawford PB, Obarzanek E, Morrison J, Sabry ZI (1994) Comparative advantage of 3-day food records over 24-hour recall and 5-day food frequency validated by observation of 9- and 10- year-old girls. *J Am Diet Assoc* 94:626–630 [PubMed: 8195550]
24. Bergman EA, Boyungs JC, Erickson ML (1990) Comparison of a food frequency questionnaire and a 3-day diet record. *J Am Diet Assoc* 90:1431–1433 [PubMed: 2212429]
25. Taylor RW, Goulding A (1998) Validation of a short food frequency questionnaire to assess calcium intake in children aged 3 to 6 years. *Eur J Clin Nutr* 52:464–465 [PubMed: 9683402]
26. Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, Flegal KM, Guo SS, Wei R, Mei Z, Curtin LR, Roche AF, Johnson CL (2000) CDC growth charts: United States. *Adv Data*:1–27
27. Warden SJ, Hill KM, Ferira AJ, Laing EM, Martin BR, Hausman DB, Weaver CM, Peacock M, Lewis RD (2013) Racial differences in cortical bone and their relationship to biochemical variables in Black and White children in the early stages of puberty. *Osteoporos Int* 24:1869–1879 [PubMed: 23093348]

28. Ferira AJ, Laing EM, Hausman DB, Hall DB, McCabe GP, Martin BR, Hill Gallant KM, Warden SJ, Weaver CM, Peacock M, Lewis RD (2016) Vitamin D supplementation does not impact insulin resistance in Black and White children. *J Clin Endocrinol Metab* 101:1710–1718 [PubMed: 26885880]
29. Macdonald H, Kontulainen S, Petit M, Janssen P, McKay H (2006) Bone strength and its determinants in pre- and early pubertal boys and girls. *Bone* 39:598–608 [PubMed: 16600704]
30. Rauch F, Schoenau E (2008) Peripheral quantitative computed tomography of the proximal radius in young subjects—new reference data and interpretation of results. *J Musculoskelet Neuronal Interact* 8:217–226 [PubMed: 18799854]
31. Pollock NK, Laing EM, Baile CA, Hamrick MW, Hall DB, Lewis RD (2007) Is adiposity advantageous for bone strength? A peripheral quantitative computed tomography study in late adolescent females. *Am J Clin Nutr* 86:1530–1538 [PubMed: 17991669]
32. Swinford RR, Warden SJ (2010) Factors affecting short-term precision of musculoskeletal measures using peripheral quantitative computed tomography (pQCT). *Osteoporos Int* 21: 1863–1870 [PubMed: 20052457]
33. Preacher KJ, Hayes AF (2008) Asymptotic and resampling strategies for assessing and comparing indirect effects in multiple mediator models. *Behav Res Methods* 40:879–891 [PubMed: 18697684]
34. Hoppe C, Molgaard C, Michaelsen KF (2000) Bone size and bone mass in 10-year-old Danish children: effect of current diet. *Osteoporos Int* 11:1024–1030 [PubMed: 11256893]
35. Iuliano-Burns S, Stone J, Hopper JL, Seeman E (2005) Diet and exercise during growth have site-specific skeletal effects: a co-twin control study. *Osteoporos Int* 16:1225–1232 [PubMed: 15782284]
36. Chevalley T, Bonjour JP, Ferrari S, Rizzoli R (2008) High-protein intake enhances the positive impact of physical activity on BMC in prepubertal boys. *J Bone Miner Res* 23:131–142 [PubMed: 17892378]
37. Ekbote VH, Khadilkar AV, Chiplonkar SA, Khadilkar VV (2011) Determinants of bone mineral content and bone area in Indian pre-school children. *J Bone Miner Metab* 29:334–341 [PubMed: 20941516]
38. Remer T, Krupp D, Shi L (2014) Dietary protein's and dietary acid load's influence on bone health. *Crit Rev Food Sci Nutr* 54:1140–1150 [PubMed: 24499146]
39. Bounds W, Skinner J, Carruth BR, Ziegler P (2005) The relationship of dietary and lifestyle factors to bone mineral indexes in children. *J Am Diet Assoc* 105:735–741 [PubMed: 15883550]
40. Vatanparast H, Bailey DA, Baxter-Jones AD, Whiting SJ (2007) The effects of dietary protein on bone mineral mass in young adults may be modulated by adolescent calcium intake. *J Nutr* 137:2674–2679 [PubMed: 18029482]
41. Berger PK, Pollock NK, Laing EM, Chertin V, Bernard PJ, Grider A, Shapses SA, Ding KH, Isaacs CM, Lewis RD (2015) Zinc supplementation increases procollagen type I amino-terminal propeptide in premenarcheal girls: a randomized controlled trial. *J Nutr* 145:2699–2704 [PubMed: 26491117]
42. Andersen CJ (2015) Bioactive egg components and inflammation. *Nutrients* 7:7889–7913 [PubMed: 26389951]
43. Agrawal M, Arora S, Li J, Rahmani R, Sun L, Steinlauf AF, Mechanick JI, Zaidi M (2011) Bone, inflammation, and inflammatory bowel disease. *Curr Osteoporos Rep* 9:251–257 [PubMed: 21935582]
44. Romas E, Gillespie MT (2006) Inflammation-induced bone loss: can it be prevented? *Rheum Dis Clin N Am* 32:759–773
45. Candow DG, Chilibeck PD, Facci M, Abeysekera S, Zello GA (2006) Protein supplementation before and after resistance training in older men. *Eur J Appl Physiol* 97:548–556 [PubMed: 16767436]
46. Kindler JM, Lewis RD, Hamrick MW (2015) Skeletal muscle and pediatric bone development. *Curr Opin Endocrinol Diabetes Obes* 22:467–474 [PubMed: 26414082]

47. Crabtree NJ, Kibirige MS, Fordham JN, Banks LM, Muntoni F, Chinn D, Boivin CM, Shaw NJ (2004) The relationship between lean body mass and bone mineral content in paediatric health and disease. *Bone* 35:965–972 [PubMed: 15454104]
48. Jackowski SA, Baxter-Jones AD, Gruodyte-Raciene R, Kontulainen SA, Erlandson MC (2015) A longitudinal study of bone area, content, density, and strength development at the radius and tibia in children 4–12 years of age exposed to recreational gymnastics. *Osteoporos Int* 26:1677–1690 [PubMed: 25740207]
49. Cassidy A (2003) Dietary phytoestrogens and bone health. *J Br Menopause Soc* 9:17–21 [PubMed: 12804308]
50. Coon KA, Tucker KL (2002) Television and children's consumption patterns. A review of the literature. *Minerva Pediatr* 54:423–436 [PubMed: 12244280]
51. Sadler LS, Pober BR, Grandinetti A, Scheiber D, Fekete G, Sharma AN, Urban Z (2001) Differences by sex in cardiovascular disease in Williams syndrome. *J Pediatr* 139:849–853 [PubMed: 11743512]
52. Alekel DL, Germain AS, Peterson CT, Hanson KB, Stewart JW, Toda T (2000) Isoflavone-rich soy protein isolate attenuates bone loss in the lumbar spine of perimenopausal women. *Am J Clin Nutr* 72:844–852 [PubMed: 10966908]
53. Arjmandi BH, Khalil DA, Smith BJ, Lucas EA, Juma S, Payton ME, Wild RA (2003) Soy protein has a greater effect on bone in postmenopausal women not on hormone replacement therapy, as evidenced by reducing bone resorption and urinary calcium excretion. *J Clin Endocrinol Metab* 88:1048–1054 [PubMed: 12629084]
54. Anderson JJ, Chen X, Boass A, Symons M, Kohlmeier M, Renner JB, Garner SC (2002) Soy isoflavones: no effects on bone mineral content and bone mineral density in healthy, menstruating young adult women after one year. *J Am Coll Nutr* 21:388–393 [PubMed: 12356779]
55. Toba Y, Takada Y, Matsuoka Y et al. (2001) Milk basic protein promotes bone formation and suppresses bone resorption in healthy adult men. *Biosci Biotechnol Biochem* 65:1353–1357 [PubMed: 11471735]
56. Kanbur NO, Derman O, Sen TA, Kinik E (2002) Osteocalcin. A biochemical marker of bone turnover during puberty. *Int J Adolesc Med Health* 14:235–244 [PubMed: 12467198]
57. Subar AF, Freedman LS, Tooze JA, Kirkpatrick SI, Boushey C, Neuhaus ML, Thompson FE, Potischman N, Guenther PM, Tarasuk V, Reedy J, Krebs-Smith SM (2015) Addressing current criticism regarding the value of self-report dietary data. *J Nutr* 145:2639–2645 [PubMed: 26468491]

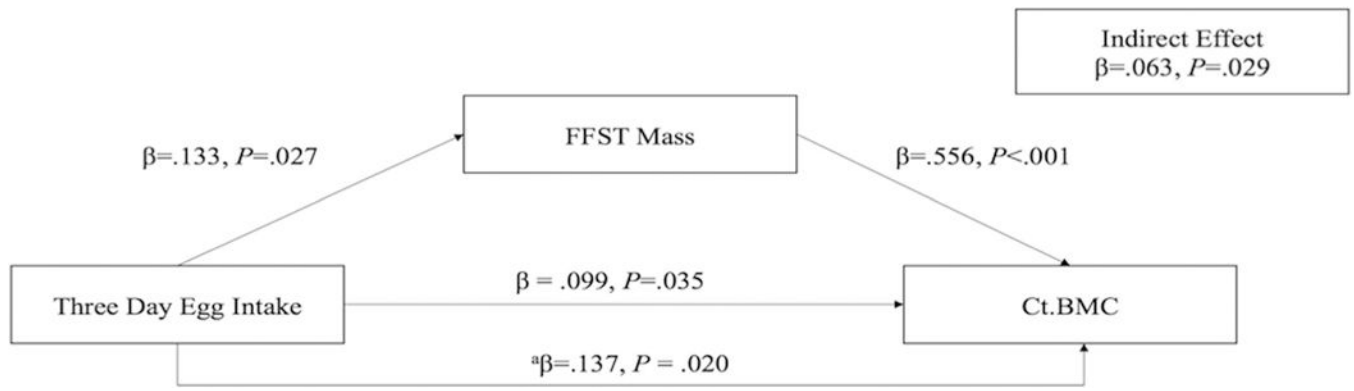


Fig. 1. FFSTmass is a partialmediator in the relationship between dietary egg intake and mid-radius Ct.BMC. Race, sex, and sexual maturation rating stage were included in this model. ^a Indicates the relationship between 3-day egg consumption and mid-radius Ct.BMC after adjusting for race, sex, and sexual maturation rating stage. FFST, fat-free soft tissue; Ct.BMC, cortical bone mineral content

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Table 1

Participant characteristics

	Total cohort N = 294	Egg consumers n = 87	Non-egg consumers n = 207	P ^a
Demographics				
Age, years	11.5 ± 1.2	11.7 ± 1.3	11.3 ± 1.2	0.004
Sex (male), n	162 (55%)	34 (12%)	128 (44%)	0.027
Race (black), n	165 (56%)	49 (17%)	116 (39%)	0.014
Sexual maturation stage (2/3)	211 (72%)/83 (28%)	50 (17%)/20 (7.0%)	161 (55%)/63 (21%)	0.040
Anthropometrics				
Height, cm	150.7 ± 9.3	153.5 ± 9.6	149.9 ± 9.1	0.002
Weight, kg	47.4 ± 12.2	53.1 ± 34.0	48.9 ± 12.5	0.002
BMI percentile	68.0 ± 29.2	69.6 ± 27.0	66.3 ± 30.5	0.352
BMI-for-age, Z-score	0.67 ± 1.1	0.75 ± 1.0	0.57 ± 1.1	0.196
Tibia length, cm	349.6 ± 35.2	360.3 ± 34.3	346.4 ± 35.6	< 0.001
Radius length, cm	245.8 ± 25.4	255.8 ± 25.3	242.5 ± 24.9	< 0.001
Body composition				
Body fat, %	31.1 ± 9.4	29.6 ± 9.0	31.5 ± 9.5	0.992
Fat mass, kg	14.7 ± 7.4	15.1 ± 7.7	14.5 ± 7.1	< 0.001
FFST mass, kg	30.4 ± 6.9	33.1 ± 7.4	29.4 ± 6.4	0.352
Serum biomarkers				
25(OH)D ₃ , nmol/L	69.9 ± 18.5	67.4 ± 18.5	70.9 ± 19.0	0.182
Physical activity				
Average 3-day EE, METS/day	62.2 ± 10.0	63.2 ± 11.1	62.0 ± 9.5	0.384
Nutrition				
Average kcal consumed, kcal/day	2004.3 ± 628.5	2097.0 ± 632.7	1975.4 ± 624.3	0.132
Average 3-day PRO intake, g/day	77.1 ± 26.3	85.0 ± 29.5	74.4 ± 24.1	0.004

	Total cohort <i>N</i> = 294	Egg consumers <i>n</i> = 87	Non-egg consumers <i>n</i> = 207	<i>p</i> ^a
Average 3-day PRO intake, gm/1000 kcal	39.9 ± 11.4	42.2 ± 11.1	38.9 ± 11.4	0.025
Average 3-day egg intake		0.7 ± 0.4		

Data presented as mean ± SD unless otherwise indicated

BMI body mass index, *FFST* fat-free soft tissue, *EE* energy expenditure, *METS* metabolic equivalents, *PRO* protein

^aTest of between-group significance based on independent samples *t* test

Table 2
Bivariate and partial correlations between dietary egg intake and cortical bone outcomes

	Tibia						Radius					
	Unadjusted			Adjusted			Unadjusted			Adjusted		
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
MCSA	0.188	0.001			0.218	<0.001						
CLvBMD	0.040	0.506	0.032	0.620	0.082	0.184	0.016	0.799				
CLBMC	0.208	<0.001	0.039	0.539	0.224	<0.001	0.138	0.031				
Tt.Ar	0.151	0.011	-0.043	0.504	0.150	0.015	0.078	0.223				
CL.Ar	0.202	0.001	0.031	0.628	0.181	0.003	0.053	0.406				
CL.Th	0.188	0.002	0.073	0.255	0.144	0.020	0.025	0.694				
PC	0.146	0.014	-0.047	0.467	0.155	0.012	0.086	0.183				
EC	0.070	0.240	-0.071	0.270	0.051	0.408	0.045	0.484				
pSSI	0.186	0.002	-0.001	0.986	0.166	0.007	0.054	0.401				

Statistically significant at $P < 0.05$; $N = 294$; adjusted for race, sex, sexual maturation rating stage, FFST, and protein intakes (g)

FFST fat-free soft tissue, MCSA muscle cross-sectional area, CLvBMD volumetric cortical bone mineral density, CLBMC cortical bone mineral content, Tt.Ar total area, CL.Ar cortical area, CL.Th cortical thickness, PC periosteal circumference, EC endosteal circumference, pSSI polar strength strain index